Causes and Risk of Stroke
The Rotterdam Study

Michiel Bos

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Causes and Risk of Stroke
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Publications and manuscripts based on the studies described in this thesis

Chapter 2.1
Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Serum uric acid is a risk factor for myocardial infarction and stroke. *Stroke 2006 Jun;37(6):1503-7*

Chapter 2.2
Bos MJ, Koudstaal PJ, Hofman A, Breteler MM. Decreased glomerular filtration rate is a risk factor for hemorrhagic but not for ischemic stroke. *Stroke 2007 Dec; 38(12):3127-32*

Chapter 2.3
Bos MJ, Koudstaal PJ, Witteman JCM, Hofman A, Breteler MMB. Transcranial Doppler hemodynamic indices and risk of stroke. *Stroke 2007 Sep;38(9):2453-8*

Chapter 2.4
Haag MDM, Bos MJ, Hofman A, Koudstaal PJ, Breteler MMB, Stricker B COX-selectivity of non-steroidal anti-inflammatory drugs and risk of stroke. *Submitted*

Chapter 3.1
van Rijn MJ, Bos MJ, Yazdanpanah M, Isaacs A, Arias-Vasquez A, Koudstaal PJ, Hofman A, Witteman JC, van Duijn CM, Breteler MM. Alpha-adducin polymorphism and the risk of atherosclerosis, cardiovascular and cerebrovascular disease. *Stroke 2006 Dec;37(12):2930-4*

Chapter 3.2
van Rijn MJE, Bos MJ, Isaacs A, Yazdanpanah M, Arias-Vásquez A, Koudstaal PJ, Witteman JC, Hofman A, Breteler MMB, van Duijn CM. Polymorphisms of the Renin-Angiotensin System and the risk of atherosclerosis, cardiovascular and cerebrovascular disease. *J Neurol Neurosurg Psychiatry 2007 Oct;78(10):1083-7*

Chapter 3.3
Bos MJ, Schuit S, Koudstaal PJ, Hofman A, Uitterlinden A, Breteler MMB. Variation in estrogen receptor alpha gene does not influence risk of stroke. *In press, Stroke*

Chapter 3.4
Bos MJ, Cheung E, Leebeek FWG, Koudstaal PJ, Breteler MMB, De Maat MP. Variation in fibrinogen FGA and FGG genes and risk of stroke. *Submitted*

Chapter 4.1
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Chapter 4.2
Bos MJ, Schipper CM, Koudstaal PJ, Witteman JC, Hofman A, Breteler MM. High serum C-reactive protein level is not an independent predictor for stroke. *Circulation 2006 Oct 10;114(15):1591-8*

Chapter 5
Bos MJ, Van Rijn MJE, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. Incidence and prognosis of transient neurological attacks. *JAMA 2007 Dec;298(24):2877-2885*

Chapter 6.1
Bos MJ, Lindén T, Koudstaal PJ, Hofman A, Skoog I, Breteler MMB, Tiemeier H. Depressive symptoms, depressive disorder, and risk of stroke. *In press, J Neurol Neurosurg Psychiatry*

Chapter 6.2
Reitz C, Bos MJ, Hofman A, Koudstaal PJ, Breteler MMB. Pre-stroke cognitive performance, incident stroke and risk of dementia. The Rotterdam Study. *Stroke 2007 Nov [epub ahead of print]*

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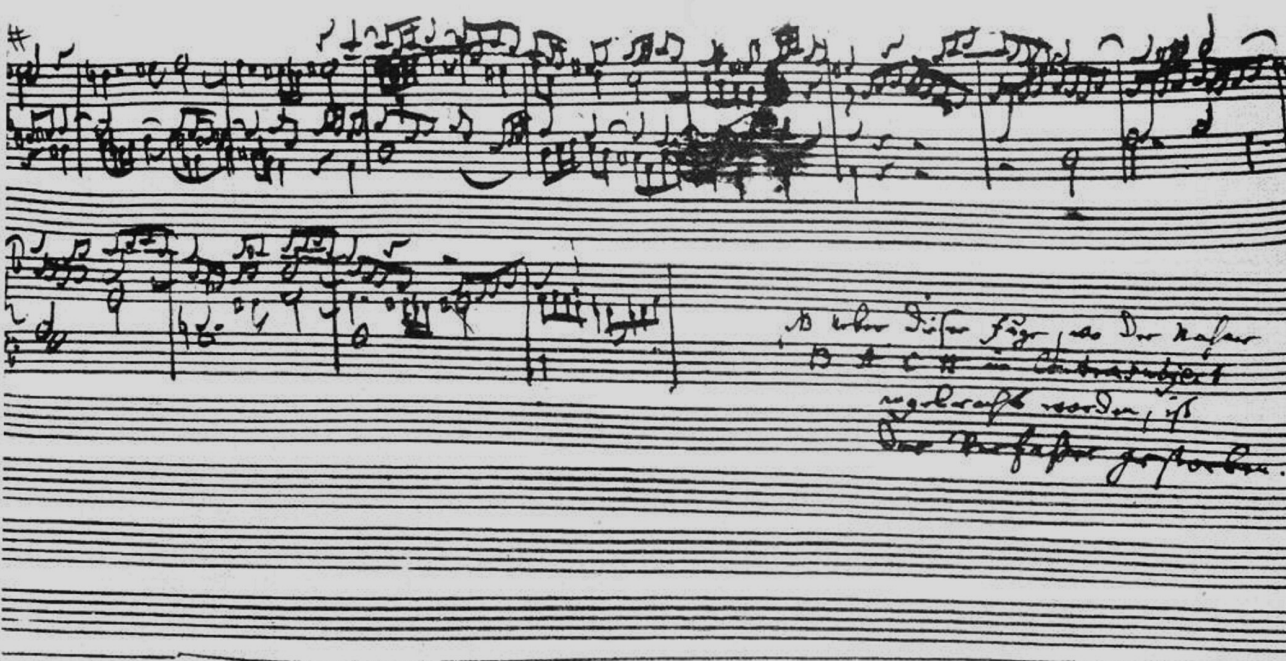
Chapter 1.

Introduction



Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, grievances, and tears. Through it we think, see, hear, and distinguish the ugly from the beautiful, the bad from the good, the pleasant from the unpleasant. [Hippocrates, Kos, ca. 460-370 BC]

It is not easy to imagine that the essence of our being is enclosed in a single organ: the brain. In addition to its miraculous function, also the immensely delicate anatomical and chemical structure of the brain is astounding. In order to maintain its structure and function, the brain needs a constant supply of blood. If the blood flow is interrupted by the occlusion of an artery, the affected part of the brain immediately stops functioning. The brain tissue becomes irreversibly damaged within seconds and the area of irreversible brain damage expands in the course of several hours, resulting in what is called an ischemic stroke (figure 1). A hemorrhagic stroke occurs when blood leaks from an artery into the brain, which has the same detrimental effect on brain tissue as ischemia.

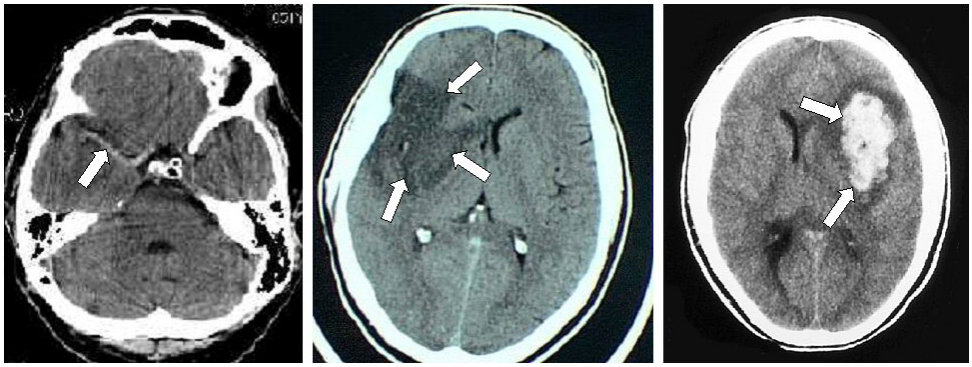


Figure 1. Non-contrast enhanced computerized tomography scans of stroke patients' brains. Left: 'dense artery:' artery occluded by thrombus. Middle: resulting ischemic stroke. Right: hemorrhagic stroke.

Strokes usually take patients completely by surprise. In the normal brain, the input from all of our senses is collected, thoughts and emotions arise, and all voluntary body actions are initiated and coordinated, therefore all these functions can be affected by a stroke. For example, a patient who has a stroke may become unable to utter or understand language, become paralyzed on one side of the body, experience half-sided blindness or difficulties with swallowing, and his or her personality may change dramatically.

Evolution of the paradigm of stroke

Although Hippocrates (Kos, 460-370 BC) and Galenus (Pergamum, 131-201 AD) already described patients who in retrospect probably suffered from strokes, they were ignorant of the pathophysiological process that caused the symptoms. Wepfer (Schaffhausen, 1620-

1695) was the first to recognize that strokes can be caused by extravasation of blood into brain tissue. Van Swieten (Leiden, 1700-1772) described that vegetations on the heart valves may embolize into the brain where they obstruct arteries and thereby 'abolish the functions of the brain.' Rostan (Paris, 1790-1866) discovered that nonhemorrhagic strokes are more frequent than hemorrhagic strokes, and Abercrombie (Edinburgh, 1780-1844) postulated that the pathological findings after nonhemorrhagic (or, as we call it today, ischemic) stroke are similar to those in gangrenous limb disease. He was the first to suspect that the cause of stroke may be found in ossified arteries. Virgow (Berlin, 1821-1902) unveiled the mechanism of arteriosclerotic thromboembolism. Miller Fisher (Waterloo, Ontario, *1913) postulated that lacunar infarcts (infarcts with diameter <15 mm, located deep within the brain) are caused by local lipohyalinosis, a vasculopathy of the deep penetrating arterioles of the brain. Ledley (New York, *1926) invented the computerized axial tomography scanner in 1975, which made it possible to diagnose ischemic and hemorrhagic strokes during life.

Today, it is commonly believed that 80% of strokes are ischemic and 20% hemorrhagic. For ischemic stroke, the pathophysiological mechanism is embolization of thrombi from large-artery atherosclerotic plaques in an estimated 50% of cases, lipohyalinotic degeneration of the deep penetrating arterioles in 25% of cases, and embolism from the heart in 20% of cases. The remaining 5% of ischemic strokes are attributable to a wide variety of rare causes.[2] Hemorrhagic strokes are believed to arise on locations that are weakened by hypertension associated lipohyalinosis, amyloid angiopathy, saccular aneurysms and other vascular malformations, vasculitis, infarctions, or tumors.

During the last decades, many factors have been identified that vary between individuals and that influence the speed of arterial degeneration (atherosclerosis and lipohyalinosis), the formation of emboli on diseased arterial/arteriolar endothelium or in the heart, or the likelihood of bleeding, thereby influencing the risk of stroke: these factors are causes of stroke. An overview of these factors can be found in tables 1[2] and 2.[3]

Table 1. Factors associated with an increased risk of hemorrhagic stroke.

Hypertension
Migraine
Anticoagulants
Antiplatelet drugs
Thrombolytic treatment
Clotting factor deficiency
Leukemia
Thrombocytopenia
Alcohol
Amphetamines
Cocaine and other drugs
Trauma

Table 2. Factors associated with an increased risk of ischemic stroke.

Established	Potential
High total cholesterol, low HDL	Metabolic syndrome
Hypertension	Alcohol abuse
Diabetes mellitus	High C-reactive protein
Obesity	Hyperhomocysteinemia
Cigarette smoking	Acute infections
Black race	C. pneumoniae
Low birth weight	Cytomegalovirus
Coronary heart disease	H. pylori
Heart failure	High IL-18
Peripheral artery disease	High Lp-PLA2
Atrial fibrillation	Anticardiolipin antibody
Sickle cell disease	Drug abuse
High sodium intake	Lupus anticoagulant
Low potassium intake	Factor V Leiden
Postmenopausal hormone therapy	prothrombin 20210 mutation
Physical inactivity	Protein C deficiency
Family history	Protein S deficiency
	Antithrombin III deficiency
	Oral contraceptive use
	Periodontal disease
	CD-40 ligand
	Migraine
	Sleep-disordered breathing

Patient care

The insights described in the previous paragraph have led to a modest but significant number of triumphs that enable us to prevent stroke, the greatest of which was celebrated in 1967: in this year it was demonstrated that the treatment of hypertension could reduce the incidence of stroke among persons with severe diastolic hypertension by more than 90 percent.[4] Later, antihypertensive treatment was also successfully offered to patients with milder hypertension. Besides antihypertensive treatment, various other interventions have been shown to reduce the incidence of stroke, both those that counterbalance a specific disease process (rigorous treatment of hyperglykemia, endarterectomy for severe symptomatic carotid stenosis, oral anticoagulant treatment for cardiac sources of emboli), and interventions that do not tackle a specific disease but do reduce stroke risk in high-risk patients (platelet inhibitors, and HMG-

CoA (3-hydroxy-3-methyl-glutaryl-Coenzyme A) reductase inhibitors – although some argue that HMG-CoA reductase inhibitors belong to the former group).

In 2001, thrombolytic drugs became the first cure for acute ischemic stroke that was approved for routine use within the European Union: these drugs decreased the proportion of patients who were death or dependent a few months after stroke from 60 percent (95% CI 56-64%) to 50 percent (95% CI 46-54%), although they increased total mortality from 19 percent (95% CI 16-22%) to 21 percent (95% CI 18-24%) when administered within 3 hours after the onset of stroke.[5]

Rationale of this thesis

In spite of the treatment and prevention options available today, still over 41 thousand strokes occur in the Netherlands annually.[6] Stroke is the third cause of death and the main cause of disability in our country. Worldwide, 5.7 million people die from stroke each year, which is about 10% of all deaths. One third of stroke victims die within a year and of those who survive, almost half are no longer able to live independently.[7]

As mentioned, however, specific interventions have been shown to decrease the risk of stroke, and indeed it has been observed that the population-wide better control of stroke risk factors over the last twenty years has been accompanied by a considerable decrease in the incidence of stroke.[8] These results encourage to continue the quest for causes and risk indicators for stroke to further decrease the incidence of stroke. This may be achieved in two ways:

1. By identifying potential targets for preventive treatment: to prevent strokes we have to intervene in the causal pathways that lead to stroke, in order to prevent that a situation in which a stroke may occur arises. Therefore, the identification of these pathways is an important step to take in finding prevention strategies for stroke.

2. By optimizing the distinction between persons at high risk and those at low risk of stroke: 16% of persons suffer from stroke at some point during life. Therefore, if everybody received preventive treatment, this would by definition be useless in 84% of treated persons and it may be started too early in others. If we could better foresee which persons will develop stroke, we would be able to treat them better and earlier because we could accept higher costs and more side effects per treated patient.

Aims and focus of this thesis

The aim of the research discribed in this thesis was to gain more insight in the pathophysiological mechanisms that lead to stroke and in the possibilities to distinguish persons who are at high

risk of stroke from those at low risk of stroke.

The vast majority of strokes at old age are caused by thrombi that grow on atherosclerotic or arteriosclerotic plaques. Atherosclerosis is thought to be initiated by the accumulation of subendothelial cholesterol and subsequently worsened by an inflammatory response.[9] This may result in the exposure of subendothelial tissue to blood, which induces the conversion of plasma fibrinogen into fibrin, leading to local thrombus formation. Etiologic cardiovascular research traditionally focused on inter-individual differences in lipid metabolism to explain differences in the atherosclerotic burden between individuals. Nowadays the attention has shifted towards inter-individual differences in the inflammatory response. Another important cause of atherosclerosis is sheer stress on the vascular wall that is exerted by the pressure of the blood. Since the volume of circulating fluid is an important determinant of blood pressure, factors that influence this volume may influence the risk of stroke. Because little is known about the relation between such factors and the risk of stroke, we investigated the relation between several factors that are involved in the homeostasis of the volume of circulating fluid, including serum markers of kidney function *(chapters 2.1 and 2.2)*, and genetic polymorphisms that influence renal sodium handling *(chapter 3.1)* and the renin-angiotensin system *(chapter 3.2)*, and the risk of stroke.

The strikingly lower age-specific incidence rate of cardiovascular diseases among women compared to men suggests a role for sex hormones in the development of atherosclerosis. Therefore, we tested whether a promising polymorphism in the estrogen receptor alpha gene is associated with the risk of stroke *(chapter 3.3)*.

As mentioned, atherosclerosis becomes a problem when it leads to thrombus formation. We investigated whether genetically determined differences in the structure of fibrinogen, the precursor of thrombi, may influence the risk of stroke *(chapter 3.4)*.

The three main sources for the emboli that cause ischemic stroke are the heart, the large extracranial arteries, and the small penetrating arterioles deep within the brain. The large intracranial arteries would logically fit into this series, but their role is generally ignored. In this thesis we investigated whether pathology in the large intracranial arteries is associated with the risk of stroke *(chapter 2.3)*.

The alarming discovery that Vioxx® (rofecoxib) strongly increases the risk of cardiovascular diseases prompted the withdrawal of the drug from the market in 2004.[10] This discovery was more or less a lucky finding because the drug was investigated in a trial that was designed to test the drug as a long-term chemoprofylaxis agent for adenomatous polyps, well after it had been introduced to the market as a non-steroid anti-inflammatory drug (NSAID). Therefore, it is not unlikely that other widely used NSAIDs have similar undiscovered effects on cardiovascular risk, which is what we investigated in *chapter 2.4*.

One of the most powerful indicators that a person is at high risk of stroke is the occurrence of a transient ischemic attack (TIA). Whereas we are well aware of the prognosis of TIAs, many patients suffer from attacks with neurological symptoms that do not fulfill

diagnostic criteria for TIA. These attacks are generally presumed to have a benign subsequent clinical course, but data to support this assumption are lacking. We describe in *chapter 5.1* whether the assumption is correct.

The scope of clinical manifestations of cerebrovascular disease may be much broader than stroke and TIA alone. We investigated the relation between presence of depressive symptoms and the risk of stroke, and relation between prestroke cognitive function, stroke, and poststroke cognitive function *(chapters 6.1* and *6.2)*.

Study population

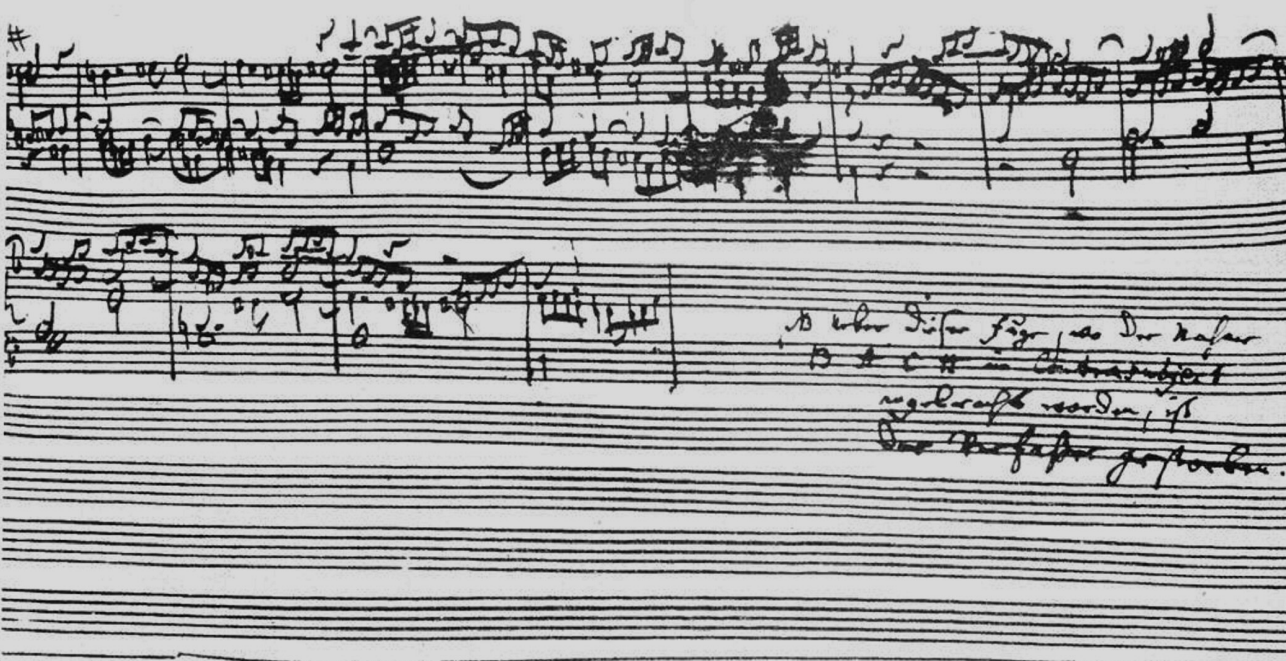
The studies described in this thesis are based on the Rotterdam Study, a prospective population-based cohort study of chronic and disabling diseases among 7,983 residents of Ommoord, a district in the city of Rotterdam, the Netherlands.[11, 12] All participants, who were 55 years of age and over at baseline, joined the study between 1990 and 1993 and have been followed for occurrence of stroke, transient ischemic attack, and other diseases up to the present day.

References

1. Bos MJ, van Goor ML, et al. Plasma homocysteine is a risk factor for recurrent vascular events in young patients with an ischaemic stroke or TIA. J Neurol. 2005;252:332-337
2. Warlow CP, Van Gijn J, et al. Stroke: A practical guide to management. Oxford: Blackwell Science Ltd; 2001.
3. Goldstein LB, Adams R, et al. Primary prevention of ischemic stroke: A guideline from the American Heart Association/American Stroke Association Stroke Council. Circulation. 2006;113:e873-923
4. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. JAMA. 1967;202:1028-1034
5. Wardlaw JM, Zoppo G, et al. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst Rev. 2003:CD000213
6. Bots ML, Berger-van Sijl M, et al. Incidentie van cerebrovasculaire ziekte in Nederland in 2000. Dutch Heart Foundation. 2006
7. Warlow C, Sudlow C, Dennis M, Wardlaw J, Sandercock P. Stroke. Lancet. 2003;362:1211-1224
8. Rothwell PM, Coull AJ, et al. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). Lancet. 2004;363:1925-1933
9. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868-874
10. Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal ad enoma chemoprevention trial. N Engl J Med. 2005;352:1092-1102
11. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
12. Hofman A, Breteler MM, et al. The Rotterdam Study: objectives and design update. Eur J Epidemiol. 2007;22:819-29.

Chapter 2.

Novel nongenetic risk factors for stroke



2.1.
Serum uric acid

Background and Purpose: *The role of uric acid as a risk factor for myocardial infarction is controversial and little is known on its role as a risk factor for stroke. Recent evidence suggests that uric acid may be an important causal agent in cardiovascular disease, for example by inducing renal disease and hence hypertension. We investigated the association between serum uric acid and coronary heart disease and stroke in a large prospective population-based study.* ***Methods:*** *The study was based on 4385 participants of the Rotterdam Study who at baseline (1990-1993) were aged 55 years or over, free from stroke and coronary heart disease, and had blood taken. Follow-up for incident stroke and myocardial infarction was complete until January 1, 2002. Data were analyzed with Cox proportional hazards models with adjustment for relevant confounders.* ***Results:*** *Average follow-up was 8.4 years. High serum uric acid levels were associated with risk of myocardial infarction and stroke; age and sex adjusted hazard ratios (95% confidence intervals) for highest versus lowest quintile of uric acid were 1.68 (1.24.2.27) for cardiovascular disease (515 cases); 1.87 (1.12-3.13) for myocardial infarction (194 cases); 1.57 (1.11-2.22) for stroke (381 cases); 1.77 (1.10-2.83) for ischemic stroke (205 cases), and 1.68 (0.68-4.15) for hemorrhagic stroke (46 cases). Adjustment for other vascular risk factors only slightly attenuated these associations. The associations were stronger in persons without hypertension than in those with hypertension.* ***Conclusions:*** *Uric acid is a strong risk factor for myocardial infarction and stroke.*

Introduction

Already in the 19th century it was known that high uric acid levels are associated with hypertension. Despite the lack of experimental studies, increased uric acid levels were commonly considered a consequence rather than a cause of cardiovascular disease. However, both animal and human studies have recently shown that high uric acid levels may impair kidney function by causing glomerular damage and preglomerular arteriolosclerosis, two effects that ultimately result in arterial hypertension.[1-7] Large cohort studies have shown that uric acid is an important independent risk factor for cardiovascular mortality.[8, 9] The role of uric acid in coronary heart disease is less clear. Some studies reported an independent association between uric acid and coronary heart disease,[10-14] but others only found an association in women[15-17] and in yet others the associations disappeared after adjustment for confounders.[15, 17-19] Little is known on the association between uric acid and stroke risk: an association was found between uric acid and stroke risk in diabetics[20] and between uric acid and fatal stroke in the general population.[21] Recently a population-based study in elderly persons also found an association between uric acid and stroke.[22]

We investigated the association between serum uric acid and coronary heart disease and stroke in a large prospective population-based cohort study in subjects aged 55 years and over who were free from stroke and coronary heart disease at baseline. We studied the associations between uric acid and cardiovascular disease in persons with and without hypertension separately because of the assumed importance of hypertension in the pathogenesis of uric acid-induced cardiovascular disease.[5, 6]

Methods

Population: The present study is part of the Rotterdam Study, a population-based cohort study on chronic and disabling diseases. All inhabitants of Ommoord, a district of the city of Rotterdam in the Netherlands, aged 55 years and over were invited. People living in homes for the elderly were included. At baseline, participants were invited in random order from the source population. Participation rate of those invited for the study was 78%; in total, 7983 subjects participated. The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were obtained from 1990 through 1993 and consisted of a home interview and 2 visits to the research center for physical examination. At the baseline visit to the research center, we sampled blood and performed carotid duplex ultrasonography and electrocardiography.

Assessment of Stroke and Myocardial Infarction: History of stroke at baseline was as-

sessed and verified as described previously.[23] A medical history of coronary heart disease was positive if a myocardial infarction, coronary artery bypass graft, or percutaneous transluminal angioplasty was reported in the baseline interview and confirmed by baseline ECG or medical records. Once subjects enter the Rotterdam Study, they are continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physicians' files are scrutinized. For reported events, additional information (including brain imaging) is obtained from hospital records. Research physicians reviewed information on all possible strokes and transient ischemic attacks; an experienced stroke neurologist (P.J.K.) verified all diagnoses blinded for uric acid status. Subarachnoid hemorrhages and retinal strokes were excluded. Ischemic strokes were diagnosed when a patient had typical symptoms and a CT or MRI, that was made within 4 weeks after the stroke occurred, ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature of the stroke. Hemorrhagic strokes were diagnosed when a relevant hemorrhage was shown on CT or MRI scan, or the subject lost consciousness permanently or died within hours after onset of focal signs. If a stroke did not match any of these criteria, it was called unspecified.

For coronary heart disease, two research physicians independently coded all reported events according to the International Classification of Diseases, 10th edition (ICD-10). Codes on which the research physicians disagreed were discussed in order to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events. Incident coronary heart disease was defined as the occurrence of a fatal or non-fatal myocardial infarction (ICD-10 code I21), a revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft), other forms of acute (I24) or chronic ischemic (I25) heart disease, sudden (cardiac) death (I46 and R96), and death due to ventricular fibrillation (I49) and congestive heart failure (I50) during follow-up. Follow-up was completed until January 1, 2002 for 97.1% of all potential person years.

Population for Analysis: Participants who had coronary heart disease (n=869), stroke (n=213), or both (n=48) before baseline were excluded. Uric acid assessments were only performed until 31 December 1992 when they were stopped due to financial constraints. Therefore, for the present analyses we excluded participants who visited the research center after this date (n=1539). After exclusion of participants who died before the first center visit, of participants who did not visit the research center due to refusal or physical inability (n=750), and of participants of whom we did not have blood available for the uric acid essays (n=204), 4385 participants were included in the analyses.

Uric Acid: Non-fasting blood was collected and centrifuged within 30 minutes the blood

was centrifuged for 10 minutes at 3000 rotations per minute. Subsequently the serum was stored at -20°C for 1 week, until uric acid activity was determined with a Kone Diagnostica reagent kit and a Kone autoanalyzer.[24] To check calibration, after every 10 samples 3 control samples were included; if the average values of the control samples of each run (100 samples) were not within 2.5% of the true value, the run was repeated. Day-by-day variation had to be within 5%.

Confounders and Effect Modifiers: Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements. Hypertension was defined as a diastolic blood pressure of at least 100 mm Hg and/or a systolic blood pressure of at least 160 mm Hg and/or use of antihypertensive medication indicated to treat high blood pressure. Total cholesterol and high-density cholesterol (HDL-cholesterol) were measured in non-fasting baseline blood with an automated enzymatic procedure. We considered diabetes mellitus to be present if a random or post-load glucose level was 11.1 mmol/L or higher, or if a person used antidiabetic medication. During the home interview smoking status (classified as current, former or never) and medication use were assessed. The waist/hip ratio was calculated by dividing the waist circumference by the hip circumference.

Statistical Analysis: We used Cox' proportional hazards models to calculate hazard ratios with 95% confidence intervals (CIs) for the associations between uric acid and cardiovascular disease after inspection of log(-log) survival curves. Hazard ratios were calculated for uric acid quintiles (relative to the lowest quintile) and per standard deviation (SD) increase in uric acid level. We adjusted for confounding by age and sex, and additionally for confounding by other putative confounders. We also did analyses in subgroups (participants not using serum uric acid influencing medication at baseline (diuretics, lipid lowering medication, antigout preparations, analgesics), men, women, hypertensives, and non-hypertensives). Missing values in confounders and effect modifiers were imputed using a linear regression model based on age and sex. Interaction, if appropriate, was examined by studying the statistical significance of interaction terms entered into the models. Analyses were performed using SPSS® 12.0.1 for Windows.

Results

The total follow-up was 36,794 person years (on average 8.4 years). During follow-up, 515 participants suffered from coronary heart disease (194 myocardial infarctions), and 381 participants suffered from a stroke (205 ischemic strokes, 46 hemorrhagic strokes, and 130 unspecified strokes). Uric acid levels were approximately normally distributed with mean

Table 1. Baseline characteristics of the study population (n=4385).

Characteristic	Median (interquartile range) or percentage
Age (yrs)	69.0 (62.5-76.2)
Female sex	64.6 %
Uric acid (µmol/l)	309 (263-364)
Hypertension	33.2 %
Systolic blood pressure (mmHg)	138 (124-153)
Cholesterol (mmol/l)	6.6 (5.8-7.4)
High-density lipoprotein (mmol/l)	1.3 (1.1-1.6)
Diabetes mellitus	10.2 %
Ever smoking	63.4 %
Diuretic use	14.8 %
Lipid lowering medication use	1.7 %
Analgesics use	23.1 %
Antigout medication use	0.5 %
Waist/hip ratio	0.90 (0.83-0.97)

Table 2. Hazard ratios for the associations between serum uric acid and coronary heart disease. N=4385.

Event	Uric acid*	Hazard ratio (95% CI)	
		Model 1†	Model 2†
Coronary heart disease	Quintile 1	1 (ref)	1 (ref)
(n=515)	Quintile 2	1.01 (0.73-1.40)	0.97 (0.70-1.34)
	Quintile 3	1.40 (1.03-1.90)	1.28 (0.94-1.75)
	Quintile 4	1.32 (0.97-1.79)	1.14 (0.83-1.56)
	Quintile 5	1.68 (1.24-2.27)	1.30 (0.96-1.78)
	Per SD	*1.27 (1.17-1.39)*	*1.17 (1.07-1.28)*
Myocardial infarction	Quintile 1	1 (ref)	1 (ref)
(n=194)	Quintile 2	1.01 (0.57-1.79)	0.95 (0.54-1.68)
	Quintile 3	1.91 (1.15-3.19)	1.69 (1.01-2.82)
	Quintile 4	1.72 (1.03-2.87)	1.40 (0.83-2.36)
	Quintile 5	1.87 (1.12-3.13)	1.38 (0.82-2.35)
	Per SD	*1.21 (1.05-1.40)*	*1.10 (0.95-1.27)*

* Cut points for quintiles are 251, 292, 327, and 381 µmol/l.
† Model 1: uric acid, age and sex. Model 2: Model 1 + systolic blood pressure, total cholesterol, high-density lipoprotein, diabetes mellitus, ever smoking, diuretic use, and waist/hip ratio.

Table 3. Hazard ratios for the associations between serum uric acid levels and risk of stroke.

Event	Uric acid*	Hazard ratio (95% CI)	
		Model 1†	Model 2†
All stroke	Quintile 1	1 (ref)	1 (ref)
(n=381)	Quintile 2	1.04 (0.72-1.50)	1.08 (0.75-1.56)
	Quintile 3	1.53 (1.09-2.15)	1.57 (1.11-2.22)
	Quintile 4	1.44 (1.02-2.03)	1.46 (1.03-2.07)
	Quintile 5	1.57 (1.11-2.22)	1.50 (1.05-2.14)
	Per SD	*1.18 (1.06-1.30)*	*1.15 (1.03-1.27)*
Ischemic stroke	Quintile 1	1 (ref)	1 (ref)
(n=205)	Quintile 2	1.08 (0.66-1.78)	1.07 (0.65-1.76)
	Quintile 3	1.41 (0.88-2.27)	1.33 (0.82-2.14)
	Quintile 4	1.68 (1.06-2.67)	1.53 (0.95-2.44)
	Quintile 5	1.77 (1.10-2.83)	1.49 (0.92-2.41)
	Per SD	*1.24 (1.08-1.42)*	*1.16 (1.00-1.34)*
Hemorrhagic stroke	Quintile 1	1 (ref)	1 (ref)
(n=46)	Quintile 2	0.71 (0.25-2.06)	0.80 (0.27-2.31)
	Quintile 3	1.47 (0.59-3.63)	1.75 (0.70-4.38)
	Quintile 4	0.70 (0.24-2.05)	0.86 (0.29-2.57)
	Quintile 5	1.68 (0.68-4.15)	2.06 (0.81-5.25)
	Per SD	*1.15 (0.86-1.55)*	*1.23 (0.91-1.66)*

† Model 1: uric acid, age and sex. Model 2: Model 1 + systolic blood pressure, total cholesterol, high-density lipoprotein, diabetes mellitus, ever smoking, diuretic use, and waist/hip ratio.

serum uric acid level 348 micromol/l for men and 302 micromol/l for women. The 5th and the 95th percentiles were 245 and 476 micromol/l for men and 198 and 453 micromol/l for women. Baseline characteristics are described in table 1.

High serum uric acid levels were associated with the risk of coronary heart disease; the age and sex adjusted hazard ratio (95% CI) for the highest versus the lowest quintile of uric acid was 1.68 (1.24-2.27) for coronary heart disease and 1.87 (1.12-3.13) for myocardial infarction alone (table 2, figure 1). High serum uric acid levels were also associated with the risk of stroke: age and sex adjusted hazard ratios (95% CIs) for the highest versus the lowest quintile of uric acid were 1.57 (1.11-2.22) for all strokes, 1.77 (1.10-2.83) for ischemic strokes, and 1.68 (0.68-4.15) for hemorrhagic strokes (table 3, figure 1). Adjustment for potential confounding (model 2; tables 2 and 3) only slightly attenuated these associations. Also exclusion of subjects receiving serum uric acid influencing medication at baseline (diuretics, analgesics, lipid lowering and antigout preparations; N=1556) slightly changed the estimated hazard ratios: age and sex adjusted hazard ratios for the highest versus the lowest quintile of uric acid were 1.37 (0.92-2.03) for coronary heart disease (n=298) and 2.06 (1.25-3.40) for

Table 4. Hazard ratios for the associations between serum uric acid and subtypes of cardiovascular disease for men and women separately. Adjusted for age.

Stratum	Uric acid*	Hazard ratio (95% CI)				
		Coronary heart disease n=258♂, 257♀	Myocardial infarctions n=110♂, 84♀	All strokes n=132♂, 249♀	Ischemic strokes n=73♂, 132♀	Hemorrhagic strokes n=16♂, 30♀
Men (n=1551)	Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Tertile 2	1.11 (0.82-1.51)	1.23 (0.77-1.96)	1.78 (1.16-2.74)	1.57 (0.88-2.79)	1.23 (0.38-4.04)
	Tertile 3	1.37 (1.01-1.84)	1.33 (0.83-2.12)	1.41 (0.90-2.23)	1.36 (0.74-2.48)	1.11 (0.32-3.83)
	Per SD	*1.21 (1.06-1.38)*	*1.15 (0.94-1.41)*	*1.15 (0.95-1.38)*	*1.18 (0.92-1.51)*	*0.97 (0.55-1.70)*
Women (n=2834)	Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Tertile 2	1.20 (0.85-1.67)	1.40 (0.78-2.54)	1.45 (1.05-2.02)	1.44 (0.91-2.27)	1.22 (0.48-3.10)
	Tertile 3	1.73 (1.27-2.36)	2.03 (1.17-3.54)	1.45 (1.05-2.01)	1.68 (1.08-2.62)	1.32 (0.53-3.26)
	Per SD	*1.30 (1.16-1.46)*	*1.27 (1.03-1.56)*	*1.18 (1.05-1.34)*	*1.26 (1.07-1.49)*	*1.23 (0.87-1.74)*

* Cut points for tertiles are 310 µmol/l and 375 µmol/l for men and 263 µmol/l and 321 µmol/l for women.

strokes (n=216); in both instances P for trend < 0.01.

The associations between uric acid and various kinds of cardiovascular disease were not significantly different for men and women (P interaction > 0.3 for all events) (table 4). The associations between uric acid and cardiovascular disease seemed stronger in persons without than in persons with hypertension, although this effect was more pronounced for cerebrovascular disease than for coronary heart disease (table 5).

Table 5. Hazard ratios for the associations between serum uric acid and subtypes of cardiovascular disease for non-hypertensives and hypertensives separately. Reported for tertiles of uric acid and per SD increase in uric acid level. Adjusted for age and sex.

Stratum	Uric acid*	Hazard ratio (95% CI)				
		Coronary heart disease	Myocardial infarctions	All strokes	Ischemic strokes	Hemorrhagic strokes
		n=286, 216†	n=112, 79†	n=192, 184†	n=107, 94†	n=21, 25†
Non-hypertensives	Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(n=2881)	Tertile 2	1.23 (0.89-1.70)	1.56 (0.89-2.72)	2.03 (1.36-3.04)	1.98 (1.16-3.39)	1.89 (0.56-6.39)
	Tertile 3	1.51 (1.10-2.09)	2.01 (1.16-3.50)	2.15 (1.41-3.26)	2.16 (1.24-3.77)	2.27 (0.65-7.95)
	Per SD	*1.25 (1.09-1.43)*	*1.33 (1.08-1.64)*	*1.30 (1.10-1.53)*	*1.33 (1.07-1.66)*	*1.08 (0.63-1.85)*
Hypertensives	Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(n=1433)	Tertile 2	1.34 (0.95-1.90)	2.33 (1.27-4.27)	0.91 (0.64-1.30)	0.81 (0.49-1.34)	0.99 (0.36-2.76)
	Tertile 3	1.37 (0.97-1.94)	1.64 (0.86-3.13)	0.93 (0.65-1.33)	0.91 (0.55-1.49)	1.37 (0.53-3.52)
	Per SD	*1.20 (1.06-1.35)*	*1.03 (0.83-1.27)*	*1.01 (0.88-1.16)*	*1.05 (0.86-1.27)*	*1.04 (0.73-1.48)*
P interaction	Hypertension yes/no	0.61	0.05	0.002	0.05	0.91

* Cut points for tertiles are 269 µmol/l and 328 µmol/l for non-hypertensives and 298 µmol/l and 374 µmol/l for hypertensives.
† Non-hypertensives and hypertensives, respectively.

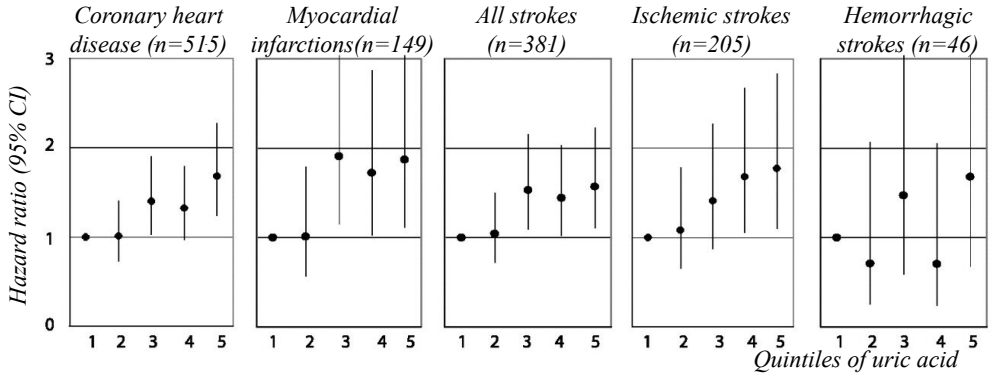


Figure 1. Hazard ratios for the associations between serum uric acid and cardiovascular disease. Circles represent consecutive quintiles of uric acid with 95% CIs. Adjusted for age and sex.

Discussion

In this community-based study in subjects aged 55 years and over who were free from stroke and coronary heart disease at baseline, we found a strong and significant association between baseline serum uric acid levels and risk of both coronary heart disease and stroke. These associations were attenuated only slightly by adjustment for other cardiovascular risk factors and were stronger in persons without than in those with hypertension.

Before these results can be interpreted, some methodological issues need to be discussed. Strengths of our study are the large study population (n=4385), the intense stroke case finding and the nearly complete follow-up (loss of potential person-years 2.9%). Our stringent stroke monitoring procedures allowed us to include also stroke patients who were not referred to a hospital. A disadvantage is that in these cases neuroimaging was often lacking (60% of our cases had neuroimaging) and examinations not thorough enough to subclassify 34% of strokes into ischemic or hemorrhagic. Uric acid examinations were stopped before all participants had visited the research center. As participants were invited in random order, we do not think that this has affected our results.

Our finding that uric acid increases risk of coronary heart disease is in line with previous studies on the association between uric acid and coronary heart disease.[11, 12, 14-19] Some of these studies found the association only in women.[15-17] In our study, too, associations seemed to be stronger in women than in men, although the differences were not statistically significant. In some previous studies the association between uric acid and coronary heart disease disappeared after adjustment for potential confounders, which led to the opinion that that uric acid has no role in the etiology of cardiovascular disease.[15, 17-19] This was legitimate because at the time that these studies were published uric acid was regarded as a biologically inert molecule. However, recent insights in the biological effects of uric acid have falsified this view,[1-7] and many epidemiological studies,[5, 6, 8-14] including our present study, found that uric acid plays a

clear and independent role in cardiovascular disease. Although we can never be sure that no residual confounding remains, we do think that the role of uric acid in cardiovascular disease has been underestimated for a long time and should be reconsidered.

There is relatively little information on the role of uric acid as a risk factor for stroke. An association between uric acid and stroke risk has been found in diabetics.[20] In the general population, an association was found between uric acid and fatal stroke.[21] We know of one published report on the relation between uric acid and (fatal and non-fatal) stroke in the general population; in this study an independent relation between uric acid and stroke was found only in subjects not using diuretics. As we may presume that nearly all subjects that use diuretics suffer from hypertension, these findings are compatible with our view that the association between uric acid and stroke is most pronounced in normotensive subjects.[22] In our study, too, this association seemed to be strongest in participants not using uric acid influencing medication (which seemed opposite for the association between uric acid and coronary heart disease). We found that the effect of uric acid on stroke risk was lower in persons with hypertension. This fits the observation in rats that, once renal disease has been established, the hypertension is driven by renal mechanisms independent of uric acid status.[4, 6]

Our data suggest that uric acid is an important cardiovascular risk factor. Additional studies are required to assess whether lowering of uric acid levels can actually reduce the risk of coronary heart disease and stroke.

References

1. Johnson RJ, Kang DH, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascu lar and renal disease? Hypertension. 2003;41:1183-1190
2. Johnson RJ, Feig DI, et al. Resurrection of uric acid as a causal risk factor in essential hypertension. Hypertension. 2005;45(1):18-20
3. Iseki K, Ikemiya Y, et al. Significance of hyperuricemia as a risk factor for developing esrd in a screened cohort. Am J Kidney Dis. 2004;44:642-650
4. Watanabe S, Kang DH, et al. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension. 2002;40:355-360
5. Sundstrom J, Sullivan L, et al. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension. 2005;45(1):28-33
6. Alper AB, Jr., Chen W, et al. Childhood uric acid predicts adult blood pressure. The Bogalusa Heart Study. Hypertension. 2005;45(1):34-38
7. Johnson RJ, Rodriguez-Iturbe B, et al. A unifying pathway for essential hypertension. Am J Hypertens. 2005;18:431-440
8. Niskanen LK, Laaksonen DE, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: A prospective cohort study. Arch Intern Med. 2004;164:1546-1551
9. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the nhanes i epidemiologic fol low-up study, 1971-1992. National Health and Nutrition Examination Survey. Jama. 2000;283:2404-2410
10. Bengtsson C, Lapidus L, et al. Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the Population Study of Women in Gothenburg, Sweden. Acta Med Scand. 1988;224:549-555
11. Goldberg RJ, Burchfiel CM, et al. Lifestyle and biologic factors associated with atherosclerotic disease in middle-aged men. 20-year findings from the Honolulu Heart Program. Arch Intern Med.

1995;155:686-694
12. Kannel WB. Metabolic risk factors for coronary heart disease in women: Perspective from the Fram ingham Study. Am Heart J. 1987;114:413-419
13. Liese AD, Hense HW, et al. Association of serum uric acid with all-cause and cardiovascular disease mortality and incident myocardial infarction in the MONICA Augsburg Cohort. Epidemiology. 1999;10:391-397
14. Persky VW, Dyer AR, et al. Uric acid: A risk factor for coronary heart disease? Circulation. 1979;59:969-977
15. Culleton BF, Larson MG, et al. Serum uric acid and risk for cardiovascular disease and death: The Framingham Heart Study. Ann Intern Med. 1999;131:7-13
16. Freedman DS, Williamson DF, et al. Relation of serum uric acid to mortality and ischemic heart dis ease. The NHANES I epidemiologic follow-up study. Am J Epidemiol. 1995;141:637-644
17. Moriarity JT, Folsom AR, et al. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk In Communities (ARIC) Study. Ann Epidemiol. 2000;10:136-143
18. Brand FN, McGee DL, et al. Hyperuricemia as a risk factor of coronary heart disease: The Framingham Study. Am J Epidemiol. 1985;121:11-18
19. Wannamethee SG, Shaper AG, Whincup PH. Serum urate and the risk of major coronary heart disease events. Heart. 1997;78:147-153
20. Lehto S, Niskanen L, et al. Serum uric acid is a strong predictor of stroke in patients with non-insulin-dependent diabetes mellitus. Stroke. 1998;29:635-639
21. Mazza A, Pessina AC, et al. Predictors of stroke mortality in elderly people from the general popula tion. The Cardiovascular Study in the Elderly. Eur J Epidemiol. 2001;17:1097-1104
22. Hozawa A, Folsom AR, et al. Serum uric acid and risk of ischemic stroke: The ARIC Study. Athero sclerosis. 2005
23. Hollander M, Bots ML, et al. Carotid plaques increase the risk of stroke and subtypes of cerebral inf arction in asymptomatic elderly: The Rotterdam Study. Circulation. 2002;105:2872-2877
24. Trivedi RC, Rebar L, et al. New enzymatic method for serum uric acid at 500 nm. Clin Chem. 1978;24:1908-1911

2.2
Glomerular filtration rate

Background and purpose: *Persons with early stages of chronic kidney disease, defined by a decreased glomerular filtration rate (GFR), have an increased risk of cardiovascular disease. It is unclear whether decreased GFR is a risk factor for stroke. We assessed the association between GFR and stroke in a prospective population-based cohort study.* ***Methods:*** *The study was based on 4937 participants of the Rotterdam Study who at baseline (1990-1993) were aged 55 years or over, free from stroke, and had serum creatinine assessment. GFR was estimated with the Cockcroft-Gault equation. Follow-up for incident cerebrovascular disease was complete until January 1, 2005. Data were analyzed with Cox proportional hazards models with adjustment for relevant confounders and results were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs).* ***Results:*** *During an average follow-up of 10.2 years, 586 strokes (338 ischemic, 44 hemorrhagic, and 204 unspecified strokes) occurred. We found no association between GFR and risk of overall stroke or risk of ischemic stroke. In contrast, with decreasing GFR the risk of hemorrhagic stroke strongly increased: the age and sex adjusted HR for hemorrhagic stroke was 4.10 (95% CI 1.25-13.42) for lowest versus highest quartile of GFR, and there was a clear and highly significant dose-effect relationship. Adjustment for other vascular risk factors only slightly attenuated this association.* ***Conclusions:*** *Decreased GFR is a strong risk factor for hemorrhagic, but not ischemic stroke.*

Introduction

The clinical spectrum of chronic kidney disease ranges from an unnoticed decrease in glomerular filtration rate (GFR) to end stage renal failure. The prevalence of chronic kidney disease, especially subclinical stages, is very high among elderly subjects.[1] It has been shown that chronic kidney disease is closely related to ischemic heart disease. On the one hand, risk factors for ischemic heart disease like hypertension, smoking, and diabetes mellitus are more prevalent among patients with chronic kidney disease.[2, 3] On the other hand, chronic kidney disease itself has numerous effects that potentially may harm the cardiovascular system, including inhibition of erythropoiesis and platelet function,[4, 5] and induction of volume overload,[6] dyslipidemia, hypertension,[7] and vascular calcification.[8] As a result, chronic kidney disease patients are at increased risk of ischemic heart disease: the ischemic heart disease death rate among end stage kidney disease patients is 10 to 30 times higher than in the general population.[9] Also persons with early stages of chronic kidney disease, usually diagnosed by means of a decreased glomerular creatinine filtration rate or increased serum cystatine C level, have an increased risk of coronary heart disease and ischemic cardiac death, which is independent of other conventional cardiovascular risk factors.[10-14] Therefore it is commonly thought that chronic kidney disease is not only a marker of presence of cardiovascular risk factors, but in addition plays a causal role in the pathophysiology or propagation of ischemic heart disease.

Despite the evidence for chronic kidney disease being a risk factor for ischemic heart disease and the overlap between ischemic heart disease and stroke risk factors, it remains uncertain whether glomerular filtration rate is also associated with risk of stroke: the few studies that reported on this association showed a small and non-significant increase in stroke risk with decreasing GFR.[13, 14] No studies reported on the association between GFR and subtypes of stroke.

The aim of our present study was to assess whether decreased glomerular filtration rate is a risk factor for ischemic and hemorrhagic stroke in the general population.

Methods

Population: The Rotterdam Study is a population-based cohort study on chronic and disabling diseases.[15] All inhabitants of Ommoord, a district of the city of Rotterdam in the Netherlands, aged 55 years and over were invited to participate. Participation rate of those invited for the study was 78%; in total, 7983 subjects participated in the first study survey (1990-1993). The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants.

Assessment of Stroke: History of stroke at baseline was positive if a stroke was reported during the baseline interview and confirmed by medical records (n=261). After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Also nursing home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes with an experienced stroke neurologist (P.J.K.) to verify all diagnoses.[16, 17] Subarachnoid hemorrhages were excluded. A stroke was subclassified as ischemic if a CT or MRI scan, made within 4 weeks after the stroke occurred, confirmed the diagnosis, or if indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature of the stroke. A stroke was subclassified as hemorrhagic if a relevant hemorrhage was shown on CT or MRI scan. Supratentorial hemorrhagic strokes were subclassified as deep or lobar based on neuroimaging. If we could not retrieve enough information to subclassify a stroke as ischemic or hemorrhagic, it was called unspecified. Follow-up was complete until January 1, 2005, for 98.7 % of potential person years.[18]

Glomerular filtration rate assessment: During the baseline center visit non-fasting blood was collected and centrifuged within 30 minutes at 3000 rotations per minute for 10 minutes. Subsequently the serum was stored at -20°C for 1 week, until serum creatinine level was assessed by a nonkinetic alkaline picrate (Jaffé) method[19] (Kone Autoanalyzer, Kone Corporation, Espoo, Finland and Elan, Merck, Darmstadt, Germany). The method was standardized against high performance liquid chromatography. The within-run precision was >98.5% and the day-by-day precision was >95.0%. Creatinine clearance was computed with the Cockcroft-Gault equation.[20] Creatinine clearance generally exceeds GFR by 10-15% due to urinary creatinine derived from tubular secretion;[22] the Cockroft Gault estimate of GFR was therefore additionally corrected with a factor of 0.9, and standardized for 1.73 m^2 body surface area using the Dubois formula:[21] GFR = (140-age[years]) (weight[kg] x 1.23) (0.85 if female) (serum creatinine [µmol/l])$^{-1}$ (1.73) (weight[kg])$^{-0.425}$ (height[cm])$^{-0.725}$ (0.007184)$^{-1}$. Since 98.5 % of our participants were Caucasian, and 99.2% were at least partly Caucasian, adjustment for ethnicity was not required.

Other Measurements: Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements in the analyses. Left ventricular hypertrophy was assessed with a 12-lead resting ECG and the Modular ECG Analysis System (MEANS)[23] implemented on an ACTA electrocardiograph (ESAOTE, Florence, Italy). We considered diabetes mellitus to be present if a random or post-load glucose level was 11.1 mmol/l or higher, or if a person used antidiabetic

medication. Total cholesterol, high-density lipoprotein, uric acid, and C-reactive protein were measured in non-fasting baseline serum with an automated enzymatic procedure. Carotid intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery. We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far wall of both the right and left common carotid artery. History of myocardial infarction was positive if a participant had reported a myocardial infarction that was confirmed by ECG or medical records. Use of medication and smoking history were assessed during a home interview. The number of pack years of smoking was calculated by multiplying the number of cigarette packs smoked per day by the number of years smoked. The proportion of missing values was ≤ 1% for all variables, with the exception of intima-media thickness (17% missing), serum C-reactive protein (7% missing), and serum uric acid (3% missing).

Population for Analysis: Of all 7983 participants who were enrolled into the Rotterdam study, 7722 were free from stroke at study baseline. Of these, 1723 participants visited the research center after 31 December 1992, when creatinine assays had been stopped due to financial constraints. For the present study, 1062 participants could not be included due to missing data: 844 did not visit the research center, 69 had unsuccessful blood sampling, 131 did not have weight or height measured, and 18 refused informed consent for retrieval of stroke follow-up data. Consequently, 4937 participants were included in the analyses.

Statistical Analysis: We used Cox' proportional hazards models to calculate hazard ratios (HRs) with 95% confidence intervals (95% CIs) for the associations between GFR and stroke after inspection of log(-log) survival curves. Hazard ratios (HRs) were calculated for GFR quartiles (relative to the lowest quartile). Subsequently, we compared the risk of stroke among participants with chronic kidney disease as defined by the internationally accepted criterion of a GFR below 60 ml/min/1.73m^2,[24] with the risk of participants without chronic kidney disease. We constructed 3 models: in model 1 we adjusted for age and sex, and in model 2 we adjusted for age, sex, systolic blood pressure, diastolic blood pressure, antihypertensive drug use, and left ventricular hypertrophy. In model 3 we adjusted for age, sex, and a propensity score: for the analyses concerning quartiles of GFR, we calculated the propensity score with a linear regression model, entering GFR as dependent variable. For the analyses concerning chronic kidney disease, we calculated a linear propensity score with a logistic regression model, entering diagnosis of chronic kidney disease as dependent variable.[25] The propensity scores were based on systolic blood pressure, diastolic blood pressure, antihypertensive drug use, diuretic use, left ventricular hypertrophy, packyears of smoking, diabetes mellitus, serum cholesterol, serum high density lipoprotein, carotid intima-media thickness, serum uric acid, serum C-reactive protein, previous myocardial infarction, previous atrial fibrillation, waist/hip ratio, antitrombotic drug use, and lipid lowering drug use. For missing values in

confounders and effect modifiers, the mean value was imputed. All analyses were performed with SPSS for Windows, Rel. 11.0.1 (SPSS Inc, Chicago, IL, 2001).

Results

The median age of the participants of the present study was 69 years and 61% were women. Other baseline characteristics are described in table 1. The 1062 participants who were not included in the present study due to missing data were older than the included participants (median age 80.3 versus 68.9 years; Mann-Whitney P<0.001) and also more likely to be female (72% versus 61%, Mann-Whitney P<0.001).

Table 1. Baseline characteristics of the study population (n=4937).

Baseline characteristic	Median* or percentage	P-value for association with GFR†
Serum creatinine, µmol/l	81 (72-91)	-
Body surface area, m²	1.8 (1.7-1.9)	-
GFR, ml/min/1.73m²	68.0 (57.3-78.8)	-
Both parents Caucasian, %	98.5	-
Age, yrs	68.9 (62.3-76.7)	<0.001
Female sex, %	61.3	0.78
Systolic blood pressure, mm Hg	138 (123-153)	0.002
Diastolic blood pressure, mm Hg	73 (66-81)	0.07
Carotid intima-media thickness, mm	0.78 (0.69-0.88)	0.68
Serum C-reactive protein, mg/l	1.87 (0.90-3.63)	0.64
Cholesterol, mmol/l	6.6 (5.8-7.4)	0.42
High-density lipoprotein, mmol/l	1.3 (1.1-1.6)	<0.001
Serum uric acid	310 (267-370)	<0.001
Diabetes mellitus, %	10.3	0.01
Left ventricular hypertrophy	5	0.01
Previous myocardial infarction, %	12.0	0.09
Atrial fibrillation	5	0.004
Waist/hip ratio, cm/cm	0.90 (0.83-0.97)	<0.001
Smoking, pack years	2.5 (0-26)	0.04
Antihypertensive medication, %	13	0.81
Diuretic use, %	16	0.17
Antithrombotic drug use	4	<0.001
Lipid lowering drug use, %	2	0.28

* with 25th and 75th percentile.

† P-values estimated with linear regression model adjusted for all other characteristics.

During on average 10.2 years of follow-up, 586 first-ever strokes occurred. Of these, 338 could be subclassified as ischemic and 44 as hemorrhagic, 204 were unspecified. The diagnosis was made without the use of neuroimaging in 9% of all ischemic stroke cases, in 0% of all hemorrhagic stroke cases, and in 96% of all unspecified stroke cases. The participants who did not have neuroimaging were older than those who did have neuroimaging (median age 78.3 versus 70.7 years; Mann-Whitney P<0.001) and also more likely to be female (69% versus 56%, Mann-Whitney P<0.01).

Inspection of log(-log) survival curves showed that the proportional hazards assumption was not violated. In an age and sex adjusted Cox model we found a slightly but non-significantly increased risk of stroke (HR 1.14; 95% CI 0.84-1.54) for the lower compared to the upper quartile of GFR (table 2). We found no association between GFR and the risk of ischemic stroke. In contrast, the hazard of hemorrhagic stroke was 4.10 (95% CI 1.25-13.42) times higher in the lowest compared with the highest quartile of GFR and showed a clear trend towards increasing hemorrhagic stroke risk with decreasing GFR (age and sex adjusted HR 2.03, 95% CI 1.31-3.15 per standard deviation decrease in GFR). None

Table 2. Hazard ratios for the association between glomerular filtration rate and risk of stroke. N=4937.

Event	GFR*	Hazard ratio (95% CI)		
		Model1†	Model 2†	Model 3†
All stroke	Quartile 4	1 (ref)	1 (ref)	1 (ref)
(n=586)	Quartile 3	0.86 (0.65-1.14)	0.88 (0.66-1.16)	0.85 (0.64-1.13)
	Quartile 2	1.15 (0.88-1.51)	1.16 (0.89-1.52)	1.10 (0.84-1.44)
	Quartile 1	1.14 (0.84-1.54)	1.15 (0.85-1.55)	1.04 (0.77-1.41)
Ischemic stroke	Quartile 4	1 (ref)	1 (ref)	1 (ref)
(n=338)	Quartile 3	0.88 (0.63-1.22)	0.89 (0.64-1.24)	0.87 (0.62-1.20)
	Quartile 2	1.13 (0.82-1.56)	1.16 (0.84-1.60)	1.09 (0.79-1.51)
	Quartile 1	0.87 (0.59-1.29)	0.90 (0.61-1.32)	0.81 (0.54-1.20)
Hemorrhagic stroke	Quartile 4	1 (ref)	1 (ref)	1 (ref)
(n=44)	Quartile 3	1.76 (0.58-5.29)	1.79 (0.59-5.38)	1.73 (0.58-5.22)
	Quartile 2	3.06 (1.06-8.86)	3.12 (1.08-9.03)	2.92 (1.00-8.48)
	Quartile 1	4.10 (1.25-13.42)	4.14 (1.27-13.54)	3.68 (1.12-12.09)

* Thresholds for increasing quartiles were 53.9, 63.1, and 72.0 ml/min/1.73m² for men, and 50.4, 60.3, and 70.1 ml/min/1.73m² for women.

† Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, antihypertensive drug use, and left ventricular hypertrophy. Model 3: adjusted for age, sex, and propensity score (systolic blood pressure, diastolic blood pressure, antihypertensive drug use, left ventricular hypertrophy, diuretic use, packyears of smoking, diabetes mellitus, serum cholesterol, serum high density lipoprotein, carotid intima-media thickness, serum uric acid, serum C-reactive protein, previous myocardial infarction, previous atrial fibrillation, waist/hip ratio, antitrombotic drug use, lipid lowering drug use).

Table 3. Hazard ratios for the association between chronic kidney disease and risk of stroke.

Event	GFR	Hazard ratio (95% CI)		
	(ml/min/1.73m²)	Model1*	Model 2*	Model 3*
All stroke	≥ 60 (N=2652)	1 (ref)	1 (ref)	1 (ref)
(n=586)	< 60 (N=2285)	1.29 (1.06-1.57)	1.28 (1.05-1.56)	1.22 (1.00-1.49)
Ischemic stroke	≥ 60 (N=2652)	1 (ref)	1 (ref)	1 (ref)
(n=338)	< 60 (N=2285)	1.25 (0.97-1.61)	1.26 (0.98-1.63)	1.19 (0.92-1.53)
Hemorrhagic stroke	≥ 60 (N=2652)	1 (ref)	1 (ref)	1 (ref)
(n=44)	< 60 (N=2285)	3.02 (1.45-6.27)	3.05 (1.46-6.35)	2.88 (1.38-6.01)

* Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, antihypertensive drug use, and left ventricular hypertrophy. Model 3: adjusted for age, sex, and propensity score (systolic blood pressure, diastolic blood pressure, antihypertensive drug use, left ventricular hypertrophy, diuretic use, packyears of smoking, diabetes mellitus, serum cholesterol, serum high density lipoprotein, carotid intima-media thickness, serum uric acid, serum C-reactive protein, previous myocardial infarction, previous atrial fibrillation, waist/hip ratio, antitrombotic drug use, lipid lowering drug use).

of the putative confounders that were tested changed the estimates of the HRs more than 4%. Adjustment for all measures of hypertension (model 2) increased all hazard ratios slightly. Adjustment for the propensity score of all potential confounders hardly attenuated the HRs. When we looked separately at subtypes of hemorrhages, the HR was 2.76 (95% CI 1.34-5.67) per SD for deep hemorrhages (n=16), and 2.77 (95% CI 1.02-7.53) per SD for lobar hemorrhages (n=8).

When we compared the risk of stroke between participants with and without chronic kidney disease,[24] we found that participants with chronic kidney disease had a slightly and nonsignificantly increased risk of ischemic stroke (HR 1.25; 95% CI 0.97-1.61; table 3) and a strongly increased risk of hemorrhagic stroke (HR 3.02; 95% CI 1.45-6.27).

Discussion

In this population-based study, we found a strong and graded inverse association between GFR and risk of hemorrhagic stroke that was independent from other vascular risk factors. We found no association between GFR and risk of ischemic stroke.

Strengths of our study are the meticulous stroke case finding and the nearly complete follow-up (loss of potential person-years, 1.3 %). To our knowledge, our study is the first that assessed the association between GFR and the risk of subtypes of stroke in the general population. Our stringent stroke monitoring procedures allowed us to include also stroke patients who had not been referred to a neurologist (31% of all stroke cases). As in these cases neuroimaging had not been performed, we could subclassify only 16% of them into ischemic or hemorrhagic. In contrast, 92% of strokes that had been referred to a neurologist

could be subclassified. As a result, we could not determine the subtype of stroke in 204 participants. It is expected that approximately 80% of these are ischemic and 20% hemorrhagic. Since participants with unspecified stroke were censored at time of stroke in the subtype analyses, they did not influence the hazard ratios for subtypes of stroke.

Not all participants of the Rotterdam Study who were stroke-free at baseline were included in the analyses. The 1723 participants who were not included in the present study because they visited the research center after the creatinine assays were stopped did not introduce bias since participants were invited to visit the research center in random order. However, 1062 participants were not included in the present study due to missing data. These participants were on average a little older, more often female, and possibly less healthy than those in the study population. Since this makes the study population more homogeneous than the source population, some associations may have been slightly underestimated.

The Cockcroft Gault equation[20] and the abbreviated Modification of Diet in Renal Disease (MDRD) equation[26] are the most widely used equations to estimate GFR. As the latter equation has been developed in a population for which 99.6% of our participants would not meet inclusion criteria, we chose to use the Cockcroft Gault equation in our present study. We measured blood pressure twice on a single day. Since we thereby ignored day-by-day variations in blood pressure, some residual confounding of the relation between GFR and risk of stroke by blood pressure may remain. However, adjustment for blood pressure did not attenuate the associations we found at all. We measured serum creatinine only once, ignoring possible intra-individual fluctuations in serum creatinine levels. This may have caused our estimates to be slightly underestimated. Furthermore, serum creatinine is influenced by nonrenal factors and additional measurement of urinary albumin might have improved the sensitivity and specificity of our assessment of kidney function. However, urinary albumin was not measured in our study. Relatively few participants developed a hemorrhagic stroke due to the low incidence rate of this disease, therefore the 95% confidence intervals are fairly wide. However, the high level of statistical significance and the strong dose response relationship show that our study had enough precision to claim that the risk of hemorrhagic stroke increases with decreasing GFR.

Few studies reported on the association between GFR and stroke in the general population and none of these studies divided stroke into ischemic and hemorrhagic. A pooled analysis of four population-based studies showed that the risk of stroke seemed increased by 17% among participants with chronic kidney disease (stage 3 or 4) compared to participants without chronic kidney disease, although this finding was not statistically significant.[14] This is of the same magnitude as our finding of a 25% non-significant increased risk of ischemic stroke in participants with GFR < 60 ml/min/1.73m² compared to participants with GFR greater than 60 ml/min/1.73m². In another study, the risk of stroke was moderately increased in the lowest GFR quintile compared to the highest GFR quintile, with a 92% increased risk among participants with GFR below the 7th percentile. This effect largely disappeared after

adjustment for confounding.[13]

In most studies, including ours, adjustment for cardiovascular risk factors did not markedly change the associations between GFR and the risk of cardiovascular disease,[10-14, 27] which means either that GFR is a better marker for vascular pathology than other vascular risk factors, or that chronic kidney disease is a causal factor in the pathogenesis of coronary heart disease and hemorrhagic stroke. On the other hand, the adjusted estimates may be underestimations of the true associations because at least part of the presumed effect of chronic kidney disease on cardiovascular disease is through the risk factors that were adjusted for.

There are at least two hypotheses that may explain how GFR can be associated with hemorrhagic stroke and not with ischemic stroke. A first explanation assumes a noncausal association between GFR and hemorrhagic stroke. Decreased GFR is often attributable to renal small vessel disease, which is presumably correlated with small vessel disease in the brain.[28] Therefore, decreased GFR may be a marker of cerebral small vessel disease, which is presumed to be the main pathophysiological mechanism in the majority of brain hemorrhages whereas it plays a much smaller role in brain infarctions. An alternative explanation assumes that chronic kidney disease has a causal role in hemorrhagic stroke pathophysiology. An important sequel of chronic kidney disease is platelet dysfunction. This becomes apparent by prolonged bleeding time, mucutaneous ecchymoses, and mucosal oozing in patients with severe chronic kidney disease.[5] It is possible that a slightly decreased GFR also influences platelet function, which might explain the increased risk of hemorrhagic stroke in persons with decreased GFR.

In conclusion, we found that GFR is a risk factor for hemorrhagic stroke and not for ischemic stroke. We think this is an important finding as our knowledge of causes of hemorrhagic stroke is very limited and we might have identified an important risk factor. This finding may provide insight into hemorrhagic stroke pathophysiology. In addition, it identifies a potentially treatable cause of this devastating disease.

References

1. Nissenson AR, Pereira BJ, et al. Prevalence and characteristics of individuals with chronic kidney disease in a large health maintenance organization. Am J Kidney Dis. 2001;37:1177-1183
2. Sarnak MJ, Levey AS, et al. Kidney disease as a risk factor for development of cardiovascular disease. Circulation. 2003;108:2154-2169
3. Foley RN, Wang C, Collins AJ. Cardiovascular risk factor profiles and kidney function stage in the US general population: The NHANES III Study. Mayo Clin Proc. 2005;80:1270-1277
4. Mezzano D, Tagle R, et al. Hemostatic disorder of uremia: The platelet defect, main determinant of the prolonged bleeding time, is correlated with indices of activation of coagulation and fibrinolysis. Thromb Haemost. 1996;76:312-321
5. Noris M, Remuzzi G. Uremic bleeding: Closing the circle after 30 years of controversies? Blood. 1999;94:2569-2574
6. Guerin AP, Adda H, et al. Cardiovascular disease in renal failure. Minerva Urol Nefrol. 2004;56:279-288
7. Toto RD. Treatment of hypertension in chronic kidney disease. Semin Nephrol. 2005;25:435-439
8. Goodman WG, Goldin J, et al. Coronary-artery calcification in young adults with end-stage renal

disease who are undergoing dialysis. N Engl J Med. 2000;342:1478-1483
9. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis. 1998;32:S112-119
10. Fox CS, Larson MG, et al. Kidney function is inversely associated with coronary artery calcification in men and women free of cardiovascular disease: The Framingham Heart Study. Kidney Int. 2004; 66:2017-2021
11. Go AS, Chertow GM, et al. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351:1296-1305
12. Henry RM, Kostense PJ, et al. Mild renal insufficiency is associated with increased cardiovascular mor tality: The Hoorn Study. Kidney Int. 2002;62:1402-1407
13. Shlipak MG, Sarnak MJ, et al. Cystatin C and the risk of death and cardiovascular events among eld erly persons. N Engl J Med. 2005;352:2049-2060
14. Weiner DE, Tighiouart H, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: A pooled analysis of community-based studies. J Am Soc Nephrol. 2004;15:1307-1315
15. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
16. Bos MJ, Schipper CM, et al. High serum C-reactive protein level is not an independent predictor for stroke: The Rotterdam Study. Circulation. 2006;114:1591-1598
17. Bos MJ, Koudstaal PJ, et al. Uric acid is a risk factor for myocardial infarction and stroke: The Rot terdam Study. Stroke. 2006;37:1503-1507
18. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;359:1309-1310
19. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem. 1992;38:1933-1953
20. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16:31-41
21. Wang Y, Moss J, Thisted R. Predictors of body surface area. J Clin Anesth. 1992;4:4-10
22. Vervoort G, Willems HL, Wetzels JF. Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric diabetic patients: Validity of a new (MDRD) prediction equation. Nephrol Dial Transplant. 2002;17:1909-1913
23. van Bemmel JH, Kors JA, van Herpen G. Methodology of the modular ECG analysis system MEANS. Methods Inf Med. 1990;29:346-353
24. Levey AS, Eckardt KU, et al. Definition and classification of chronic kidney disease: A position state ment from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2005;67:2089-2100
25. Kurth T, Walker AM, et al. Results of multivariable logistic regression, propensity matching, pro pensity adjustment, and propensity-based weighting under conditions of nonuniform effect. Am J Epidemiol. 2006;163:262-270
26. Levey AS, Bosch JP, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461-470
27. Brugts JJ, Knetsch AM, et al. Renal function and risk of myocardial infarction in an elderly population: The Rotterdam Study. Arch Intern Med. 2005;165:2659-2665
28. O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: Cause and logic of therapy. Hypertension. 2005;46:200-204

2.3.
Transcranial Doppler hemodynamic parameters

Background and purpose: *We explored the association between transcranial Doppler (TCD) hemodynamic parameters and the risk of stroke in the general population.* ***Methods:*** *At baseline we assessed mean flow velocity, peak systolic flow velocity, end diastolic flow velocity, and vasomotor reactivity (VMR) with TCD in 2022 Rotterdam Study participants of age 61 years and over in both middle cerebral arteries. All participants, who at baseline were free from previous stroke, were subsequently followed for occurrence of stroke (average follow-up time 5.1 years). We calculated hazard ratios (HRs) for the association between hemodynamic parameters and risk of stroke using Cox proportional hazards models with adjustment for age, sex, systolic blood pressure, antihypertensive drug use, diabetes mellitus, ever smoking, current smoking, carotid intima-media thickness, and carotid distensibility.* ***Results:*** *Risk of stroke (n=122) and ischemic stroke (n=89) increased with increasing middle cerebral artery flow velocity: when comparing the tertile with highest velocity to the tertile with lowest velocity, the HR was 1.74 (95% CI 1.09-2.77) for the association between mean flow velocity and stroke, 1.63 (95% CI 1.03-2.58) for end diastolic flow velocity and stroke, and 1.33 (95% CI 0.86-2.08) for peak systolic flow velocity and stroke. These estimates increased 10-26% when only ischemic strokes were included. The side of highest flow velocity was not associated with the side of stroke. We found no associations between vasomotor reactivity and risk of stroke.* ***Conclusions:*** *Risk of stroke increased strongly with increasing middle cerebral artery flow velocity as measured with TCD in the general population.*

Introduction

The technique of transcranial Doppler ultrasonography, which became available in 1982, enables assessment of hemodynamic parameters including flow velocity in intracranial arteries. The mechanisms that influence intracranial flow velocity are incompletely understood. However, local strong increases or decreases in flow velocity have been found to be associated with high-grade local stenosis.[1] A milder and more generalized increased flow velocity could reflect intracerebral atherosclerosis or arterial narrowing in response to systemic hypertension. The ability of the flow velocity to adapt to stimuli like hypercapnia is called vasomotor reactivity (VMR), which is another hemodynamic parameter. Possible mechanisms that may account for exhausted VMR include chronic hypoperfusion of the brain that leads to continuous maximal dilation of cerebral vasculature, and arterial wall stiffening due to atherosclerosis. Despite the limited understanding of the mechanisms that influence intracranial flow velocity, the technique of TCD is frequently used in clinical praxis and research.[2]

The role of hemodynamic parameters in relation to stroke has been assessed in specific patient groups: Sacco et al. demonstrated with TCD that in 8% of 438 ischemic stroke patients intracerebral atherosclerosis was the most probable cause of the stroke.[3] Internal carotid and middle cerebral artery TCD flow velocity were good predictors of stroke risk in children with sickle cell disease.[4] Also progression of symptomatic middle cerebral artery occlusion monitored with TCD was associated with an increased risk of (recurrent) stroke.[5] In patients with severe carotid artery stenosis, impaired VMR was shown to be associated with risk of subsequent stroke and transient ischemic attack.[6-8] However, it remains to be determined whether cerebral hemodynamic parameters are risk factors for stroke in the general population.

The aim of this study was to assess the association between various middle cerebral artery hemodynamic parameters measured with TCD and the risk of stroke in a population-based setting.

Methods

Population: The Rotterdam Study is a population-based cohort study on chronic and disabling diseases.[9, 10] All inhabitants of Ommoord, a district of the city of Rotterdam in the Netherlands, aged 55 years and over were invited to participate. Participation rate of those invited for the study was 78%; in total, 7983 subjects participated in the first study survey (1990-1993). Baseline measurements for the present study were done during the third survey of the Rotterdam Study (1997-1999); at this time participants were 61 years of age and over. The third study survey included only participants who had also participated in the first survey. The Medical Ethics Committee of Erasmus University Rotterdam approved of the study.

Written informed consent to retrieve information from treating physicians was obtained from all participants. Follow-up was from TCD assessment to first-ever stroke, death, study end or loss to follow-up (whichever occurred first).

Assessment of Stroke: History of stroke at time of enrollment into the first Rotterdam Study survey (1990-1992) was positive if a stroke was reported during the interview and confirmed by medical records. After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. Subarachnoid hemorrhages were excluded. A stroke was subclassified as ischemic when a CT or MRI scan, made within 4 weeks after the stroke occurred, ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature of the stroke. Follow-up was complete until January 1, 2005, for 99.0% of potential person years.[11]

Transcranial Doppler assessment: All participants underwent transcranial Doppler ultrasonography monitoring in one session during the third Rotterdam Study survey, which was the baseline measurement for the present study. This monitoring (Multi-Dop X-4, DWL, Sipplingen, Germany) was performed to measure the cerebral blood flow velocity in the middle cerebral artery on both sides. End-diastolic, peak systolic and mean cerebral blood flow velocities were recorded automatically. If automatic recording was not possible, the participant was excluded from the study. All velocities were measured at a depth of 50 mm, or as close as possible to this depth. The mean cerebral blood flow velocity was defined as: 1/3 * (peak systolic flow velocity + 2 x end-diastolic flow velocity). If flow velocity was available for both sides the average value was used. Cerebrovascular CO_2 reactivity measurement was performed as follows: the cerebral blood flow velocity was measured continuously and the participants first breathed room air through an anesthetic mask, tightly fit over mouth and nose, until a steady expiratory end tidal CO_2 was obtained. Participants were then asked to inhale a mixture of 5% carbon dioxide in 95% oxygen for 2 minutes. Cerebrovascular CO_2 reactivity was defined as the percentage increase in mean cerebral blood flow velocity during inspiration of 5% CO2/the absolute increase in end-tidal CO_2 in the same time period (%/kPa). End-tidal CO_2 pressure (kPa) was recorded continuously with a CO_2 analyser (Multinex, Datascope, Hoevelaken, The Netherlands). End-expiratory CO_2 was assumed to reflect arterial CO_2.[12] All measurements were performed with the participant in supine position after 5 min of rest. TCD-8 DWL special software (VMR- CO_2) was used. All transcranial Doppler

data were stored on hard disk for off-line analysis. The TCD studies were performed by one of 3 technicians who were especially trained for the present study.

Other Measurements: Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements in the analyses. We considered diabetes mellitus to be present if a fasting glucose level was 7.0 mmol/L or higher, or if a person used antidiabetic medication. Smoking status was assessed during a home interview. Carotid intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery.[13] We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far wall of both the right and left common carotid artery. Common carotid distensibility was assessed with a duplex scanner connected to a vessel wall movement detector system in the right common carotid artery as described previously.[14]

Population for Analysis: TCD flow velocity assessment was implemented in the protocol of the third Rotterdam Study survey starting April 8, 1997. From this date onwards, 4215 participants visited the study center. Because of lacking technical support over a prolonged period, transcranial Doppler measurements were only offered to 3118 participants. We excluded all participants who had previous stroke at time of TCD assessment (n=110), which left 3008 participants eligible for TCD assessment. Of these, 986 participants had window failure on both sides (n=741), or difficulty to participate because of a variety of other reasons, most importantly restlessness, anxiety, and discomfort (n=245). This left 2022 stroke-free participants eligible for the analyses. VMR assessment was only performed from July 1, 1997, leaving 1695 participants eligible for VMR analyses.

Statistical Analysis: We used Cox' proportional hazards models to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for the associations between TCD parameters and risk of stroke. Hazard ratios were calculated for sex specific TCD parameter tertiles (relative to the lowest tertile) and per standard deviation (SD) increase in TCD parameter. We performed the analyses per participant, averaging parameters over both middle cerebral arteries if available, and associated this with risk of stroke. We looked separately at TCD mean flow velocity > 100 cm/s as this cutoff has been found to correspond well with stenosis of > 50%.[15] We adjusted for confounding by age and sex, and additionally for confounding by other putative confounders (systolic blood pressure, antihypertensive drug use, diabetes mellitus, ever smoking, current smoking, carotid intima-media thickness, and carotid distensibility). Missing values in carotid intima-media thickness (n=163) and carotid distensibility (n=546) were imputed with a linear regression model based on age and sex. Analyses were performed with SPSS® 11.0.1 for Windows.

Results

Baseline characteristics of the study population are described in table 1. Flow velocity was available for both sides in 1354 participants and for 1 side in 668 participants. Measurements failed in 986 participants. TCD measurement failure occurred more often in women and in older participants who possibly had more diabetes mellitus and smoked less.

Average follow-up from time of TCD measurement to stroke, death, or censoring was 5.1 years. During this period, 122 strokes occurred; 89 of these were subclassified as ischemic strokes. Among people with VMR measurements, 87 strokes (69 ischemic strokes) occurred during on average 5.0 years of follow-up.

A strong association was found between mean cerebral blood flow velocity and the risk of stroke: the age and sex adjusted HR (95% CI) for upper versus lower tertile of flow velocity was 1.74 (1.05-1.49) for the risk of stroke and 2.21 (1.26-3.88) for the risk of ischemic stroke (table 2). End diastolic and peak systolic flow velocities were somewhat weaker but positively associated with the risk of stroke and ischemic stroke, although not statistically significant at α=0.05: HRs for highest versus lowest tertile of end diastolic flow velocity were

Table 1. Baseline characteristics of participants who were eligible for TCD measurements.

Characteristic	Study population* (N=2022)	Participants with TCD measurement failure* (N=986)	P for difference†
Age (yrs)	70.2 (65.8-75.5)	72.9 (68.5-77.9)	<0.001
Female sex	47%	79%	<0.001
Systolic blood pressure (mmHg)	141 (128-155)	144 (131-158)	0.77
Antihypertensive drug use	34%	37%	0.88
Diabetes mellitus	13%	15%	0.08
Ever smoking	73%	56%	0.10
Current smoking	19%	16%	0.33
Carotid intima-media thickness (mm)	0.85 (0.76-0.95)	0.86 (0.77-0.97)	0.24
Carotid distensibility	10.59 (7.89-13.77)	9.04 (6.77-12.27)	0.37
Measurement depth (mm)	50 (50-51)	-	-
Mean flow velocity (cm/s)	56 (46-65)	-	-
End diastolic flow velocity (cm/s)	32 (26-38)	-	-
Peak systolic flow velocity (cm/s)	85 (73-99)	-	-
Vasomotor reactivity (%/kPa)	39 (28-54)	-	-

* Values are presented as median (interquartile range) or percentage

† P-values for differences are calculated with logistic regression model adjusted for all other baseline characteristics

Table 2. Middle cerebral artery flow velocity (N=2022) and vasomotor reactivity (N=1695) and risk of stroke.

TCD hemodynamic parameter*	Hazard ratios (95% CIs)			
	Model 1†		Model 2†	
	All strokes (n=122)	Ischemic strokes (n=89)	All strokes (n=122)	Ischemic strokes (n=89)
Mean flow velocity				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	1.60 (1.03-2.48)	2.04 (1.19-3.49)	1.60 (1.03-2.48)	2.04 (1.19-3.49)
Tertile 3	1.75 (1.10-2.77)	2.21 (1.26-3.88)	1.74 (1.09-2.77)	2.20 (1.25-3.87)
Per SD	*1.25 (1.05-1.49)*	*1.38 (1.13-1.67)*	*1.24 (1.04-1.49)*	*1.37 (1.12-1.68)*
End diastolic flow velocity				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	1.09 (0.70-1.69)	1.23 (0.73-2.06)	1.12 (0.72-1.75)	1.28 (0.76-2.16)
Tertile 3	1.47 (0.93-2.32)	1.58 (0.92-2.72)	1.63 (1.03-2.58)	1.80 (1.04-3.11)
Per SD	*1.17 (0.98-1.40)*	*1.22 (0.99-1.50)*	*1.20 (1.01-1.43)*	*1.25 (1.02-1.53)*
Peak systolic flow velocity				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	1.20 (0.77-1.86)	1.51 (0.89-2.55)	1.16 (0.74-1.80)	1.45 (0.85-2.45)
Tertile 3	1.42 (0.91-2.19)	1.69 (1.00-2.87)	1.33 (0.86-2.08)	1.58 (0.92-2.69)
Per SD	*1.17 (0.99-1.38)*	*1.28 (1.06-1.54)*	*1.14 (0.97-1.35)*	*1.24 (1.02-1.50)*
Vasomotor reactivity‡				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	0.73 (0.43-1.24)	0.80 (0.43-1.47)	0.75 (0.44-1.27)	0.81 (0.44-1.51)
Tertile 3	1.04 (0.63-1.72)	1.17 (0.66-2.09)	1.04 (0.62-1.74)	1.15 (0.64-2.08)
Per SD	*1.03 (0.83-1.28)*	*1.10 (0.88-1.38)*	*1.01 (0.82-1.25)*	*1.07 (0.85-1.33)*

*Cut points for tertiles in men: 48 and 60 cm/s (mean flow velocity), 28 and 35 cm/s (end diastolic flow velocity), 76 and 92 cm/s (peak systolic flow velocity), 27 and 34 %/kPa (VMR). Cut points for tertiles in women: 52 and 65 cm/s (mean flow velocity), 29 and 37 cm/s (end diastolic flow velocity), 79 and 96 cm/s (peak systolic flow velocity), 31 and 46 %/kPa (VMR).

† Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, antihypertensive drug use, diabetes mellitus, ever smoking, current smoking, carotid intima-media thickness, carotid distensibility.

‡ The vasomotor reactivity analyses included 87 incident strokes and 67 incident ischemic strokes.

1.47 (0.93-2.32) for stroke and 1.58 (0.92-2.72) for ischemic stroke. HRs (95% CIs) for highest versus lowest tertile of peak systolic flow velocity were 1.42 (0.91-2.19) for stroke and 1.69 (1.00-2.87) for ischemic stroke. VMR was not associated with the risk of stroke (table 2). Adjustment for putative confounders had only a small effect on the observed associations: either the strength of the associations increased or the associations slightly decreased by no more than 27%.

We found no difference in the associations between TCD parameters and stroke when we compared participants of different ages (P interaction > 0.33 for all parameters). We also found no difference in the associations between TCD parameters and stroke when we compared men to women (P interaction = 0.05 for interaction between end diastolic blood pressure and sex on risk of ischemic stroke, for all other parameters P interaction > 0.10).
In twenty-seven participants (10 males, 17 females, median age 68.9 years) the mean flow velocity in the middle cerebral artery exceeded 100 cm/s; this was an unilateral finding in 24 participants and bilateral in 3 participants. Two strokes occurred in these participants: both strokes were ischemic and in the hemisphere contralateral to the artery with velocity > 100 cm/s.

For most participants the left mean flow velocity was very similar to the right mean flow velocity (Pearson's correlation coefficient 0.79, P<0.001). In 96% of participants the difference between left and right mean flow velocity was less than 1 SD of the mean flow velocity distribution. Of all participants with a difference > 1 SD (N=134), 4 had a stroke at the side with highest flow velocity, 8 had a stroke at the side with lowest flow velocity, and 1 had a vertebrobasilar stroke. In addition, we found that the difference between left and right flow velocity was neither associated with risk of right hemispherical stroke nor with risk of left hemispherical stroke in Cox models (P>0.25).

Discussion

In this population-based study in stroke-free subjects aged 61 years and over, we found a strong, significant, and independent association between middle cerebral artery blood flow velocity measured with TCD and the risk of stroke and particularly ischemic stroke. Differences between left and right flow velocities were very small, and the side of highest flow velocity was not associated with the side of stroke. No associations were found between VMR and the risk of stroke.

Before these results can be interpreted, some methodological issues need to be discussed. Strengths of our study are the meticulous stroke case finding and the nearly complete follow-up (loss of potential person-years, 1.0 %). To our knowledge, ours is the first study that assessed TCD parameters in the general population. Our stringent stroke monitoring procedures allowed us to include also stroke patients who were not referred to a hospital. A

disadvantage is that in these cases neuroimaging was often lacking so that 21% of strokes could not be subclassified into ischemic or hemorrhagic. A limitation of the technique of TCD is window failure. We had bilateral window failure in 33% of participants, which is comparable to other studies,[16, 17] although some studies had a lower failure rate.[6-8] It is possible that healthy participants in a population study are less inclined to endure the discomfort of TCD investigations than hospital patients. Our decision to restrict ourselves to automated velocity recording increased measurement standardization but could have increased failure rate. As expected, window failure, which primarily depends on temporal bone thickness, occurred most frequently in older persons and in women. As we found no difference in the associations between TCD parameters and stroke when we compared older to younger participants or when we compared men to women, we think the relative underrepresentation of older women in our study population compared to our source population has not biased our results. Several participants (n=245) did not have successful TCD assessments because of anxiety or restlessness. To the extent that this could have reflected cerebrovascular dysfunction, it could have led to an underestimation of the true associations. TCD ultrasonography is not the gold standard method to assess intracranial atherosclerosis but it is very well suited for population-based research due to its non-invasiveness and its strong association with gold standard measurements.[18, 19]

We cannot directly compare our findings regarding the association between middle cerebral artery flow velocity and the risk of stroke with previous studies: in children with sickle cell disease high flow velocity also predicted a high stroke risk,[4] but the pathophysiological mechanism of stroke in sickle cell disease greatly differs from that in our study population. Another study reported that progression of symptomatic middle cerebral artery stenosis measured with TCD was associated with an increased risk of (recurrent) stroke.[5] Even though this study was conducted in stroke and TIA patients rather than in stroke-free subjects and looked at progression of flow velocity rather than at flow velocity itself, we can regard the findings of this study as support for our finding that increased middle cerebral artery flow velocity is associated with an increased risk of stroke.

It has been shown that middle cerebral artery flow velocity can locally be strongly increased by atheroma: in a study among patients with symptomatic intracranial stenosis it was concluded that a mean flow velocity of > 100 cm/s corresponds well with a middle cerebral artery stenosis of 50%.[15] In our study population, 99% of participants had flow velocities < 100 cm/s, therefore high-grade middle cerebral artery stenoses probably do not account for the associations between flow velocity and stroke that we observed. However, it is possible that a more subtle increase in flow velocity reflects a more subtle, and likely a more generalized, arterial narrowing caused by atherosclerosis: Poiseuille's law shows that an atherosclerotic decrease in arterial diameter (D) leads to a strong drop in total blood flow (which is proportional to D^4) and to a milder drop in flow velocity (which is proportional to D^2), unless the effect of arterial narrowing is counterbalanced by an increase in the pressure

gradient over the stenosed trajectory. This pressure gradient can be increased by a decrease in pressure downstream to the stenosis or by an increase in blood pressure. If the increase in pressure gradient is large enough to result in a constant total flow, it results in an increased flow velocity. As such, increased flow velocity may be a marker of subtle generalized atherosclerosis. An alternative explanation for our findings is based on the observation that cerebral blood flow is kept constant over a wide blood pressure range by compensatory constriction or dilatation of cerebral arteries and arterioles.[20] Because this compensatory mechanism is balanced to keep total flow constant and, as described above, its effect on total flow is much larger than on flow velocity, the cerebral vasomotor response to increasing blood pressure leads to increasing flow velocity. As such, increased flow velocity may be a marker of increased blood pressure. However, adjusting for systolic blood pressure did not attenuate the observed associations between flow velocity and stroke, which is not in line with the latter hypothesis but could be attributable to residual confounding.

Left and right flow velocity were very similar and the side of highest flow velocity was not associated with the side of stroke. These observations support the hypothesis that moderately increased flow velocity reflects a generalized process rather than a local atheroma causing stenosis and embolus or poor distal flow.

Whereas we found no association between VMR and stroke risk, previous studies reported that patients with impaired vasomotor reactivity were at increased stroke risk.[6-8] In one of these studies[8] arterial CO_2 was raised with a method similar to the one we used, whereas in others[6, 7] arterial CO_2 was raised with breath holding. However, all these studies were conducted in patients with severe steno-occlusive carotid disease, and VMR is strongly influenced by steno-occlusive carotid disease: the mean VMR was 16%/kPa in a previous study in 117 patients with >70% carotid stenosis or occlusion,[8] whereas in the general elderly population we found a mean VMR of 44%/kPa (median VMR 39%/kPa). Therefore, the contrast between our findings and previous findings might be explained by the much more severe exhaustion of VMR in previous studies among patients with carotid stenosis compared to that in our study population.

In conclusion, middle cerebral artery flow velocity is strongly and independently associated with the risk of stroke in the general population. The pathophysiological mechanisms that underlie this association are most likely mild diffuse middle cerebral artery atherosclerosis or middle cerebral artery vasoconstriction in response to systemic hypertension. Whereas exhausted VMR is a risk factor for stroke in patients with carotid artery disease, it is not in our study in the general population.

References

1. Tang SC, Jeng JS, et al. Transcranial color-coded sonography for the detection of middle cerebral artery stenosis. J Ultrasound Med. 2005;24:451-457; quiz 459-460

2. Sloan MA, Alexandrov AV, et al. Assessment: Transcranial Doppler ultrasonography: Report of the therapeutics and technology assessment subcommittee of the American Academy of Neurology. Neurology. 2004;62:1468-1481
3. Sacco RL, Kargman DE, et al. Race-ethnicity and determinants of intracranial atherosclerotic cerebral infarction. The Northern Manhattan Stroke Study. Stroke. 1995;26:14-20
4. Adams RJ, McKie VC, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998;339:5-11
5. Arenillas JF, Molina CA, et al. Progression and clinical recurrence of symptomatic middle cerebral artery stenosis: A long-term follow-up transcranial Doppler ultrasound study. Stroke. 2001;32:2898-2904
6. Silvestrini M, Vernieri F, et al. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. JAMA. 2000;283:2122-2127
7. Vernieri F, Pasqualetti P, et al. Outcome of carotid artery occlusion is predicted by cerebrovascular reactivity. Stroke. 1999;30:593-598
8. Markus H, Cullinane M. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. Brain. 2001;124:457-467
9. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
10. Barendregt JJ, Ott A. Consistency of epidemiologic estimates. Eur J Epidemiol. 2005;20:827-832
11. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;359:1309-1310
12. Diehl RR, Berlit P. Dopplerfunktionstests. Funktionelle Dopplersonographie in der Neurologie. Berlin: Springer; 1996:41-78.
13. Bots ML, Hoes AW, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. Circulation. 1997;96:1432-1437
14. Mattace-Raso FU, van der Cammen TJ, et al. Arterial stiffness and risk of coronary heart disease and stroke: The Rotterdam Study. Circulation. 2006;113:657-663
15. Stroke outcome and neuroimaging of intracranial atherosclerosis (SONIA): Design of a prospective, multicenter trial of diagnostic tests. Neuroepidemiology. 2004;23:23-32
16. Heun R, Knappertz V, Kraemer G. Transcranial Doppler sonography in dementia of Alzheimer type. Dementia. 1994;5:327-333
17. Sugimori H, Ibayashi S, et al. Can transcranial Doppler really detect reduced cerebral perfusion states? Stroke. 1995;26:2053-2060
18. Rorick MB, Nichols FT, Adams RJ. Transcranial Doppler correlation with angiography in detection of intracranial stenosis. Stroke. 1994;25:1931-1934
19. Babikian VL, Feldmann E, et al. Transcranial Doppler ultrasonography: Year 2000 update. J Neuroim aging. 2000;10:101-115
20. Paulson OB, Waldemar G, et al. Cerebral circulation under normal and pathologic conditions. Am J Cardiol. 1989;63:2C-5C

2.4.
COX-selectivity of non-steroidal anti-inflammatory drugs

Background and purpose: *In clinical trials, cyclo-oxygenase (COX)-2 selective non-steroidal anti-inflammatory drugs (NSAIDs) were associated with an increased risk of thromboembolic events. We studied the association between use of NSAIDs and risk of stroke in the prospective, population-based Rotterdam Study.* ***Methods:*** *We followed 7,636 persons without a history of stroke for incident stroke until September 2004. Data on all filled prescriptions came from pharmacy records. Using Cox-regression models, we calculated crude and adjusted hazard ratios (HR) of stroke with time-dependent current use compared to never use of NSAIDs grouped according to COX-selectivity (COX1-selective, non-selective and COX2-selective) and individual NSAIDs.* ***Results:*** *During 70,063 person-years of follow-up, 807 persons developed a stroke. Current users of non-selective (HR 1.66 [95%CI 1.19-2.31]) and COX2-selective NSAIDs (HR 2.58 [1.20-5.56]) had an increased risk of stroke compared to non-users, but not those who used COX1-selective NSAIDs (HR 1.17 [0.48-2.85]). HRs for ischemic stroke were 1.70 [1.08-2.69] for non-selective NSAIDs and 4.40 [2.01-9.66] for COX2-selective NSAIDs. For individual NSAIDs, current use of the non-selective naproxen (HR 2.32 [1.27-4.26]) and the COX2-selective rofecoxib (HR 3.22 [1.41-7.35]) were associated with an increased risk of stroke. Diclofenac (HR 1.49 [0.93-2.39]), ibuprofen (HR1.56 [0.83-2.95]), and celecoxib (HR 3.40 [0.47-24.78]) users had an increased risk of stroke, but not statistically significantly.* ***Conclusions****: In the general population, we found an increased risk of stroke with current use of non-selective and COX2-selective NSAIDs. The increased risk of stroke is not limited to the use of COX2-selective NSAIDs.*

Introduction

Clinical trials have shown that the use of cyclo-oxygenase-(COX)-2 selective non-steroidal anti-inflammatory drugs (NSAIDs) is associated with an increased risk of cardiovascular events and death.[1-5] It is generally assumed that selective inhibition of the COX2-enzyme by the COX2-selective NSAIDs induces a prothrombotic state, whereas NSAIDs that inhibit COX2 to a lesser degree (and additionally inhibit COX1) do not have this effect.[11-13]

However, considerable uncertainty on the relation between NSAID use and the risk of cardiovascular diseases remains: the cardiovascular risk observed for the various COX2-selective NSAIDs has not been consistent in all clinical trials.[14] In addition, although several observational studies have confirmed that NSAIDs differ in their potential to cause cardiovascular events,[15-17] in a recent placebo-controlled trial the non-COX2-selective NSAID naproxen was also associated with an increased cardiovascular risk.[4] In all, it remains unclear whether the increased risk of cardiovascular events is specific for COX2-selective NSAIDs or whether other (class) pharmacological properties of NSAIDs could cause these detrimental effects. Lastly, not only trials but also observational studies reported on combined cardiovascular endpoints and not on stroke specifically.[6-10]

We investigated the association between use of NSAIDs and the risk of incident stroke in a large prospective, population-based cohort study, comparing NSAIDs based on their COX-inhibiting properties.

Methods

Study population: The Rotterdam Study is a prospective, population-based cohort study of age related disorders.[18] The medical ethics committee of the Erasmus Medical Center approved the study. Between 1990 and 1993 all persons aged 55 years or older living in Ommoord, a district of Rotterdam, were invited to participate. Of the 10,275 eligible persons, 7,983 (78%) signed informed consent. Of these, 7,722 were free of stroke at baseline. The cohort was continuously monitored for major disease outcomes and death through linkage with records from general practitioners and bimonthly updates from the municipality records. This resulted in a virtually complete follow-up for stroke. Nearly all persons (99.7%) were registered at one or more of seven automated pharmacies serving the Ommoord area. Of these pharmacies, records of all filled prescriptions were available as of January 1st 1991. To ensure at least six months of medication history, we excluded 86 persons for whom follow-up ended before July 1st 1991. Consequently, the study population included 7,636 persons. The end of the study period for this analysis was September 30th 2004, the date on which rofecoxib was withdrawn from the market after clinical trial data had shown an increased risk of thromboembolic events in the rofecoxib treatment arm.

Drug Exposure: Complete information on all filled prescriptions for all persons was obtained in automated format from the pharmacies. This included the product name; international non-proprietary name; Anatomical Therapeutic Chemical code; total number of delivered units (e.g. tablets/capsules); prescribed daily number of units; date of delivery and drug dosage. The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. Drug dosage was defined by the defined daily dose (DDD), the recommended daily dosage of a drug taken by adults for the main indication of the drug.

NSAIDs were classified as COX1-selective, non-selective and COX2-selective according to their relative selectivity for the COX1 and COX2 enzymes at therapeutic dosages, based on data from in vitro and clinical studies (Table 1).[19-24] The classification of COX-selectivity used in this study complies with the generally accepted labelling of NSAID selectivity. Nevertheless, some debate exists regarding the COX-selective properties of diclofenac and naproxen. Some have argued that diclofenac is COX2-selective since diclofenac would not differ much from celecoxib in terms of its ability to inhibit COX2.[25] However, therapeutically relevant COX2-selectivity will be difficult to attain as the concentration of diclofenac necessary to achieve 80% inhibition of COX2 is expected to cause similar inhibition of COX1.[20] For naproxen, relative selectivity for COX1 has been suggested since naproxen causes near-maximal inhibition of platelet aggregation similar to aspirin.[24, 26] However, clinical evidence does not suggest that relative COX1-selectivity is achieved.[26] Hence, both diclofenac and naproxen were classified as non-selective. For some NSAIDs, COX-selectivity is unknown or equivocal (benzydamine, tiaprofenic acid, tolfenamic acid, phenylbutazone, tenoxicam, aceclofenac).

Table 1. Classification of NSAIDs according to COX-selective properties

COX1-selective NSAIDs	Non-selective NSAIDs	COX2-selective NSAIDs
Indometacine	Diclofenac*	Rofecoxib
Piroxicam	Naproxen	Celecoxib
Ketoprofen	Ibuprofen	Meloxicam
Dexketoprofen	Nabumeton	Etoricoxib
Flurbiprofen	Sulindac	Valdecoxib
Azapropazon		

*Includes combination products of diclofenac

Salicylates (i.e. acetylsalicylic acid and carbasalate calcium) are pharmacologically related to NSAIDs and inhibit platelet aggregation via COX1, though irreversible contrary to NSAIDs.27 On these grounds salicylates could be regarded as COX1-selective NSAIDs. However, they are mostly prescribed as platelet inhibitors for prevention of cardiovascu-

lar disease and stroke in a low dose. We did not include salicylates in the COX1-selective NSAID group because they are indicated for the prevention of stroke. NSAID use is cautioned in persons already using salicylates due to the increased risk of gastrointestinal bleeding, and some NSAIDs possibly antagonize the platelet inhibition induced by salicylates,[28] which might obscure the protective effect of salicylates. Because of these potential sources of confounding by salicylates, all analyses were adjusted for the current use of salicylates and the effect of salicylates on the association between NSAID use and stroke was studied through stratification.

Diagnosis of stroke: A history of stroke at the time of enrolment into the Rotterdam Study was assessed by asking 'did you ever suffer from a stroke, diagnosed by a physician?' Positive answers to this question were verified by the review of medical records. A history of transient ischemic attack (TIA) was also assessed during the baseline interview. After enrolment into the Rotterdam Study, participants were continuously monitored for strokes and TIA through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes and TIAs with an experienced neurologist to verify all diagnoses while blinded to drug exposure. Subarachnoid hemorrhages were excluded. A stroke was subclassified as ischemic when a CT or MRI scan, that was made within 4 weeks after the stroke occurred, ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) indicated the ischemic nature of the stroke. Hemorrhagic stroke was diagnosed when a relevant hemorrhage was shown on CT or MRI scan or when the patient permanently lost consciousness or died within hours after onset of focal signs. If a stroke could not be subclassified as ischemic or hemorrhagic as a consequence of a lack of the data mentioned above, it was classified as 'unspecified'.[29]

Other covariates: Potential confounders were chosen a priori. Baseline covariates included age, sex, and cardiovascular disease (myocardial infarction, silent cardiac infarcts, coronary artery bypass graft, PTCA, previous TIA and atrial fibrillation; data unavailable for 3.5% of the participants) and systolic blood pressure. Time-dependent covariates included diabetes mellitus, heart failure and current use of antihypertensives, salicylates and anti-thrombotics. Diabetes mellitus was defined as non-fasting serum glucose level exceeding 11.1 mmol/l or the use of oral blood glucose lowering drugs or insulin.

Statistical analysis: For all subjects, we calculated the duration of follow-up between start of the study and date of death, diagnosis of stroke (the index date), or end of the study period,

whichever came first. We calculated the hazard ratios (HRs) and 95% confidence intervals (CIs) of stroke with a Cox proportional hazards model (SPSS 11.01 software) in which calendar-time was used as the time-axis. Separate analyses were performed for all stroke, ischemic stroke and hemorrhagic stroke. All analyses were complete-case analyses, adjusted for age and sex (model 1). In a second model, we adjusted for the potential confounders described above (model 2).

Use of NSAIDs was represented by time-dependent covariates. We defined NSAID use of cases and controls on the index date as either never, current or past use of an NSAID, categorized into NSAID-groups according to COX-selectivity and as individual NSAIDs. Current use was defined as use of an NSAID on the index date as determined by the date of filling of the prescription and duration of use. Simultaneous current use of 2 or more NSAIDs was rare (0.1%) and excluded from the analyses. Past use was defined as any use prior to the index-date if the person was not a current user of an NSAID. Never use was defined as non-use during the whole study period prior to the index-date. Never use was the reference for all analyses to allow comparability of the results to the findings of placebo-controlled trials.
For the individual NSAIDs we investigated a dose-effect relationship by dichotomizing the average dose of current use as ≤1DDD and >1 DDD.

We performed several subanalyses. First, we included a 14-day 'past'-period in the current-use exposure category to take into account the possibility that, as a consequence of non-compliance, the duration of a prescription could in reality have been longer. Second, the analysis was performed in a subcohort with a history of at least 1 NSAID prescription during follow-up to study the role of potential confounding by indication or contraindication. Third, previous studies have shown that COX2-selective NSAIDs are preferentially prescribed to persons with substantial comorbidity.[30] To investigate whether this played a role in our study population, we determined for all COX-selective NSAID groups whether a history of use was related to the risk of stroke. Finally, the effect of concomitant use of salicylates could affect our results for reasons described previously. We thus performed an analysis stratified by current use of salicylates.

Results

Baseline characteristics of the cohort at risk are shown in Table 2. During 70,063 person years of follow-up, 807 persons developed a stroke. Of these individuals, 460 were diagnosed with an ischemic stroke, 74 with a hemorrhagic stroke while for 273 individuals the type of stroke could not be specified. The mean follow-up was 9.2 years. In our study population, 61 persons who experienced a stroke were current user of any NSAID at the time of the event whereas 290 persons with a stroke had never used an NSAID during the study period.

Current use of any NSAID was associated with an increased risk of stroke compared

Table 2. Demographic and Clinical Characteristics of the Study Cohort

Characteristic	Mean (SD) or percentage
Age, y	70.2 (9.6)
Female sex	61.3 %
Ever smoking	63.5 %
Osteoarthritis in hand, knee or hip	7.8 %
Diabetes mellitus	10.4 %
Cardiovascular disease	26.3 %
Heart failure	3.0 %
Body Mass Index, kg/m2	26.3 (3.7)
Systolic blood pressure, mm Hg	139 (22)

to never use (sex and age adjusted HR 1.58 [95% CI 1.19-2.08]). Adjustment for confounders resulted in higher estimates (adjusted HR 1.70 [95% CI 1.26-2.29]) (Table 3). Associations were stronger if only ischemic strokes were considered (Table 3). Use of any NSAID was related to the risk of hemorrhagic stroke (HR 1.82 [95% CI 0.73-4.54]), albeit non-significant.

Current users of non-selective NSAIDs and of COX2-selective NSAIDs had a higher risk of stroke than never users (adjusted HR for non-selective NSAIDs 1.66 (95% CI 1.19-2.31); for COX2-selective NSAIDs 2.58 [95% CI 1.20-5.56]) (Table 3). We found no association for COX1-selective NSAID use with the risk of stroke (adjusted HR 1.17 [95% CI 0.48-2.85]). Current use of NSAIDs with unknown COX-selectivity on the index date was infrequent (0.02%) and thus not studied further. All associations were stronger if only ischemic strokes were considered (Table 3). There were no exposed cases in the class of COX2-selective NSAIDs for hemorrhagic stroke precluding comparison of the effect of different NSAIDs on risk of hemorrhagic stroke.

For individual NSAIDs, current use of the non-selective NSAIDs naproxen (adjusted HR 2.32 [95% CI 1.27-4.26]) and the COX2-selective rofecoxib (adjusted HR 3.22 [95% CI 1.41-7.35]) were associated with a statistically significant increased risk of stroke (Table 4). All but one person in the COX2-selective group were users of rofecoxib. Although HRs for current use of diclofenac (HR 1.49 [95% CI 0.93-2.39]), ibuprofen (adjusted HR 1.56 [95% CI 0.83-2.95]) and celecoxib (adjusted HR 3.24 [95% CI 0.45-23.58]) were above one, neither of them reached the level of conventional statistical significance. The low number of events for celecoxib (n=1), as well as for all other individual NSAIDs, prohibited further investigation of these exposure categories. There was no clear dose-response effect as doses ≤1DDD and >1DDD were both associated with an increased risk of stroke. However, analyses were compromised by low case numbers.

As for our subanalyses, extension of the risk window with a 14-day period after cessation of drug use attenuated the risk estimates (adjusted HRs of stroke for current use of any

Table 3. Hazard ratios for all and ischemic stroke with current use of NSAIDs

Drug Exposure	All stroke				Ischemic stroke			
	Model 1*		Model 2*		Model 1*		Model 2*	
	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)
Never use	290	1 (ref)	235	1 (ref)	156	1 (ref)	129	1 (ref)
Any NSAID	61	1.58 (1.19-2.08)	55	1.70 (1.26-2.29)	34	1.74 (1.20-2.53)	32	1.92 (1.29-2.84)
Non-selective NSAID	48	1.58 (1.16-2.15)	42	1.66 (1.19-2.31)	24	1.58 (1.02-2.44)	22	1.70 (1.08-2.69)
COX1-selective NSAID	5	0.95 (0.39-2.31)	5	1.17 (0.48-2.85)	2	0.78 (0.19-3.16)	2	0.94 (0.28-3.81)
COX2-selective NSAID	7	2.40 (1.12-5.14)	7	2.58 (1.20-5.56)	7	4.20 (1.93-9.13)	7	4.40 (2.01-9.66)

* Model 1: Adjusted for age and sex. Model 2: additionally adjusted for prevalent cardiovascular disease, systolic blood pressure, and as time dependent variables diabetes, heart failure and current use of antihypertensives, salicylates and anti-thrombotics.

Table 4. Hazard ratios of all stroke and ischemic stroke with current use of individual NSAIDs

Drug Exposure*	All stroke				Ischemic stroke			
	Model 1†		Model 2†		Model 1†		Model 2†	
	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)
Never use	290	1 (ref)	235	1 (ref)	156	1 (ref)	129	1 (ref)
Use of diclofenac	20	1.35 (0.86-2.13)	19	1.49 (0.93-2.39)	12	1.61 (0.89-2.90)	10	1.70 (0.91-3.15)
Use of ibuprofen	11	1.32 (0.72-2.42)	10	1.56 (0.83-2.95)	3	0.76 (0.24-2.40)	3	0.96 (0.30-3.03)
Use of naproxen	15	2.67 (1.58-4.48)	11	2.32 (1.27-4.26)	7	2.37 (1.11-5.06)	6	2.37 (1.04-5.39)
Use of rofecoxib	6	3.24 (1.42-7.37)	6	3.22 (1.41-7.35)	6	5.73 (2.48-13.2)	6	5.55 (2.39-12.9)

* Diclofenac, ibuprofen, and naproxen are non-selective, rofecoxib is COX2-selective.

† Model 1: Adjusted for age and sex. Model 2: additionally adjusted for prevalent cardiovascular disease, systolic blood pressure, and as time dependent variables diabetes, heart failure and current use of antihypertensives, salicylates and anti-thrombotics.

current NSAID 1.55 [95% CI 1.17-2.05], COX1-selective NSAIDs 1.08 [95% CI 0.44-2.61], non-selective NSAIDs 1.49 [95% CI 1.08-2.04] and COX2-selective NSAIDs 2.54 [95% CI 1.18-5.48]). If we performed the analysis in a subcohort with at least 1 NSAID prescription during follow-up (517 events) we observed little change in HRs compared to the analyses in which never use was defined as reference (data not shown). Past use of COX2-selective NSAIDs compared to never use was associated with an increased risk of stroke (adjusted HR 1.93 [95% CI 1.27-2.92]); no such association was observed for a history of any NSAID-use (HR 1.07 [95% CI 0.91-1.27]) or for the other COX-selective groups (HRs for COX1 selective NSAIDs 1.15 [95% CI 0.91-1.45] and non-selective NSAIDs 1.07 [95% CI 0.90-1.28]). Notably, almost all users of COX2 had used other types of NSAIDs earlier during follow-up and were, in general, chronic users of NSAIDs.

Only 2 cases were concurrent current users of salicylates and NSAIDs, hence stratification on concomitant use of salicylates could not be performed.

Discussion

In the general population, we found an overall increased risk of stroke with use of NSAIDs, especially in the categories of non-selective NSAIDs and COX2-selective NSAIDs.

Strengths of our study design are its prospective design, large number of participants, long follow-up and a general population-based setting which makes selection bias unlikely. Information bias was prevented by prospectively collected and complete automated pharmacy records of all filled prescriptions and blinded adjudication of cerebrovascular events. Certain limitations of our study, however, deserve comment. First, as with most of the clinical trials and observational studies performed to date, inferences must be interpreted in the context of small numbers despite an average follow-up of more than 9 years. Second, although in the Netherlands chronic use of NSAIDs was fully reimbursed until the beginning of 2004, some misclassification might have occurred due to intermittent use of 'over the counter' (OTC) NSAIDs. If this biased our results, however, it will have led us to underestimate an effect rather than overestimate the risk of stroke. Finally, 'preferential prescribing' might have played a role in the COX2-selective NSAIDs group since we observed a higher risk of stroke for past-users of COX2-selective NSAIDs. This channelling bias with COX2-selective NSAIDs has been described previously, also in the Dutch patient population setting.[30] However, since the risk estimates were higher for current use of COX2-selective NSAIDs than for past use, this type of confounding cannot fully explain the increased risk of stroke.

Our results are largely in agreement with the currently available data from randomized clinical trials. In the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT) use of naproxen was associated with a similar 2-fold increased risk of stroke compared to placebo which is in striking accordance with the results of the present study.[4] The

same study did not report an effect of celecoxib on the risk of stroke. In our study, no statistically significant effect was found for celecoxib either although the effect size was similar to that of rofecoxib. Because of low numbers, however, an effect of celecoxib on the risk of stroke cannot be excluded. The similar occurrence of ischemic cerebrovascular events for the rofecoxib and the naproxen treatment arm of the VIOXX GI Outcomes Research (VIGOR) study complies with our finding of an increased risk of stroke for both these NSAIDs. However, since VIGOR did not include a placebo treatment it does not provide evidence for the direction of the association.[1] More compelling data consistent with a true increase in the risk of stroke with rofecoxib use is provided by the results of the 'Adenomatous Polyp Prevention on Vioxx trial' in which a 2-fold increased risk for cerebrovascular events was observed compared to placebo after 36 months of follow-up.[2]

The results of our study suggest that an effect of NSAIDs on the risk of stroke is not restricted to the COX2-selective compounds. Primarily this can be inferred from the observation that the risk of stroke was increased for both the use of COX2-selective NSAIDs and non-selective NSAIDs. Our results do not necessarily exclude the possibility of an effect through a COX-related mechanism. Because selective inhibition of COX2 causes platelet aggregation, use of COX2-selective NSAIDs could, as suggested previously, cause a prothrombotic state.[11-13] But since both COX1 and COX2 are involved in vascular homeostasis, any pharmacological inhibition of the COX-enzymes could be expected to disturb the thrombotic equilibrium, which would explain our observations. Alternatively, other COX-mediated processes relevant to the pathophysiology of cerebrovascular events might be involved, such as inflammatory response and renovascular physiology.[31, 32] This is also supported by our observation that NSAID use was associated with an increased risk of both ischemic and hemorrhagic stroke.

In conclusion, our study confirms that not only COX2-selective but also COX-non-selective NSAIDs are associated with an increased risk of stroke. Threrefore, these drugs should be used reluctantly.

References

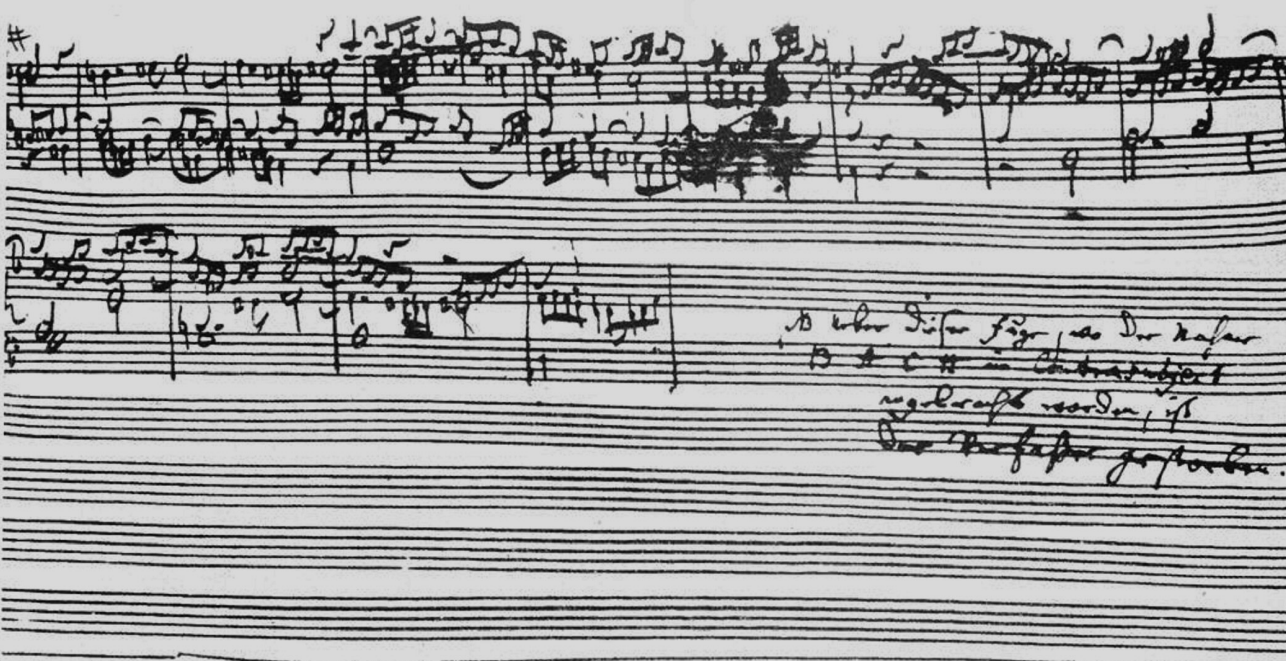
1. Bombardier C, Laine L, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med. 2000;343(21):1520-1528.
2. Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352(11):1092-1102.
3. Solomon SD, McMurray JJ, et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med. 2005;352(11):1071-1080.
4. ADAPT Research Group. Cardiovascular and Cerebrovascular Events in the Randomized, Controlled Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT). PLoS Clin Trials. 2006;1(7):e33.
5. Lenzer J. FDA advisers warn: COX 2 inhibitors increase risk of heart attack and stroke. Bmj. 2005;330(7489):440.
6. Thrift AG, McNeil JJ, et al. Risk of primary intracerebral haemorrhage associated with aspirin and non-steroidal anti-inflammatory drugs: case-control study. Bmj. 1999;318(7186):759-764.

7. Bak S, Andersen M, Tsiropoulos I et al. Risk of stroke associated with nonsteroidal anti-inflammatory drugs: a nested case-control study. Stroke. 2003;34(2):379-386.
8. Johnsen SP, Pedersen L, Friis S et al. Nonaspirin nonsteroidal anti-inflammatory drugs and risk of hospitalization for intracerebral hemorrhage: a population-based case-control study. Stroke. 2003;34(2):387-391.
9. Juvela S. Nonsteroidal anti-inflammatory drugs as risk factors for spontaneous intracerebral hemorrhage and aneurysmal subarachnoid hemorrhage. Stroke. 2003;34(6):e34-36.
10. Andersohn F, Schade R, et al. Cyclooxygenase-2 selective nonsteroidal anti-inflammatory drugs and the risk of ischemic stroke: a nested case-control study. Stroke. 2006;37(7):1725-1730.
11. Catella-Lawson F, Crofford LJ. Cyclooxygenase inhibition and thrombogenicity. Am J Med. 2001;110 Suppl 3A28S-32S.
12. Antman EM, DeMets D, Loscalzo J. Cyclooxygenase inhibition and cardiovascular risk. Circulation. 2005;112(5):759-770.
13. Crofford LJ, Breyer MD, et al. Cardiovascular effects of selective COX-2 inhibition: is there a class effect? The International COX-2 Study Group. J Rheumatol. 2006;33(7):1403-1408.
14. Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA. 2001;286(8):954-959.
15. Mamdani M, Rochon P, et al. Effect of selective cyclooxygenase 2 inhibitors and naproxen on short-term risk of acute myocardial infarction in the elderly. Arch Intern Med. 2003;163(4):481-486.
16. Graham DJ, Campen D, et al. Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: nested case-control study. Lancet. 2005;365(9458):475-481.
17. Hippisley-Cox J, Coupland C. Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. Bmj. 2005;330(7504):1366.
18. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7(4):403-422.
19. Cryer B, Feldman M. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. Am J Med. 1998;104(5):413-421.
20. Warner TD, Giuliano F, et al. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci U S A. 1999;96(13):7563-7568.
21. Giuliano F, Ferraz JG, et al. Cyclooxygenase selectivity of non-steroid anti-inflammatory drugs in humans: ex vivo evaluation. Eur J Pharmacol. 2001;426(1-2):95-103.
22. de Leval X, Delarge J, et al. Evaluation of classical NSAIDs and COX-2 selective inhibitors on purified ovine enzymes and human whole blood. Prostaglandins Leukot Essent Fatty Acids. 2001;64(4-5):211-216.
23. Stichtenoth DO, Wagner B, Frolich JC. Effects of meloxicam and indomethacin on cyclooxygenase pathways in healthy volunteers. J Investig Med. 1997;45(2):44-49.
24. Van Hecken A, Schwartz JI, et al. Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. J Clin Pharmacol. 2000;40(10):1109-1120.
25. Hinz B, Dormann H, Brune K. More pronounced inhibition of cyclooxygenase 2, increase in blood pressure, and reduction of heart rate by treatment with diclofenac compared with celecoxib and rofecoxib. Arthritis Rheum. 2006;54(1):282-291.
26. Shah AA, Thjodleifsson B, et al. Selective inhibition of COX-2 in humans is associated with less gastrointestinal injury: a comparison of nimesulide and naproxen. Gut. 2001;48(3):339-346.
27. Catella-Lawson F, Reilly MP, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med. 2001;345(25):1809-1817.
28. Konstantinopoulos PA, Lehmann DF. The cardiovascular toxicity of selective and nonselective cy clooxygenase inhibitors: comparisons, contrasts, and aspirin confounding. J Clin Pharmacol. 2005;45(7):742-750.
29. Bos MJ, Schipper CM, et al. High serum C-reactive protein level is not an independent predictor for stroke: the Rotterdam Study. Circulation. 2006;114(15):1591-1598.
30. Mosis G, Stijnen T, et al. Channeling and prevalence of cardiovascular contraindications in users of cyclooxygenase 2 selective nonsteroidal antiinflammatory drugs. Arthritis Rheum. 2006;55(4):537-542.
31. Krum H, Aw TJ, et al. Blood pressure effects of COX-2 inhibitors. J Cardiovasc Pharmacol. 2006;47 Suppl 1S43-48.

32. Catella-Lawson F, McAdam B, et al. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J Pharmacol Exp Ther. 1999;289(2):735-741.

Chapter 3.

Genetic risk factors for stroke



3.1
Polymorphisms of the alpha-adducin gene

Background and purpose: *Carriers of the 460Trp allele of the α-adducin gene (ADD1) show higher rates of sodium reabsorption compared with homozygous carriers of the Gly460 allele and were found to have an increased risk of hypertension and cardiovascular disease. We studied the association between the Gly460Trp polymorphism and atherosclerosis, cardiovascular and cerebrovascular disease.* ***Methods:*** *Intima-media thickness of the carotid artery (CCA IMT), as well as incident stroke and myocardial infarction (MI) were studied within 6471 subjects of the Rotterdam Study. Within 1018 subjects of the Rotterdam Scan Study, prevalent silent brain infarcts (SBI) and cerebral white matter lesions (WML) were studied. Subjects were grouped into 460Trp carriers (variant carriers) and homozygous carriers of the Gly460 allele (reference).* ***Results:*** *CCA IMT was 0.80 mm in variant carriers compared with 0.79 mm in the reference group (p=0.04). Variant carriers had an increased risk of any stroke (HR 1.22, 95% CI: 1.02-1.45), of ischemic stroke (HR 1.29, 95% CI: 1.02-1.63), and of MI (HR 1.33, 95% CI: 1.05-1.69). For any and ischemic stroke, there was a significant interaction between the Gly460Trp polymorphism and hypertension. Variant carriers more often had a SBI (OR 1.36, 95% CI: 0.98-1.88) and had more subcortical WML than the reference group (1.45 ml vs 1.24 ml, p=0.22).* ***Conclusions:*** *The Gly460Trp polymorphism is associated with atherosclerosis, cardiovascular and cerebrovascular disease, especially in hypertensive subjects.*

Introduction

Hypertension is a major risk factor for atherosclerosis, cardiovascular and cerebrovascular disease.[1-4] Recently it was found that hypertension and stroke, but also hypertension and myocardial infarction (MI), cluster within families.[5,6] This suggests that overlapping genetic factors, alone or in conjunction with environmental factors, influence susceptibility to hypertension, stroke and MI.

Adducin is a cytoskeleton protein consisting of a α- and β- subunit. It favors the binding of actin to spectrin and may affect ion transport through the actin cytoskeleton and modulation of the NA+-K+ pump activity.[7,8] Carriers of the 460Trp allele (variant allele) of the α-adducin gene (ADD1), located on chromosome 4p16.3, show a higher NA+-K+ pump activity and therefore higher rates of renal tubular sodium reabsorption compared with homozygous carriers of the Gly460 allele (wild type allele).[9,10] The Gly460Trp polymorphism has been associated with blood pressure levels and the risk of hypertension in many, but not all populations.[11]

Furthermore, this polymorphism has been associated with salt-sensitivity,[12] which reportedly is a risk factor for cardiovascular events.[13] Indeed, carriers of the 460Trp allele were found to have an increased risk of cardiovascular disease, although findings have not been consistent.[11,14] So far, no association was found between ADD1 and ischemic stroke.[15,16]

We studied the Gly460Trp polymorphism in relation to atherosclerosis, myocardial infarction and cerebrovascular disease. Also, we studied the interaction between hypertension and the Gly460Trp polymorphism with respect to all above-mentioned outcomes.

Methods

Study populations: The Rotterdam Study is an ongoing prospective population-based cohort study on chronic and disabling diseases in the elderly. Baseline examinations were done between 1990 and 1993. A total of 7983 subjects (age 55 years and over) participated in this study. In 6471 (81.1%) participants, the Gly460Trp polymorphism was successfully genotyped.

The Rotterdam Scan Study was designed to study the etiology and natural history of age-related brain changes in the elderly. Baseline examinations, which included brain MRI scanning, were performed in 1995 and 1996 in 1077 participants (aged 60 to 90 years).[17] In 1018 (94.5%) participants, the Gly460Trp polymorphism was successfully genotyped. The medical ethics committee of Erasmus Medical Center, Rotterdam, approved both studies and all participants gave written informed consent and permission to retrieve information from treating physicians.

Measurements: Body mass index (BMI) was calculated from height and weight. Blood pressure was measured twice using a random-zero sphygmomanometer. The average of two measurements was used for analyses. Hypertension was defined as a systolic blood pressure of 160 mmHg or higher or a diastolic blood pressure of 100mmHg or higher (grade 2 and 3 of the 1999 WHO criteria)[18] or use of blood pressure lowering medication. Information on smoking habits was obtained during a home interview.

We collected non-fasting blood samples from all participants. We defined diabetes mellitus as a random glucose level of 11.1 mmol/l or higher or use of oral anti-diabetics or insulin. Total serum cholesterol and HDL-cholesterol were determined by means of an automated enzymatic method.

Measurements of atherosclerosis: Intima-media thickness of the common arotid artery (CCA IMT) was assessed by duplex scan ultrasonography over an average distance of 10 mm.[19] We used the average of the measurements of three still images of both the left and right arteries. CCA IMT was determined as the mean of the mean IMT of near and far wall measurements of both the left and right arteries. These measurements were done in 5643 participants. For 5083 participants, genotypic information was available.

Stroke and myocardial infarction: Stroke and MI were assessed as part of the Rotterdam Study. A prevalent stroke or MI was determined during the baseline interview and verified by checking medical records. Incident stroke and MI were determined by continuously monitoring subjects for major events. Research physicians reviewed information on all possible strokes and transient ischemic attacks (TIAs) with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. Subarachnoid hemorrhages and retinal strokes were excluded. A stroke was classified ischemic when a patient had typical symptoms and a CT or MRI, that was made within 4 weeks after the stroke occurred, ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours or atrial fibrillation in absence of anticoagulants) pointed to an ischemic nature of the stroke. A stroke was classified hemorrhagic when a relevant hemorrhage was shown on CT or MRI scan, or the subject lost consciousness permanently or died within hours after the onset of focal signs. If a stroke did not match any of these criteria, it was classified unspecified. During follow-up, 637 first ever strokes occurred. Genotype data was available for 498 of these individuals.

Two research physicians independently coded all reported cardiovascular events according to the International Classification of Diseases, 10th Edition (ICD-10).[20] Incident MI was defined as the occurrence of a fatal or non-fatal MI (ICD-10 code I21) after the baseline examination. Follow-up started at baseline and lasted until January 1st 2002 for stroke and until January 1st 2003 for MI. Of all participants 2.6% were lost to follow-up. For these subjects, the follow-up time was computed until the last date of contact. We ascertained 371 incident MI cases. For 272 cases, genotypic data was available.

Silent brain infarcts and white matter lesions: Presence of silent brain infarcts (SBI) was assessed in the Rotterdam Scan Study. Infarcts were defined as focal hyperintensities on T2-weighted images, 3 to 20 mm in size. SBI were defined as evidence for one or more infarcts on MRI, without a history of a (corresponding) stroke or TIA.[21] We observed SBI in 217 participants. Genotypic data was available for 119 cases.

White matter lesions (WML) were scored present if visible as hyperintense on proton-density and T2-weighted images, without prominent hypointensity on T1-weighted scans and, according to their location, as periventricular or subcortical.[22] Periventricular WML were rated semi-quantitatively (range 0-9). A total volume of subcortical WML was approximated based on number and size of lesions (volume range 0-29.5 ml).

Genotyping: Genotyping was performed using TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA).[23] Forward primer sequence was 5'-GAGAAGACAAGATGGCTGAACTCT-3' and reverse primer sequence 5'-GTCTTCGACTTGGGACTGCTT-3'. The minor groove binding probes were 5'-VIC-CATTCTGCCCTTCCTC-NFQ-3' and 5'-FAM-ATTCTGCCATTCCTC-NFQ-3'. We used the reverse strand design. The assays utilized 5 nanograms of genomic DNA and 5 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 minutes at 95 °C preceded 40 cycles of denaturation at 95 °C for 15 seconds and annealing and extension at 50 °C for 60 seconds. Allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA). Based on the analysis of blind duplicates, there was a 98% concordance in genotyping.

Statistical analyses: Hardy-Weinberg equilibrium proportions were tested using GENEPOP-package.[24] Baseline characteristics were compared using univariate ANOVA or Chi^2 statistics. Univariate analyses of variance were used to assess the relation between the Gly460Trp polymorphism and CCA IMT and WML. Cox proportional hazards regression analysis was used to assess the association between the Gly460Trp polymorphism and stroke and MI. For the analyses on incident stroke, we excluded prevalent strokes, for the analyses on incident MI, we excluded prevalent MI at baseline from the analyses. We performed a binary logistic regression analysis to study the relation between the Gly460Trp polymorphism and SBI. All analyses were adjusted for age and sex and additionally for hypertension, BMI, total cholesterol, diabetes mellitus and smoking and performed in SPSS version 11.0.

Results

Genotype frequencies were in Hardy-Weinberg equilibrium in both study populations. Table

1 shows the baseline characteristics stratified by ADD1 genotype. No significant differences were observed between the genotype groups with the exception of smoking. Within the Rotterdam Study, there were significantly more smokers among the variant carriers, compared with wild type homozygotes. After adjusting for age and sex, no association between the Gly460Trp polymorphism and blood pressure or hypertension was found in either of the two study populations (not shown).

Figure 1 shows that overall, adjusted for age and sex, variant carriers had an increase in mean CCA IMT (0.80 mm) compared with wild type homozygotes (0.79 mm, p=0.04). This finding did not remain statistically significant after additional adjustment for smoking and other cardiovascular risk factors. Within hypertensive subjects, mean CCA IMT was 0.85 mm in variant carriers, compared with 0.83 mm in wild type homozygotes (p=0.03). After additional adjustment for smoking and other cardiovascular risk factors, this finding remained significant. No significant differences were observed within normotensive subjects. The interaction term hypertension*ADD1 was not a significant predictor of CCA IMT (p=0.07) in the model including age, sex, hypertension and ADD1.

We classified 291 stroke cases as ischemic stroke, 47 as hemorrhagic stroke and

Table 1. General characteristics stratified by ADD1 genotype

	ADD1 Gly460Trp	
	Wild type homozygotes (GG)	Variant carrier (GT/TT)
Rotterdam Study		
Number of subjects (%)	4018 (62.1)	2453 (37.9)
Age, y	69.6 ± 9.3	69.3 ± 8.9
Sex, % men	40.0	41.1
BMI (kg/m^2)	26.3 ± 3.7	26.3 ± 3.8
Current smoking, %	21.8	24.1*
Hypertension, %	34.3	32.8
Total Cholesterol (mmol/)	6.6 ± 1.2	6.6 ± 1.2
Diabetes Mellitus, %	10.3	9.6
Rotterdam Scan Study		
Number of subjects	649 (63.8)	369 (36.2)
Age, y	72.2 ± 7.5	72.1 ± 7.2
Sex, % men	50.7	45.3
BMI (kg/m^2)	26.7 ± 3.6	26.7 ± 3.7
Current smoking, %	18.3	15.0
Hypertension, %	51.2	53.1
Total Cholesterol (mmol/)	5.9 ± 1.0	5.9 ± 1.0
Diabetes Mellitus, %	7.4	5.7

All values are percentages or means ± SD, *p<0.05 compared with wild type homozygotes.

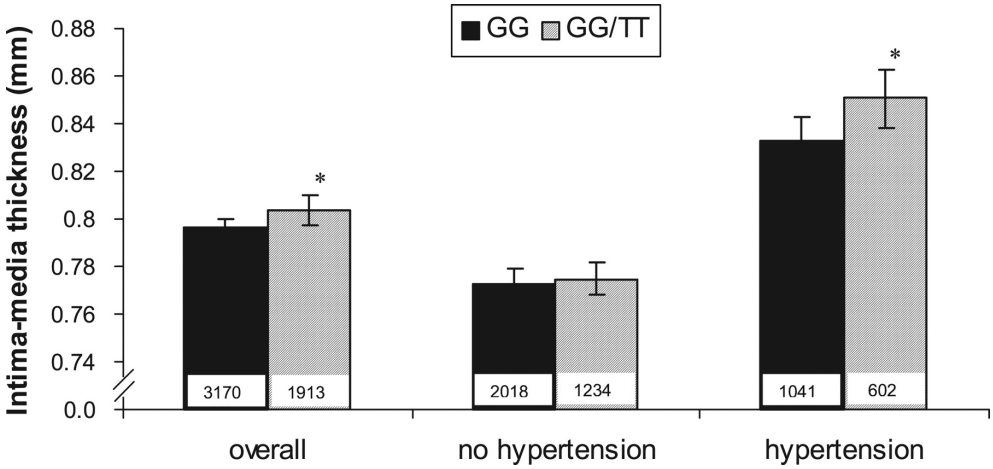


*Figure 1. Association between the ADD1 polymorphism and common carotid intima-media thickness in overall, non-hypertensive and hypertensive subjects. *p<0.05 compared with GG genotype.*

160 as unspecified stroke. Table 2 shows the hazard ratio (HR) for incident stroke and MI, by genotype adjusted for age and sex. Variant carriers were found to have a an increased risk of any stroke (HR 1.22, 95% CI: 1.02-1.45), ischemic stroke (HR 1.29, 95% CI: 1.02-1.63) and MI (HR 1.33, 95% CI: 1.05-1.69), compared with wild type homozygotes. Findings remained significant after additional adjustment for smoking and other cardiovascular risk factors. No association was found between the Gly460Trp polymorphism and hemorrhagic stroke.

Table 3 shows that for variant carriers, after adjusting for age and sex, we observed an increased revalence of SBI (OR 1.36, 95% CI: 0.98-1.88). There was no difference in mean periventricular WML grade between variant carriers and wild type homozygotes (Table 3). There was an increase in mean subcortical WML volume for variant carriers (1.45 ml +/- 0.14) compared with wild type homozygotes (1.24 ml +/- 0.10), but this difference was not significant (p=0.22).

Table 2. The Rotterdam Study: Risk of incident stroke and myocardial infarction in relation to the ADD1 polymorphism.

ADD1 Gly460Trp	n	HR (95% CI)	
		GG	GT/TT
Any incident stroke	498	1 (ref)	1.22 (1.02-1.45)*
Incident ischemic stroke	291	1 (ref)	1.29 (1.02-1.63)*
Incident hemorrhagic stroke	47	1 (ref)	1.07 (0.59-1.92)
Incident MI	272	1 (ref)	1.33 (1.05-1.69)*

All hazard ratios are adjusted for age and sex. For incident stroke, all prevalent strokes were excluded, for incident MI, all prevalent MI's were excluded, n=absolute number of cases, MI=myocardial infarction, HR=hazard ratio. *p<0.05 compared with the GG genotype.

Table 3. Rotterdam Scan Study: Association of the ADD1 polymorphism with SBI and white matter lesions.

ADD1 Gly460Trp	SBI		Periventricular WML		Deep subcortical WML	
	n	OR (95 % CI)	n	Mean ± SE	n	Mean ± SE
GG	119	1 (ref)	649	2.36 ± 0.08	647	1.24 ± 0.10
GT/TT	85	1.36 (0.98-1.88)	369	2.37 ± 0.10	367	1.45 ± 0.14

All odds ratios and means are adjusted for age and sex, n=absolute number of cases, SBI=silent brain infarction, OR=odds ratio, SE=standard error, WML=white matter lesions.

Within the Rotterdam study, we investigated the interaction between hypertension and the Gly460Trp polymorphism in relation to atherosclerosis (see figure 1), stroke and MI. Table 4 shows the interaction between hypertension and the Gly460Trp polymorphism in relation to stroke and MI. We found an increased risk for variant carriers with hypertension for any stroke (HR 2.18, 95% CI: 1.70-2.79), ischemic stroke (HR 2.32, 95% CI: 1.68-3.21), hemorrhagic stroke (HR 2.48, 95% CI: 1.13-5.42) and MI (HR 1.81, 95% CI: 1.26-2.62), compared with wild type homozygotes without hypertension, adjusted for age and sex. Adjusting for smoking and other cardiovascular risk factors did not alter these results. The interaction term hypertension*ADD1 was a significant predictor of any stroke (p=0.04) in the model including age, sex, hypertension and ADD1 and in the full model including age, sex, hypertension, ADD1, BMI, total cholesterol, diabetes mellitus and smoking (p=0.04). This interaction term was also significant for ischemic stroke in the model including age, sex, ADD1 and hypertension (p=0.05), but only borderline in the full model (p=0.06). No interaction was found between hypertension and SBI or WML.

Discussion

In the Rotterdam Study, we found an increased mean CCA IMT and a higher risk for any stroke, ischemic stroke and MI for carriers of the 460Trp allele. Consistent with these findings, we found in the Rotterdam Scan Study that variant carriers had an increased risk of SBI and an increase in mean subcortical WML volume, but these findings were not significant. We found a significant interaction between the Gly460Trp polymorphism and hypertension in relation to any and ischemic stroke. To our best knowledge, this is the first study to find an association between the Gly460Trp polymorphism and CCA IMT, ischemic stroke and MI.

The strengths of our study are the size of our two study populations and the fact that we were able to study the effect of the ADD1 in relation with both clinical stroke and SBI and white matter lesions. SBI and WML have previously been found to be risk factors for stroke.[21, 25]

An early marker of ischemic stroke, CCA IMT[26] was increased in variant carriers in

Table 4. The Rotterdam Study: Interaction between hypertension and the ADD1 polymorphism in relation to incident stroke and myocardial infarction.

Hypertension, ADD1	Any stroke		Ischemic stroke		Hemorrhagic stroke		Myocardial infarction	
	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)
HT-, GG	154	1 (ref)	91	1 (ref)	15	1 (ref)	90	1 (ref)
HT-, GT/TT	94	1.02 (0.79-1.31)	57	1.03 (0.74-1.43)	7	0.77 (0.31-1.88)	67	1.21 (0.88-1.66)
HT+, GG	130	1.46 (1.15-1.84)*	69	1.42 (1.03-1.94)*	13	1.59 (0.75-3.36)	56	1.33 (0.95-1.86)
HT+, GT/TT	104	2.18 (1.70-2.79)**	62	2.32 (1.68-3.21)**	11	2.48 (1.13-5.42)*	42	1.81 (1.26-2.62)*
p for interaction		0.04		0.05		0.3		0.6

All hazard ratios are adjusted for age and sex, HT- = hypertension absent, HT+ = hypertension present, HR=hazard ratio.
*p<0.05, **p<0.001 compared with the reference group HT-, GG.

this study, especially in hypertensive subjects. Also, we observed significant associations between the Gly460Trp polymorphism and any and ischemic stroke and MI. Hypertension has found to be a strong risk factor for all of these outcomes.[1-3, 13, 27] Also, it has been reported that variant carriers have an increased left ventricular mass[28] and are at increased risk of coronary heart disease.[14] Recently, it was found that hypertension and stroke, as well as hypertension and MI coaggregate strongly within families.[5,6] This suggests that overlapping genetic factors

influence susceptibility to hypertension, stroke and MI.

We did not observe an association between the Gly460Trp polymorphism and hemorrhagic stroke. This may be due to small numbers. Also, the higher case fatality in patients with hemorrhagic stroke compared with ischemic stroke patients may have impaired the subclassification of hemorrhagic stroke patients.[29,30] Especially in hemorrhagic stroke patients with high blood pressure on admission, prognosis was found to be poor.[31]

Consistent with the association between stroke and SBI and WML,[21,25] and the association between the Gly460Trp polymorphism and stroke in our study, we observed an association between the Gly460Trp polymorphism and SBI and subcortical WML. The lack of statistical significance may have been due to lack of power.

We found a significant interaction between the Gly460Trp polymorphism and hypertension with respect to any and ischemic stroke, suggesting that hypertension is an effect modifier in the development of these diseases. This does not mean that the effect of ADD1 on these outcomes may be solely attributed to an effect on blood pressure. We did not observe a relationship between the Gly460Trp polymorphism and blood pressure or hypertension. Also, when adjusting for hypertension or blood pressure, the associations between the Gly460Trp polymorphism and IMT, stroke and MI remained significant, suggesting that hypertension and blood pressure are not part of the intermediate pathway. A potential pathway could be salt sensitivity, which was found to be a risk factor for cardiovascular events, independent of blood pressure.[13]

It has previously been reported that there is an interaction between the 460Trp allele and diuretic therapy.[15] Hypertensive subjects carrying the variant allele and treated with diuretics were at lower risk of stroke and MI compared with other antihypertensive therapies. We found that in hypertensive subjects carrying the variant allele of the Gly460Trp polymorphism, the risk of stroke was significantly increased. Therefore, it is of great importance to optimize treatment of hypertension for these patients. When selecting anti-hypertensive medication, the genetic profile of a patient may need to be taken into account in the future.

References

1. Antikainen R, Jousilahti P, Tuomilehto J. Systolic blood pressure, isolated systolic hypertension and risk of coronary heart disease, strokes, cardiovascular disease and all-cause mortality in the middle-aged population. J Hypertens. 1998;16:577-583
2. Mancia G. Prevention and treatment of stroke in patients with hypertension. Clin Ther. 2004;26:631-648
3. Su TC, Jeng JS, et al. Hypertension status is the major determinant of carotid atherosclerosis: A community-based study in Taiwan. Stroke. 2001;32:2265-2271
4. van Dijk EJ, Breteler MM, et al. The association between blood pressure, hypertension, and cerebral white matter lesions: Cardiovascular Determinants of Dementia study. Hypertension. 2004;44:625-630
5. Schulz UG, Flossmann E, Rothwell PM. Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. Stroke. 2004;35:819-824
6. Kondo T, Toyoshima H, et al. Familial aggregation and coaggregation of history of hypertension and

stroke. J Hum Hypertens. 2005;19:119-125
7. Manunta P, Barlassina C, Bianchi G. Adducin in essential hypertension. FEBS Lett. 1998;430:41-44
8. Hughes CA, Bennett V. Adducin: A physical model with implications for function in assembly of spectrin-actin complexes. J Biol Chem. 1995;270:18990-18996
9. Glorioso N, Filigheddu F, et al. Alpha-adducin 460trp allele is associated with erythrocyte Na transport rate in North Sardinian primary hypertensives. Hypertension. 2002;39:357-362
10. Manunta P, Cusi D, et al. Alpha-adducin polymorphisms and renal sodium handling in essential hypertensive patients. Kidney Int. 1998;53:1471-1478
11. Bianchi G, Ferrari P, Staessen JA. Adducin polymorphism: Detection and impact on hypertension and related disorders. Hypertension. 2005;45:331-340
12. Beeks E, Kessels AG, et al. Genetic predisposition to salt-sensitivity: A systematic review. J Hypertens. 2004;22:1243-1249
13. Morimoto A, Uzu T, et al. Sodium sensitivity and cardiovascular events in patients with essential hypertension. Lancet. 1997;350:1734-1737
14. Morrison AC, Bray MS, et al. Add1 460w allele associated with cardiovascular disease in hypertensive individuals. Hypertension. 2002;39:1053-1057
15. Psaty BM, Smith NL, et al. Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. JAMA. 2002;287:1680-1689
16. Morrison AC, Doris PA, et al. G-protein beta3 subunit and alpha-adducin polymorphisms and risk of subclinical and clinical stroke. Stroke. 2001;32:822-829
17. Breteler MM. Vascular involvement in cognitive decline and dementia. Epidemiologic evidence from the Rotterdam Study and the Rotterdam Scan Study. Ann N Y Acad Sci. 2000;903:457-465
18. 1999 World Health Organization-International Society of Hypertension guidelines for the management of hypertension. Guidelines subcommittee. J Hypertens. 1999;17:151-183
19. Bots ML, Hoes AW, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. Circulation. 1997;96:1432-1437
20. WHO. International statistical classification of diseases and related health problems, 10th revision. Geneva, 1992.
21. Vermeer SE, Hollander M, et al. Silent brain infarcts and white matter lesions increase stroke risk in the general population: The Rotterdam Scan Study. Stroke. 2003;34:1126-1129
22. de Leeuw FE, de Groot JC, et al. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-833
23. Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Res. 1993;21:3761-3766
24. Raymond M, Rousset F. Genepop-package, version 1.2. Montpelier, France, Laboratoire de Genetique et Environment. 1995
25. Kobayashi S, Okada K, et al. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke. 1997;28:1932-1939
26. Hollander M, Hak AE, et al. Comparison between measures of atherosclerosis and risk of stroke: The Rotterdam Study. Stroke. 2003;34:2367-2372
27. Zanchetti A, Bond MG, et al. Risk factors associated with alterations in carotid intima-media thickness in hypertension: Baseline data from the European lacidipine study on atherosclerosis. J Hypertens. 1998;16:949-961
28. Winnicki M, Somers VK, et al. Alpha-adducin gly460trp polymorphism, left ventricular mass and plasma renin activity. J Hypertens. 2002;20:1771-1777
29. Kiyohara Y, Kubo M, et al. Ten-year prognosis of stroke and risk factors for death in a Japanese community: The Hisayama Study. Stroke. 2003;34:2343-2347
30. Dennis MS. Outcome after brain haemorrhage. Cerebrovasc Dis. 2003;16 Suppl 1:9-13
31. Okumura K, Ohya Y, et al. Effects of blood pressure levels on case fatality after acute stroke. J Hypertens. 2005;23:1217-1223

3.2.
Polymorphisms of the renin-angiotensin system

Background and purpose: *The renin-angiotensin system is involved in the development of hypertension, atherosclerosis and cardiovascular disease. We studied the association between the M235T polymorphism of the angiotensinogen gene (AGT) and the C573T polymorphism of the angiotensin II type 1 receptor (AT1R) and blood pressure, carotid atherosclerosis and cerebrovascular disease.* ***Methods:*** *We genotyped over 6000 subjects of the Rotterdam Study and over 1000 subjects of the Rotterdam Scan Study. We used logistic regression and univariate analyses, adjusting for age and sex, with for AGT, the MM, and for AT1R, the TT genotype as reference.* ***Results:*** *We found that AGT-235T increased systolic (p for trend=0.03) and diastolic blood pressure (p for trend=0.04). The prevalence of carotid plaques was 1.25 fold increased (95% CI: 1.02-1.52) in AGT-TT carriers. There was a significant increase in mean volume deep subcortical white matter lesions (WML) for AGT-TT carriers (1.78 ml versus 1.09 ml in the reference group, p=0.008). A significant interaction was found between AGT and AT1R, further increasing the effect on periventricular and subcortical WML (p for interaction=0.02). We found a non-significant increased risk of silent brain infarction for AGT-TT carriers and AT1R-CC carriers, but no effect on stroke.* ***Conclusions:*** *We found an association between AGT and blood pressure, atherosclerosis and WML. Also, we found synergistic effects between AGT and AT1R on the development of WML. These findings raise the question whether the RAS may be a therapeutical target for the prevention of cerebral white matter pathology.*

Introduction

The renin-angiotensin system (RAS) regulates blood pressure, cardiovascular homeostasis and vascular tone.[1] Polymorphisms in genes that encode for the proteins of the RAS are candidate genes for hypertension, cardiovascular and cerebrovascular diseases. The angiotensin (AGT) and the angiotensin II type I receptor (AT1R) genes are two key players in the AGT protein metabolism.

The AGT-M235T polymorphism encodes the substitution of methionine by threonine at residue 235 of the AGT protein, increasing plasma AGT levels in 235T homozygotes.[2] A haplotype at the AGT promotor, which was in complete linkage disequilibrium with the M235T polymorphism, was found to increase transcriptional activity in astrocytes.[3] AGT has been associated with hypertension, carotid atherosclerosis, cardiovascular and cerebrovascular disease, although findings have been inconsistent.[4-7] AGT has been associated consistently with cerebral small vessel disease.[8-10]

The AT1R gene has been associated with hypertension, cardiovascular and cerebrovascular disease.[10-14] The AT1R-C573T polymorphism, which is in linkage disequilibrium with the frequently studied A1166C polymorphism,[10, 14] has been associated with blood pressure and vascular complications in hypertensive patients.[15, 16]

Hypertension, atheroclerosis and cerebrovascular diseases are all complex diseases. For these traits, a network of interactions between genetic factors can be supposed.[17] Both the AGT and the AT1R gene products are part of the RAS. So far, an interaction has not been reported. We studied the AGT-M235T and the AGT-C573T polymorphisms in relation to blood pressure, carotid atherosclerosis and small- and large-vessel cerebral pathology. Also, we studied the interaction between AGT and AT1R with respect to all above-mentioned outcomes.

Methods

Study populations: The Rotterdam Study is an ongoing prospective population-based cohort study on chronic and disabling diseases in the elderly.[18] Baseline examinations were done between 1990 and 1993. A total of 7983 subjects (age 55 years and over) participated in this study. In 6,444 (80.7%) and 6,367 (79.8%) participants, the M235T polymorphism of AGT and the C573T polymorphism of AT1R, respectively, were successfully genotyped. No DNA was available for 1455 subjects and there was a genotyping failure in 84 (AGT) and 161 (AT1R) subjects.

The Rotterdam Scan Study was designed to study the etiology and natural history of age-related brain changes in the elderly, using a similar protocol (baseline between 1995).

A total of 1,077 non-demented elderly persons (age 60 years and over) participated in this study. In 1995/1996, subjects aged between 60 and 90 years were selected randomly in strata of age (5 years) and sex, from the Zoetermeer Study[19] and the Rotterdam Study. In 1,048 (97.3%) participants, the M235T polymorphism of AGT and in 1011 (93.9%) participants, the C573T polymorphism of AT1R, were successfully genotyped. There was a genotyping failure in 29 (AGT) and 66 (AT1R) subjects. The medical ethics committee of the Erasmus Medical Center, Rotterdam, approved both studies and all participants gave written informed consent and permission to retrieve information from treating physicians.

Measurements: Height and weight were measured and body mass index (BMI in kg/m^2) was calculated. Blood pressure was based on the average of two measurements with a random-zero sphygmomanometer. Hypertension was defined as a systolic blood pressure (SBP) of at least 140 mmHg and/or a diastolic blood pressure (DBP) of at least 90 mmHg and/or use of anti-hypertensives. For 1184 (14.8%), blood pressure measurements were not available. Information on smoking habits was obtained.

We collected non-fasting blood samples from all participants. We defined diabetes mellitus as a random glucose level of at least 11.1 mmol/l and/or use of oral anti-diabetics or insulin. Total serum cholesterol and HDL-cholesterol were determined using an automated enzymatic method.[20]

Measurements of atherosclerosis and stroke: As part of the Rotterdam study, the total number of plaques was assessed by duplex scan ultrasonography.[21] Plaques were defined as focal widening of the vessel wall with protrusion into the lumen. The total plaque score reflected the total number of sites with plaques ranging from 0-6 (left and right sided, common carotid arteries, bifurcation, and internal carotid arteries). This score was dichotomized (0,1 or 2 versus >2). For 2372 (29.7%) participants the number of plaques could not be assessed.

Incident stroke was also assessed as part of the Rotterdam Study. A prevalent stroke was determined during the baseline interview. Research physicians reviewed information on all possible strokes with an experienced stroke neurologist to verify all diagnoses. Subarachnoid hemorrhages and retinal strokes were excluded. A stroke was classified ischemic when a patient had typical symptoms and a CT or MRI ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours or atrial fibrillation in absence of anticoagulants) pointed to an ischemic nature of the stroke. A stroke was classified hemorrhagic when a relevant hemorrhage was shown on CT or MRI scan, or the subject lost consciousness permanently or died within hours after the onset of focal signs.

Cerebral infarcts and white matter lesions: As part of the Rotterdam Scan Study, we obtained axial T1, T2 and proton-density MRI scans of the brain. Infarcts were defined as focal hyperintensities on T2-weighted images, 3 to 20 mm in size. Silent brain infarctions (SBI)

were defined as evidence for infarcts on MRI, without a history of a (corresponding) stroke or TIA.[22]

White matter lesions (WML) were scored as hyperintense on proton-density and T2-weighted images, without prominent hypointensity on T1-weighted scans and, according to their location, as periventricular or subcortical.[23] Periventricular WML were rated semi-quantitatively (range 0-9). A total volume of subcortical WML was approximated based on number and size of lesions (volume range 0-29.5 ml). In 4 participants (0.004%), sub cortical WML could not be measured, due to the quality of the MRI scans.

Genotyping: Genotyping of the AGT M235T polymorphism and the AT1R C573T polymorphism was performed using TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA). Based on the analysis of blind duplicates (326 control pairs), there was a 99.4 % concordance in genotyping AGT and 100 % concordance in genotyping AT1R. The 2 discordant pairs were set to missing.

Statistical analyses: Hardy-Weinberg equilibrium proportions of the M235T and the C573T polymorphisms were tested using the GENEPOP-package. Baseline characteristics were compared using univariate ANOVA or Chi^2 statistics. Analyses of variance was used to assess the relation between AGT and AT1R and SBP and DBP, as well as WML. In order to obtain a normal distribution of WML, we used the natural logarithm transformation. Cox proportional hazards regression analysis was used to assess the relative risk of stroke. For this analysis, we excluded prevalent stroke. We used logistic regression to assess the odds ratio for carotid artery plaques and SBI for AGT and AT1R (SPSS version 11.0). All analyses were adjusted for age and sex (model 1) and additionally adjusted for SBP, DBP, BMI, total cholesterol, diabetes mellitus and smoking (model 2). The analyses on blood pressure levels were also adjusted use of anti-hypertensives. A p-value <0.05 was considered statistically significant.

Results

Genotype frequencies were in Hardy-Weinberg-Equilibrium for both study populations. Table 1 shows the baseline characteristics stratified by AGT and AT1R genotype. No significant differences were observed between genotype groups and baseline characteristics.

In Table 2 we show that for AGT, SBP (p for trend=0.03) and DBP (p for trend=0.04) increased with the number of AGT-235T alleles. In the fully adjusted model, including anti-hypertensive medication use, these findings remained significant. Also, a (borderline) significant increase in prevalence of hypertension was found. AT1R was not associated with blood pressure or hypertension.

Figure 1 shows that carriers of the AGT-235T allele had an increased risk of plaques.

Table 1. Baseline characteristics stratified by AGT and AT1R genotype.

AGT M235T	MM	MT	TT
Rotterdam Study, n (%)	2341 (36.3)	3084 (47.9)	1019 (15.8)
Age, y	69.4 ± 9.1	69.7 ± 9.2	69.0 ± 9.1
Sex, % men	39.6	41.0	40.7
BMI, kg/m^2	26.4 ± 3.8	26.3 ± 3.7	26.2 ± 3.6
Current smoking, %	21.9	23.1	23.2
Total Cholesterol, mmol/l	6.6 ± 1.2	6.6 ± 1.3	6.6 ± 1.2
Diabetes Mellitus, %	10.1	10.2	8.9
Rotterdam Scan Study, n (%)	386 (36.8)	503 (48.0)	159 (15.1)
Age, y	72.2 ± 7.3	72.2 ± 7.4	72.4 ± 7.6
Sex, % men	46.4	50.3	42.8
BMI, kg/m^2	26.7 ± 3.6	26.7 ± 3.6	26.5 ± 3.6
Current smoking, %	18.1	16.1	17.7
Total Cholesterol, mmol/l	5.9 ± 1.1	5.9 ± 1.0	5.9 ± 1.1
Diabetes Mellitus, %	5.2	8.3	7.5
AT1R C573T	TT	CT	CC
Rotterdam Study, n (%)	1484 (23.3)	3126 (49.1)	1755 (27.6)
Age, y	69.5 ± 9.3	69.4 ± 8.9	69.5 ± 9.4
Sex, % men	38.8	41.3	40.6
BMI, kg/m^2	26.2 ± 3.7	26.3 ± 3.7	26.4 ± 3.8
Current smoking, %	21.6	23.9	21.4
Total Cholesterol, mmol/l	6.6 ± 1.2	6.6 ± 1.2	6.6 ± 1.2
Diabetes Mellitus, %	9.4	10.0	10.8
Rotterdam Scan Study, n (%)	219 (21.7)	504 (49.9)	288 (28.5)
Age, y	72.6 ± 7.5	72.1 ± 7.1	71.8 ± 7.2
Sex, % men	46.1	49.8	48.3
BMI, kg/m^2	26.7 ± 4.2	26.8 ± 3.6	26.4 ± 3.3
Current smoking, %	17.4	15.7	19.9
Total Cholesterol, mmol/l	5.9 ± 1.0	5.9 ± 1.0	5.9 ± 1.1
Diabetes Mellitus, %	5.5	7.3	7.3

All values are percentages or means ± SD.

The OR for the AGT-MT genotype was 1.16 (95% CI: 1.00-1.34, p=0.05), and for the TT genotype 1.25 (95% CI: 1.02-1.52, p=0.03). In the fully adjusted model, the OR for the AGT-MT genotype was 1.17 (95% CI: 1.01-1.37, p=0.04), and for the TT genotype 1.27 (95% CI: 1.03-1.57, p=0.03). We did not find an association between AT1R and carotid artery plaques.

Periventricular WML were present in 219 (20.3%), deep sub cortical WML in 84

(7.8%) participants. No association was found between AGT or AT1R and periventricular WML. Participants with the TT genotype of AGT had an increased volume of deep subcortical WML (1.78 ml versus 1.09 ml for the reference group, p=0.008; figure 2). Participants with the CT genotype of the AT1R genotype also showed an increased volume (1.45 ml versus 0.99 ml for the reference group, p=0.03). Findings remained significant in the fully adjusted model (AGT-TT 1.81 ml versus 1.08 ml for the reference group, p=0.004 and AT1R-CT 1.39 ml versus 0.98 ml for the reference group, p=0.05).

We observed 217 (20.2%) participants with a SBI (Rotterdam Scan Study) and 637 (8.0%) with incident stroke (Rotterdam Study). The prevalence of SBI was increased in AGT-TT carriers (OR=1.44 (95% CI: 0.89-2.33, p=0.14), and AT1R-CC carriers (OR=1.43 (95% CI: 0.89-2.30, p=0.14), model 2), however, findings were not statistically significant. No significant association was found between AGT or AT1R and overall stroke (AGT-TT HR=0.99 (95% CI: 0.75-1.30), AT1R-CC HR=1.19 (95% CI: 0.91-1.56), model 2), ischemic stroke (AGT-TT HR =1.05 (95% CI: 0.73-1.50), AT1R-CC HR=1.34 (95% CI: 0.94-1.92), model 2), or hemorrhagic stroke (AGT-TT HR=1.19 (95% CI: 0.50-2.80), AT1R-CC HR=1.14 (95% CI: 0.52-2.52), model 2).

Finally, we studied the interaction between AGT and AT1R. Within the Rotterdam Study, SBP and DBP were highest in participants carrying both the TT genotype of AGT and the CC genotype of AT1R (n=234) compared with the reference group of participants with MM genotype of AGT and the CC genotype of AT1R (n=593) (model 1: SBP 141.9 mmHg

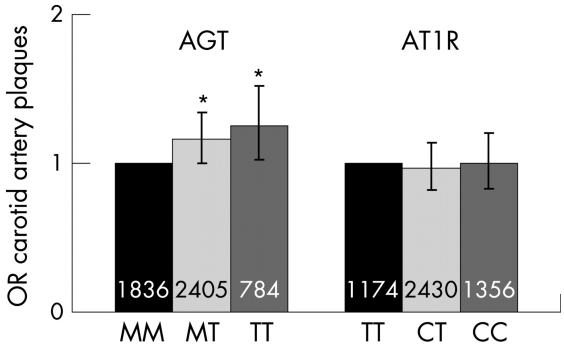


Figure 1. The Rotterdam Study: Association between the AGT and AT1R genotypes and carotid artery plaques. From a total plaque score of 0-12, plaques were scored low if the total plaquescore was 0, 1 or 2, and high if the total plaque score was greater than 2.
**p<0.05 compared with TT genotype, adjusted for age and sex. OR=odds ratio.*

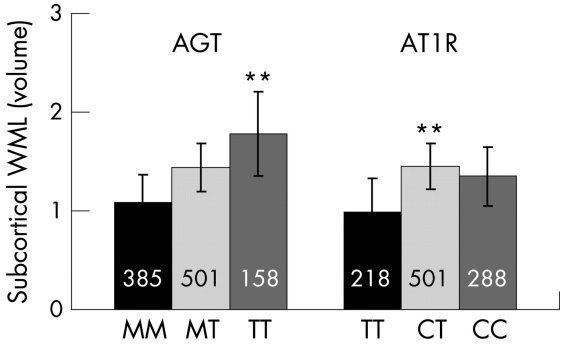


Figure 2. The Rotterdam Scan Study: Association of the AGT and the AT1R genotypes with subcortical white matter lesions (WML).
***p<0.01 compared with the MM genotype for AGT and the TT genotype for AT1R, adjusted for age and sex.*

versus 137.3 mmHg, p=0.007, model 2: SBP 141.7 mmHg versus 137.2 mmHg, p=0.007 and model 1: DBP 74.8 mmHg versus 72.7 mmHg, p=0.02 and model 2: DBP 75.0 mmHg versus 72.6 mmHg, p=0.005).

For periventricular WML, we found the highest degree in participants with both the TT genotype of AGT and the CC genotype of AT1R (model 1: 3.06 (n=37), versus 2.06, (n=105), in the reference group, p=0.008, model 2: 2.93 versus 2.09, p=0.03), as well as for deep sub cortical WML (model 1: 2.60 ml (n=37), versus 0.93 ml (n=105) in the reference group, p=0.001, model 2: 2.67 ml versus 0.98 ml, p=0.001). The p for interaction was significant for both periventricular (p=0.02, both models) and deep sub cortical WML (p=0.02, both models). No interaction was found for carotid artery plaques or SBI.

Discussion

Within the Rotterdam Study, we found that mean systolic and diastolic blood pressure levels increased with the number of T alleles of the M235T polymorphism of AGT, as did the risk for the prevalence of carotid artery plaques. Within the Rotterdam Scan Study, we found that subjects with the TT genotype of AGT and the CT genotype of AT1R, had an increase in mean volume deep subcortical WML. A statistically significant interaction between AGT and AT1R was found for periventricular and deep subcortical WML.

We believe the strength of our study lies in the size of our study populations and the follow-up of patients over time. Also, we were able to study the effect of AGT and AT1R in two study populations, which made it possible to study both clinical and subclinical stroke (SBI), as well as white matter lesions of the brain and atherosclerosis. Results on plaques and incident stroke were obtained from the Rotterdam Study, while results on SBI and WML were obtained from the Rotterdam Scan Study. None of these outcomes were available in both cohorts. Participants of the Rotterdam Scan Study were randomly selected from the Rotterdam Study and the Zoetermeer Study in strata of age (>60 years) and sex. Participants of the Rotterdam Study and the Rotterdam Scan Study therefore party overlap. Both cohorts consist of elderly participants, living in the Netherlands, who are participants of two large prospective population-based studies, and are therefore comparable cohorts.

For genotyping measurements, as well as measurements of blood pressure and plaques, there was missing data. We did not find differences between participants with and without a genotype with regard to demographic or cardiovascular characteristics. Participants with missing data on blood pressure and plaques, were significantly older and more often males. As there were no genotype differences between these participants, this will most likely not have biased our results.

According to previous studies, carriers of the 235T allele of the AGT gene have increased plasma angiotensinogen levels,[2] hypertension and atherosclerosis.[5, 6] In line with

Table 2. The Rotterdam Study: Association between the M235T and the C573T polymorphism with SBP and DBP and hypertension.

AGT M235T	SBP (mmHg) Mean ± SE			DBP (mmHg) Mean ± SE			Hypertension OR (95% CI)		
	n	Model 1	Model 2	n	Model 1	Model 2	n	Model 1	Model 2
MM	2198	138.2 ± 0.5	138.2 ± 0.5	2197	73.4 ± 0.2	73.4 ± 0.2	2206	1.0 (ref)	1.0 (ref)
MT	2910	139.5 ± 0.4*	139.4 ± 0.4*	2910	73.7 ± 0.2	73.7 ± 0.2	2927	1.13 (1.01-1.27)*	1.15 (1.02-1.30)*
TT	955	139.6 ± 0.7	139.6 ± 0.7	955	74.2 ± 0.4*	74.2 ± 0.4*	964	1.17 (1.00-1.36)	1.20 (1.02-1.41)*
P for trend		0.03	0.03		0.04	0.04			
AT1R C573T									
TT	1397	139.4 ± 0.6	139.6 ± 0.6	1396	73.7 ± 0.3	73.8 ± 0.3	1409	1.0 (ref)	1.0 (ref)
CT	2942	139.3 ± 0.4	139.2 ± 0.4	2942	73.9 ± 0.2	73.9 ± 0.2	2952	1.04 (0.91-1.18)	1.02 (0.89-1.17)
CC	1657	138.6 ± 0.5	138.4 ± 0.5	1657	73.4 ± 0.3	73.4 ± 0.3	1669	0.95 (0.82-1.10)	0.92 (0.79-1.07)
P for trend		0.3	0.1		0.4	0.3			

All means and ORs are adjusted in model 1 for: age and sex, in model 2 for: age, sex, body mass index, total cholesterol, diabetes mellitus, smoking (and use of anti-hypertensive medication in SBP and DBP analyses), n=absolute number of subjects, SBP=systolic blood pressure, DBP=diastolic blood pressure, OR=odds ratio, SE=standard error, *p<0.05 compared with the MM genotype .

these findings, we found an increase in SBP and DBP in AGT-235T allele carriers in the Rotterdam Study and a (borderline) significant increased risk of hypertension. Also, we observed a significant association between AGT and carotid artery plaques. Previously, an association between AGT-M235T and carotid intima-media thickness (IMT) was reported.[5, 24] Even though we found an association between AGT-M235T and SBP and DBP, the observed association with carotid artery plaques may not be solely attributable to the effect on blood pressure. After adjusting for blood pressure, the association between the M235T polymorphism and carotid artery plaque remained significant, suggesting that blood pressure levels may not be part of the intermediate pathway.

Within the Rotterdam Scan Study, we found that the risk of SBI was increased in AGT-TT and AT1R-CC carriers, although not statistically significant. AGT was significantly associated with deep sub cortical WML. We did not find an association with periventricular WML. We used two different scales to define periventricular and deep subcortical WML, categorical and volumetric, respectively. As the scale to define subcortical WML was quantitative and the scale to define periventricular WML semi-quantitatively, the power to detect an effect is most likely higher for subcortical WML. This may explain why a significant association was only observed for subcortical WML in this study.

So far, three studies found an association between AGT and small-vessel disease and periventricular hyperintensity grade.[8-10] This finding has been explained by an increase in plasma AGT levels, which may lead to increased formation of angiotensin II, which has several proatherogenic effects,[25] and may also explain the effect we found on carotid atherosclerosis. However, the lack of a convincing association with SBI or stroke, does not support this pathway. Another mechanism explaining our findings and these of others, may be related to the fact that an independent renin-angiotensin system exists in the brain, which might amplify cerebrovascular pathology, in particular WML.[26] Also, a haplotype in at the AGT-promotor has been found to increase transcriptional activity in astrocytes.[3]

As plaques and WML are precursors of stroke,[22, 27] they are less heterogeneous compared with (sub)clinical stroke. This may also explain why we did not find a significant association for stroke and SBI, not even after subtyping stroke in order to increase homogeneity. As genes involved in so-called complex diseases, such as stroke, usually have small effects, they may be difficult to detect, even in large study populations. Studying intermediate phenotypes increases homogeneity as they focus on a specific pathohysiological pathway.

An interaction was observed between AT1R and AGT. Cross talk between genes is plausible as both the AGT and the AT1R gene products are part of the RAS. This is the first study addressing the interaction between AGT and AT1R. In complex traits, such as WML, one may expect joint effects of multiple genes. Other genes of the RAS may also be of interest in relation to small vessel pathology.

There is increasing interest in the association between cognitive decline and depression, and severe WML.[28, 29] The consistent association between AGT and WML, and

the interaction with AT1R reported here, raises the question whether the RAS may be a therapeutical target for the prevention of cerebral small vessel pathology. Previously, subjects with hypertension and the DD genotype of the insertion/deletion polymorphisms of the angiotensin converting enzyme (ACE) gene, also part of the RAS, showed a relative resistance to ACE-inhibitor therapy and therefore an increase in cardiovascular mortality.[30] In addition, several other studies found that this polymorphism may influence antihypertensive response, particularly when using ACE inhibitors.[31] Also, AGT was found to be an independent predictor of blood pressure response to ACE inhibititors[32] and a protective association between ACE inhibitor use and nonfatal stroke was found among 235T allele carriers of AGT.[4]

In conclusion, we found that the AGT-235T allele was associated with increased blood pressure levels and carotid artery plaques. With respect to WML, we found evidence for interaction between AGT and AT1R. As no significant evidence was found for an association with SBI or stroke, the effect of AGT and AT1R may be specific for small vessel pathology, perhaps related to blood pressure early in life.

References

1. MacGregor GA, Markandu ND, et al. Maintenance of blood pressure by the renin-angiotensin system in normal man. Nature. 1981;291:329-331
2. Paillard F, Chansel D, et al. Genotype-phenotype relationships for the renin-angiotensin-aldosterone system in a normal population. Hypertension. 1999;34:423-429
3. Schmidt H, Aulchenko YS, et al. Angiotensinogen promoter B-haplotype associated with cerebral small vessel disease enhances basal transcriptional activity. Stroke. 2004;35:2592-2597
4. Bis JC, Smith NL, et al. Angiotensinogen met235thr polymorphism, angiotensin-converting enzyme inhibitor therapy, and the risk of nonfatal stroke or myocardial infarction in hypertensive patients. Am J Hypertens. 2003;16:1011-1017
5. Bozec E, Fassot C, et al. Angiotensinogen gene m235t polymorphism and reduction in wall thickness in response to antihypertensive treatment. Clin Sci (Lond). 2003;105:637-644
6. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: A meta-analysis. Arterioscler Thromb Vasc Biol. 2003;23:1269-1275
7. Um JY, Moon KS, et al. Polymorphism of angiotensin-converting enzyme, angiotensinogen, and apoli poprotein e genes in Korean patients with cerebral infarction. J Mol Neurosci. 2003;21:23-28
8. Schmidt H, Fazekas F, et al. Angiotensinogen gene promoter haplotype and microangiopathy-related cerebral damage: Results of the Austrian Stroke Prevention Study. Stroke. 2001;32:405-412
9. Schmidt R, Schmidt H, et al. Angiotensinogen polymorphism m235t, carotid atherosclerosis, and small-vessel disease-related cerebral abnormalities. Hypertension. 2001;38:110-115
10. Takami S, Imai Y, et al. Gene polymorphism of the renin-angiotensin system associates with risk for lacunar infarction. The Ohasama Study. Am J Hypertens. 2000;13:121-127
11. Kainulainen K, Perola M, et al. Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. Hypertension. 1999;33:844-849
12. Bonnardeaux A, Davies E, et al. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension. 1994;24:63-69
13. Tiret L, Bonnardeaux A, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. Lancet. 1994;344:910-913
14. Rubattu S, Di Angelantonio E, et al. Gene polymorphisms of the renin-angiotensin-aldosterone system and the risk of ischemic stroke: A role of the A1166c/AT1 gene variant. J Hypertens. 2004;22:2129-2134

15. Poirier O, Georges JL, et al. New polymorphisms of the angiotensin II type 1 receptor gene and their associations with myocardial infarction and blood pressure: The ECTIM study. Etude Cas-Temoin de l'Infarctus du Myocarde. J Hypertens. 1998;16:1443-1447
16. Chaves FJ, Pascual JM, et al. Angiotensin II AT1 receptor gene polymorphism and microalbuminuria in essential hypertension. Am J Hypertens. 2001;14:364-370
17. Szolnoki Z, Melegh B. Gene-gene and gene-environment interplay represent specific susceptibility for different types of ischaemic stroke and leukoaraiosis. Curr Med Chem. 2006;13:1627-1634
18. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
19. Hofman A, van Laar A, et al. Coffee and cholesterol. N Engl J Med. 1983;309:1248-1250
20. van Gent CM, van der Voort HA, et al. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chim Acta. 1977;75:243-251
21. Bots ML, Hoes AW, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. Circulation. 1997;96:1432-1437
22. Vermeer SE, Hollander M, et al. Silent brain infarcts and white matter lesions increase stroke risk in the general population: The Rotterdam Scan Study. Stroke. 2003;34:1126-1129
23. de Leeuw FE, de Groot JC, et al. A follow-up study of blood pressure and cerebral white matter lesions. Ann Neurol. 1999;46:827-833
24. Tabara Y, Kohara K, et al. Risk factor-gene interaction in carotid atherosclerosis: Effect of gene poly morphisms of renin-angiotensin system. J Hum Genet. 2001;46:278-284
25. Burnier M, Brunner HR. Angiotensin II receptor antagonists. Lancet. 2000;355:637-645
26. Bunnemann B, Fuxe K, Ganten D. The brain renin-angiotensin system: Localization and general significance. J Cardiovasc Pharmacol. 1992;19 Suppl 6:S51-62
27. Hollander M, Hak AE, et al. Comparison between measures of atherosclerosis and risk of stroke: The Rotterdam Study. Stroke. 2003;34:2367-2372
28. Steffens DC, Krishnan KR, et al. Cerebrovascular disease and evolution of depressive symptoms in the Cardiovascular Health Study. Stroke. 2002;33:1636-1644
29. Minett TS, Dean JL, et al. Subjective memory complaints, white-matter lesions, depressive symptoms, and cognition in elderly patients. Am J Geriatr Psychiatry. 2005;13:665-671
30. Bleumink GS, Schut AF, et al. Mortality in patients with hypertension on angiotensin-I converting enzyme (ACE)-inhibitor treatment is influenced by the ACE insertion/deletion polymorphism. Pharmacogenet Genomics. 2005;15:75-81
31. Baudin B. Angiotensin I-converting enzyme gene polymorphism and drug response. Clin Chem Lab Med. 2000;38:853-856
32. Hingorani AD, Jia H, et al. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. J Hypertens. 1995;13:1602-1609

3.3.
Variation in the estrogen receptor alpha gene

Background and purpose: *Variations in the –397T>C (rs2234693) and –351A>G (rs9340799) single nucleotide polymorphisms (SNPs) of the estrogen alpha receptor (ESR1) gene were found to be strongly associated with risk of ischemic heart disease, although not all studies could replicate this finding. One study also reported an association with stroke. We assessed whether variations in the ESR1 gene are associated with the risk of stroke in the general population.* ***Methods:*** *This prospective population-based study was based on 6,229 Rotterdam Study participants who at baseline (1990-1993) were aged 55 years or over, free from stroke, and had assessment of the ESR1 rs2234693 and rs9340799 SNPs. Follow-up for incident stroke was complete until January 1, 2005. Data were analyzed with Cox proportional hazards models for men and women separately with adjustment for age.* ***Results:*** *During an average follow-up time of 10.1 years, 659 strokes occurred, of which 386 were ischemic. Three common haplotypes were identified: -397T/-351A (carried by 78% of all participants), -397C/-351G (carried by 57%), and -397C/-351A (carried by 22%). Although we had at least 89% power to detect a relative risk of 1.5 (with alpha=0.05) in all subgroups, we did not find any association between ESR1 haplotype carriership and risk of stroke and ischemic stroke.* ***Conclusions:*** *We have not been able to replicate the previously reported association between variations in the ESR1 gene and risk of stroke.*

Introduction

The relatively low incidence rate of ischemic heart disease in premenopausal women has drawn attention to the role of estrogen in cardiovascular disease. Estrogen exerts its effect through estrogen receptors α and β. Previous research has demonstrated that the estrogen receptor α is involved in atherosclerosis,[1] and several epidemiological studies found associations between variations in the –397T>C (rs2234693) and –351A>G (rs9340799) single nucleotide polymorphisms (SNPs) of the estrogen α receptor (ESR1) gene and risk of ischemic heart disease,[2-5] which could not be replicated by two other studies, however.[6, 7] Studies on the association between variations in the –397T>C SNP and risk of stroke are also contradictive.[7, 8]

We assessed whether variations in the ESR1 gene are associated with the risk of stroke in the general population.

Methods

The present study was part of the Rotterdam Study, a prospective study on chronic and disabling diseases in 7,983 Caucasian Dutch (98.5%) community-dwelling participants of age 55 years and over (participation rate 78%).[9] The Medical Ethics Committee of Erasmus University Rotterdam approved of the study. Written informed consent to retrieve information from treating physicians was obtained from all participants.

All Rotterdam Study participants who at baseline (1990-1993) were free from stroke and had successful assessment of both ESR1 SNPs were included in the present study (N=6,229; 2,511 men). After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Strokes were subclassified as ischemic strokes, hemorrhagic strokes, and unspecified strokes, as described previously.[9] Follow-up was complete until January 1, 2005, for 97.1 % of potential person years.

All participants were genotyped for the –397T>C (rs2234693) and –351A>G (rs9340799) polymorphisms as described previously.[2] We used the genotype data for each of the two polymorphisms to infer the haplotype alleles present in the population by using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes.[10] Haplotype alleles were numbered in order of decreasing prevalence.

We calculated hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the associations between ESR1 haplotypes (dominant model) and risk of stroke and ischemic stroke using Cox' proportional hazards models with SPSS for Windows, Rel. 11.0.1.

Results

During an average follow-up time of 10.1 years, 659 strokes occurred, of which 386 were ischemic, 62 hemorrhagic, and 211 could not be subclassified. Baseline characteristics are presented in Table 1. Neither in women nor in men were variations in individual SNPs associated with risk of stroke and ischemic stroke (P>0.37). Of all participants, 78% carried haplotype 1 (-397T/-351A; 29% homozygous), 57% carried haplotype 2 (-397C/-351G; 12% homozygous), 22% carried haplotype 3 (-397C/-351A; 1.3% homozygous), and 1 participant carried haplotype 4 (-397C/-351A). For women there was no association between ESR1 haplotypes and risk of stroke and ischemic stroke (Table 2). For men, risk of stroke and ischemic stroke might be slightly increased in haplotype 1 carriers compared to non-carriers (HR 1.21, 95% CI 0.89-1.64 for stroke and HR 1.40, 95% CI 0.94-2.09 for ischemic stroke), but this was not statistically significant at α=0.05. Further adjustment did not change the associations.

Table 1. Baseline characteristics of the study population (n=6,229).[9]

	Women (N=3,718)	Men (N=2,511)
Age, yrs	69.1 (62.2-76.7)	66.7 (61.6-73.0)
Systolic blood pressure, mm Hg	138 (123-154)	136 (123-152)
Diabetes mellitus, %	10	10
Ever smoking, %	46	92
Antihypertensive medication, %	12	14
Previous myocardial infarction, %	9	18

Values are median (25th and 75th percentile) if appropriate.

Discussion

ESR1 haplotypes were not associated with risk of stroke in our population-based cohort.

For men, our power (with α=0.05) to detect a relative risk of 1.3 in haplotype carriers compared to non-carriers ranged from 50% for haplotype 1 to 80% for haplotype 2, the power to detect a relative risk of 1.4 ranged from 74% (H1) to 96% (H2); and the power to detect a relative risk of 1.5 ranged from 89% (H1) to 100% (H2). For women, the power to detect a relative risk of 1.3 ranged from 66% for H1 to 83% for H2, the power to detect a relative risk of 1.4 ranged from 88% (H1) to 97% (H2); and the power to detect a relative risk of 1.5 ranged from 98% (H3) to 100% (H2). Therefore, we cannot completely rule out the possibility that the risk of stroke might be slightly increased (with hazards ratio < 1.5) in male haplotype 1 carriers compared to noncarriers; similarly, the risk of ischemic stroke might be slightly increased in male haplotype 3 carriers compared to noncarriers.

Table 2. ESR1 haplotypes and risk of stroke and ischemic stroke (dominant model).

Haplotype	Haplotype carrier	HR (95% CI), adjusted for age					
		Women			Men		
		N	Strokes (n=388)	Ischemic strokes (n=211)	N	Strokes (n=271)	Ischemic strokes (n=175)
Haplotype 1	No	811	1 (ref)	1 (ref)	547	1 (ref)	1 (ref)
(-397T/-351A)	Yes (model 1)*	2,907	0.98 (0.77-1.25)	0.95 (0.69-1.31)	1,964	1.21 (0.89-1.64)	1.40 (0.94-2.09)
	Yes (model 2)*	2,907	0.96 (0.76-1.22)	0.93 (0.67-1.28)		1.22 (0.90-1.66)	1.10 (0.94-2.09)
Haplotype 2	No	1,598	1 (ref)	1 (ref)	1,055	1 (ref)	1 (ref)
(-397C/-351G)	Yes (model 1)*	2,12	0.94 (0.77-1.15)	0.89 (0.68-1.16)	1,456	0.97 (0.76-1.23)	0.91 (0.68-1.23)
	Yes (model 2)*	2,12	0.95 (0.78-1.16)	0.90 (0.69-1.19)		0.95 (0.75-1.21)	0.89 (0.66-1.21)
Haplotype 3	No	2,903	1 (ref)	1 (ref)	1,932	1 (ref)	1 (ref)
(-397C/-351A)	Yes (model 1)*	815	1.03 (0.81-1.31)	0.89 (0.63-1.24)	579	1.24 (0.94-1.62)	0.93 (0.65-1.34)
	Yes (model 2)*	815	1.05 (0.83-1.33)	0.90 (0.64-1.26)		1.22 (0.93-1.60)	0.94 (0.66-1.35)

*Model 1: adjusted for age. Model 2; adjusted for age, systolic blood pressure, antihypertensive drug use, current smoking, former smoking, diabetes mellitus, serum C-reactive protein, previous myocardial infarction, atrial fibrillation, waist-hip ratio.

Strengths of our study are the large study population (N=6229), the intense stroke case finding, and the nearly complete follow-up (loss of potential person-years 2.9%). Our stringent stroke monitoring procedures enabled the ascertainment of stroke cases that were not referred to a hospital. Since in these cases neuroimaging was often lacking, 32% of the total number of strokes could not be subclassified into ischemic or hemorrhagic.

Four large studies with a total of 7,134 participants found an association between variation in the ESR1 gene and the risk of ischemic heart disease,[2-5] whereas one study with 4,868 participants could not replicate this finding.[6] According to a study in 2,709 men, those with the –397CC genotype were at higher risk of stroke than those with –397CT or TT (adjusted HR 1.92, 95% CI 1.06-3.48).[8] A study in 5063 participants found no relation between this polymorphism and risk of stroke.[7] We found no associations between variations in the ESR1 gene and risk of stroke, neither in 3,718 women nor in 2,511 men.

In conclusion, we have not been able to replicate the previously reported association between variations in the ESR1 gene and risk of stroke.

References

1. Losordo DW, Kearney M, et al. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. Circulation. 1994;89:1501-1510
2. Schuit SC, Oei HH, et al. Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. JAMA. 2004;291:2969-2977
3. Shearman AM, Cooper JA, et al. Estrogen receptor alpha gene variation is associated with risk of myocardial infarction in more than seven thousand men from five cohorts. Circ Res. 2006;98:590-592
4. Shearman AM, Cupples LA, et al. Association between estrogen receptor alpha gene variation and cardiovascular disease. JAMA. 2003;290:2263-2270
5. Lu H, Higashikata T, et al. Association of estrogen receptor-alpha gene polymorphisms with coronary artery disease in patients with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2002;22:817-823
6. Koch W, Hoppmann P, et al. No replication of association between estrogen receptor alpha gene polymorphisms and susceptibility to myocardial infarction in a large sample of patients of European descent. Circulation. 2005;112:2138-2142
7. Kjaergaard AD, Ellervik C, et al. Estrogen receptor alpha polymorphism and risk of cardiovascular disease, cancer, and hip fracture. Circulation. 2007;115:861-871
8. Shearman AM, Cooper JA, et al. Estrogen receptor alpha gene variation and the risk of stroke. Stroke. 2005;36:2281-2282
9. Bos MJ, Schipper CM, et al. High serum C-reactive protein level is not an independent predictor for stroke: The Rotterdam Study. Circulation. 2006;114:1591-1598
10. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001;68:978-989

3.4.
Variation in fibrinogen FGA and FGG genes

Background and purpose: *Haplotypes of the fibrinogen gamma and alpha (FGG and FGA) genes are associated with the structure of the fibrin network and may therefore influence the risk of stroke. We investigated the relationship between common variation in these genes with ischemic and hemorrhagic stroke.* ***Methods:*** *The study was based on 6275 participants of the prospective population-based Rotterdam Study who at baseline (1990-1993) were aged 55 years or over, free from stroke, and had successful assessment of at least one FGG or FGA SNP. Common haplotypes were estimated using seven tagging SNPs across a 30 kb region containing the FGG and FGA genes. Follow-up for incident stroke was complete until January 1, 2005. Associations between constructed haplotypes and risk of stroke were estimated with an age and sex adjusted logistic regression model.* ***Results:*** *We observed 669 strokes, of which 393 were ischemic and 62 hemorrhagic, during a median follow-up time of 10.1 years. FGG+FGA haplotype 3 (H3) was associated with an increased risk of ischemic stroke (odds ratio (OR) 1.31, 95% confidence interval (CI) 1.05-1.63) and a decreased risk of hemorrhagic stroke (OR 0.69, 95% CI 0.45-1.06) compared to the most frequent H1. The FGG and FGA genes were not associated with stroke or its subtypes when analyzed separately.* ***Conclusions:*** *Risk of ischemic stroke was higher in FGG + FGA haplotype H3 than in H1; this association was opposite for hemorrhagic stroke. Confirmation of these findings is warranted.*

Introduction

Fibrinogen is an important coagulation factor and its levels increase 2-4 times during acute phase reactions. The fibrinogen molecule comprises two identical disulphide-linked halves, each consisting of three polypeptide chains termed A-alpha, B-beta and gamma,[1] which are encoded by the fibrinogen alpha (FGA), fibrinogen beta (FGB) and fibrinogen gamma (FGG) genes, respectively, on chromosome 4.[2] At the final stage of the coagulation cascade, thrombin cleaves fibrinopeptide A and B from the amino termini of the fibrinogen A-alpha and B-beta chains, converting the soluble fibrinogen into fibrin monomers, which then polymerize to an insoluble fibrin clot.

Many prospective studies have confirmed that high total fibrinogen levels are associated with an increased risk of ischemic heart disease and stroke,[3-5] but it is still unclear whether fibrinogen is only an inflammatory marker of underlying atherosclerosis or whether it also directly contributes to the progression of cardiovascular disease (CVD) via effects on coagulation and/or atherogenesis.[6] One of the mechanisms by which fibrinogen can influence the pathogenesis of atherosclerosis is the structure of the fibrin clot.[7-8]

To determine whether fibrinogen is causally involved in stroke risk can be done using the approach of Mendelian randomization.[9] Briefly, when a variable is genetically determined and also associated with disease, an association between genetic variation and disease is expected. It has already been reported that the fibrin clot structure is partly determined by genetic factors,[10-11] that a number of single nucleotide polymorphisms (SNPs) in the FGG and FGA genes are associated with fibrinogen levels, functions and clot structure,[7, 12-15] which are variables that have been associated with CVD.[16-18] Therefore, studies on genetic variation provide an opportunity to further investigate the involvement of fibrinogen in stroke. Since variations in both the FGG and the FGA gene are reported to be associated with fibrin clot structure, we focused this research project on the variation in the FGG and FGA genes.

The aim of our study was to investigate the association between common genetic variations in the fibrinogen FGG and FGA genes and risk of stroke in the Rotterdam Study, a large, prospective, population-based cohort study.

Methods

Study population: The present study is part of the Rotterdam Study, a population-based cohort study on chronic and disabling diseases. The design of this study has been described in detail previously.[19] All inhabitants of Ommoord, a district of the city of Rotterdam in the Netherlands, aged 55 years and over were invited to participate. Participation rate for the study was 78% and a total of 7983 subjects participated in the study. The study was approved by the Medical Ethics Committee of Erasmus University Rotterdam and was performed in

accordance with the Declaration of Helsinki. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were obtained from 1990 through 1993 and consisted of a home interview and 2 visits to the research center for measurements and collection of blood.

Single nucleotide polymorphisms (SNPs) selection and genotyping: The SeattleSNPs program for Genomic Applications (http://pga.mbt.washington.edu/) has identified three haplotype-tagging SNPs in FGG and four SNPs in FGA, which tag the total common genetic variation in these genes in Caucasians. By genotyping these seven haplotype tagging SNPs, we were able to infer haplotypes and describe the common variation within the FGG and the FGA genes and also across the FGG and FGA genes. We performed haplotype analysis that covers both the FGG and FGA regions because high linkage disequilibrium between the two genes complicates assigning the risk to one of the genes. We genotyped the FGG 4288G>A (rs2066860), FGG 6326G>A (rs2066861), FGG 7792T>C (rs1049636), FGA -58G>A (rs2070011), FGA 1374G>A (rs2070014), FGA 1526T>C (rs2070016) and the FGA 4253A>G (rs6050) polymorphisms that tag haplotypes covering the total common variation in the FGG and FGA genes. Annotation of the SNPs is in accordance with the nomenclature recommendations of the Human Genome Variation Society using GenBank Accession Number AF350254 for FGG and AF361104 for FGA as reference with nucleotide +1 being the A of the ATG translation initiation codon.[20] These polymorphisms have also been described at http://www.ncbi.nlm.nih.gov/SNP.

DNA was isolated using standard salting-out procedures and genotyping was performed using baseline samples stored at -80º Celsius. Genotypes were determined in 2 ng genomic DNA with the 5' nuclease/Taqman allelic discrimination assay (Applied Biosystems, Foster City, California, USA). Primer and probe sequences were designed using the SNP assay-by-design service of Applied Biosystems and are available upon request. Polymerase chain reactions were performed with fluorescent allele-specific oligonucleotide probes on GeneAmp PCR System 9700 thermal cyclers with 384 wells format in 2 μL of reaction volume (Applied Biosystems), and fluorescence clustered endpoint reading for allelic discrimination was read on an ABI Prism 7900HT sequence detection system (Applied Biosystems).

Assessment of stroke: History of stroke at baseline was positive if a stroke was reported during the baseline interview and confirmed by medical records. After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Also nursing home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes with an experienced stroke neurologist to verify all diagnoses.

A stroke was subclassified as ischemic if a computed tomography (CT) or magnetic resonance imaging (MRI) scan, made within 4 weeks after the stroke occurred, ruled out other diagnoses, or if indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants treatment) pointed at an ischemic nature of the stroke. A stroke was subclassified as hemorrhagic if a relevant hemorrhage was shown on CT or MRI scan, or if the subject lost consciousness permanently or died within hours after onset of focal signs. If a stroke did not match any of these criteria, it was called unspecified. Follow-up was complete until January 1, 2005, for 98.7 % of potential person years.[21]

Population for analysis: Of all 7983 Rotterdam Study participants, 261 had had a stroke before baseline and 176 refused informed consent for retrieval of stroke follow-up data. In addition, no blood was available for 1217 participants and the SNP assays failed on all seven SNPs in 54 participants, leaving 6275 participants eligible for the present study.

Haplotype construction and data analysis: We assessed the associations between individual SNPs and risk of stroke, ischemic stroke, and hemorrhagic stroke with Cox proportional hazards models, adjusting for age and sex. Hardy-Weinberg equilibrium of each fibrinogen polymorphism was tested using Chi square analysis. When a minor homozygotes group contains <10 subjects (e.g. FGG4288), we combined the minor homozygotes with the heterozygotes. Haplotype alleles present in the population were inferred by means of the *haplo.em* function of the *Haplo Stats* suite implemented in the *R* package (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html),[22-24] which computes maximum likelihood estimates of haplotype probabilities, accounting for missing genotype data by including in the analysis the likelihood for all possible genotypes for the incomplete loci. Only haplotypes with frequencies of at least 5% were used in the analysis. The haplotypes were numbered in order of decreasing frequency in the population.

We calculated odds ratios (OR) for stroke in fibrinogen haplotypes relative to the risk of stroke in the most common fibrinogen haplotype using the *Haplo Stats* suite. The probability for each haplotype pair in each individual was assigned and then an individual's phenotype was directly modeled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. An estimate of the haplotype frequencies was based on the expectation-maximization (EM) algorithm. The association between fibrinogen haplotypes and risk of stroke was examined by means of the *haplo.glm* function of *Haplo Stats*.[23] This approach is based on the binary family of generalized linear models, and computes the regression of a trait on haplotypes and other covariates. In these analyses the most frequent haplotype was used as the reference category. We adjusted for age and sex.

Results

During on average 10.1 years of follow-up, 668 strokes occurred, of which 393 were classified as ischemic, 62 as hemorrhagic, and in 213 patients the stroke class was not specified. The median age at start of follow-up was 68 years and 59% of participants were female (table 1). The median age of the study population was 68 years and 59% of the participants were female. The genotypic distributions for all 7 haplotype-tagging SNPs were in Hardy-Weinberg equilibrium. 6.7% of subjects had 1 or 2 missing FGG or FGA SNPs and were included in the haplotype analysis, 1.8% of subjects were excluded because they had 3-6 missing SNPs. Analysis of the single SNPs in the FGG and FGA showed a slightly increased risk for ischemic stroke in carriers of the A-allele of the FGG4288, but no association was seen between the other variants and risk of stroke (Table 2).

Three common haplotypes were inferred in FGG. The most common FGG haplotype (H1), which was estimated to be present in 41% of participants, was G-G-T (table 3). Two other FGG haplotypes (H2: G-G-C and H3: G-A-T) were present in more than 5% of the participants. Inferring haplotypes resulted in five common haplotypes in FGA, of which the most common FGA haplotype (H1: G-G-T-A) was estimated to be present in 31% of participants. Four other FGA haplotypes (H2-H5) had a prevalence of at least 5%.

The risk of stroke was similar for the various FGG haplotypes: the OR for stroke for FGG-H2 and FGG-H3 were 0.96 (95% CI 0.83-1.11) and 1.09 (95% CI 0.94-1.26), respectively, compared with FGG-H1 (Table 4). Likewise, risk of stroke was similar for the FGA haplotypes. When we studied only ischemic stroke, the risks in all haplotypes were slightly increased when compared with FGG-H1 or FGA-H1 (Table 4). Since the linkage disequilibrium between the FGG SNPs and the FGA SNPs was very high (D' of 0.94), we also constructed extended haplotypes covering both the FGG and FGA genes. Haplotype

Table 1. Baseline characteristics of study population (n=6275).

Characteristic	Median (interquartile range) or percentage
Age, yrs	68 (62-75)
Female sex, %	59
Both parents Caucasian, %	98.5
Antihypertensive drug use, %	13
Systolic blood pressure, mmHg	137 (123-153)
C-reactive protein, mg/l	1.85 (0.89-3.60)
Diabetes mellitus, %	15
Ever smokers, %	65
Current smokers, %	23
Atrial fibrillation, %	5
Previous myocardial infarction, %	12

reconstruction resulted in five FGG+FGA haplotypes with a frequency >5%, and together these five haplotypes covered 93% of the total genetic variation of this region (Table 3). The total risk of stroke was similar in all FGG+FGA haplotypes. However, when we studied only ischemic stroke we observed for FGG+FGA-H3 an association with an OR of 1.31 (95% CI 1.05-1.63) compared with the reference haplotype FGG+FGA-H1 (table 4). When we studied only hemorrhagic stroke, we observed an opposite effect with an OR of 0.69 (95% CI 0.45-1.06) and the 95% confidence intervals for ischemic and hemorrhagic stroke in H3 did not overlap. Furthermore, a slightly increased risk estimate for ischemic stroke was seen for FGG+FGA-H2 (OR 1.18, 95% CI 0.96-1.43). To account for multiple testing, permutation p-values (1000 permutations) were computed. The p-value for the extended FGG+FGA haplotypes was 0.07 for the ischemic stroke group and larger for the other analyses.

Table 2. Association between individual SNPs in FGG and FGA and risk of stroke.

SNP	genotype	Frequency (strokes/N)	Stroke	Ischemic stroke	Hemorrhagic stroke
FGG4288	GG	594/5677	1 (ref)	1 (ref)	1 (ref)
	GA&AA	55/456	1.14 (0.87-1.50)	1.41 (1.01-1.96)	1.37 (0.59-3.18)
FGG6326	GG	338/3330	1 (ref)	1 (ref)	1 (ref)
	GA	266/2365	1.08 (0.92-1.27)	1.10 (0.89-1.36)	0.94 (0.57-1.56)
	AA	46/405	1.14 (0.84-1.55)	0.87 (0.55-1.36)	
FGG7792	TT	337/3045	1 (ref)	1 (ref)	1 (ref)
	TC	268/2595	0.95 (0.81-1.11)	1.19 (0.96-1.46)	0.60 (0.69-1.01)
	CC	49/506	0.88 (0.65-1.19)	1.00 (0.68-1.48)	
FGA-58	GG	228/2261	1 (ref)	1 (ref)	1 (ref)
	GA	327/2966	1.08 (0.91-1.28)	1.09 (0.88-1.36)	1.14 (0.68-1.94)
	AA	100/909	1.12 (0.88-1.41)	1.03 (0.76-1.41)	
FGA1374	GG	441/4184	1 (ref)	1 (ref)	1 (ref)
	GA	187/1734	1.00 (0.84-1.19)	1.20 (0.96-1.48)	0.71 (0.39-1.26)
	AA	15/184	0.84 (0.50-1.40)	0.98 (0.52-1.85)	
FGA1526	TT	503/4649	1 (ref)	1 (ref)	1 (ref)
	TC&CC	152/1469	0.96 (0.81-1.15)	0.95 (0.75-1.20)	1.39 (0.81-2.39)
FGA4253	AA	312/3156	1 (ref)	1 (ref)	1 (ref)
	AG	275/2422	1.14 (0.97-1.34)	1.20 (0.97-1.48)	0.75 (0.51-1.44)
	GG	53/455	1.21 (0.90-1.62)	0.92 (0.60-1.41)	

Presented are hazard ratios (Cox regression), adjusted for age and sex.

Table 3. Frequency of fibrinogen FGG and FGA haplotypes separate and in combination with each other. Only haplotypes with frequency > 5% were studied.

Fibrinogen chain	Haplotype	4288G>A rs2066860	6326G>A rs2066861	7792T>C Rs1049636	-58G>A rs2070011	1374G>A rs2070014	1526T>C rs2070016	4253A>G rs6050	Frequency in study
FGG	H1	G	G	T					40.8 %
	H2	G	G	**C**					29.4 %
	H3	G	**A**	T					26.1 %
FGA	H1				G	G	T	A	30.5 %
	H2				**A**	G	T	**G**	27.1 %
	H3				G	**A**	T	A	17.3 %
	H4				G	G	**C**	A	13.0 %
	H5				**A**	G	T	A	11.8 %
FGG + FGA	H1	G	G	T	G	G	T	A	26.4 %
	H2	G	**A**	T	**A**	G	T	**G**	25.6 %
	H3	G	G	**C**	G	**A**	T	A	16.8 %
	H4	G	G	T	G	G	**C**	A	12.2 %
	H5	G	G	**C**	**A**	G	T	A	11.5%

Table 4. Association between fibrinogen haplotypes and risk of any, ischemic and hemorrhagic stroke. N=6275; 668 strokes, 393 ischemic and 62 hemorrhagic strokes were observed.

FGG Haplotype	Stroke	Ischemic stroke	Hemorrhagic stroke
H1 (GGT)	1 (ref)	1 (ref)	1 (ref)
H2 (GG**C**)	0.96 (0.83-1.11)	1.15 (0.96-1.38)	0.71 (0.45-1.12)
H3 (G**A**T)	1.09 (0.94-1.26)	1.11 (0.92-1.34)	0.87 (0.56-1.36)
FGA Haplotype			
H1 (GGTA)	1 (ref)	1 (ref)	1 (ref)
H2 (**A**GT**G**)	1.09 (0.94-1.27)	1.10 (0.91-1.34)	0.92 (0.59-1.44)
H3 (G**A**TA)	0.97 (0.81-1.15)	1.19 (0.96-1.48)	0.65 (0.36-1.16)
H4 (GG**C**A)	0.97 (0.79-1.18)	1.04 (0.81-1.34)	1.13 (0.66-1.92)
H5 (**A**GTA)	0.95 (0.77-1.16)	1.07 (0.82-1.38)	0.98 (0.55-1.76)
FGG + FGA Haplotype			
H1 (GGT-GGTA)	1 (ref)	1 (ref)	1 (ref)
H2 (G**A**T-**A**GT**G**)	1.10 (0.94-1.30)	1.18 (0.96-1.43)	0.97 (0.67-1.40)
H3 (GG**C**-G**A**TA)	0.99 (0.82-1.19)	1.31 (1.05-1.63)	0.69 (0.45-1.06)
H4 (GGT-GG**C**A)	0.99 (0.81-1.22)	1.14 (0.89-1.47)	1.23 (0.81-1.89)
H5 (GG**C**-**A**GTA)	0.94 (0.76-1.16)	1.13 (0.87-1.46)	0.88 (0.60-1.31)

Discussion

In this population-based cohort study, we observed that the risk of ischemic stroke was associated with FGG+FGA-Haplotype 3 and that this relationship was opposite for ischemic and hemorrhagic stroke. This finding strengthens the hypothesis that fibrin structure may be a causal factor in the development of stroke.

Strengths of our study are the prospective inclusion of the stroke cases and the meticulous and nearly complete follow-up (loss of potential person-years 1.3 %). To our knowledge, this is the first study that assessed the association between FGG and FGA haplotypes and risk of stroke in the general population. Our stringent stroke monitoring procedures allowed us to include also stroke patients who had not been referred to a neurologist. In our study population, 31% of all stroke cases had not been referred. As neuroimaging had not been performed in these non-referred cases, we could subclassify only 16% of them into ischemic or hemorrhagic. In contrast, 92% of strokes that had been diagnosed by a neurologist could be subclassified. In total, 32% of all stroke cases could not be subclassfied.

In this study, we focused on the FGA and FGG genes, since these genes determine the fibrin clot structure, which is suggested to directly affect atherosclerosis and thrombotic disease.[7,25] Although the FGG and FGA gene encode for different peptides, we decided to also perform a combined haplotype analysis because both the alpha and gamma chains are part of one single protein, fibrinogen, and their effects on fibrin structure cannot be separated.

In addition, variants in both genes are reported to be associated with fibrin clot structure and functionality has been proposed for variants in both genes.[17,18,26] Because there is high linkage disequilibrium between the two genes the effects cannot be assigned to one of the genes. In our study the strongest association with stroke risk was seen when we combined variants in both genes.

The FBG promoter SNPs have been much studied previously, since increased plasma fibrinogen levels have been identified as a consistent risk indicator for cardiovascular disease and stroke[3-5] and the fibrinogen beta chain synthesis is rate limiting step in the production of mature fibrinogen in vitro.[27] Indeed, several studies reported that FGB promoter SNPs contributed to the regulation of plasma fibrinogen concentration,[28] but no clear and consistent association was found between these SNPs and stroke.[29-31] We did not include the FGB gene in this study because it has not been shown to be associated with fibrin structure and because it is generally considered not to be associated with thrombosis risk.[29] In the Rotterdam study, the FGB promoter SNP rs1800787 (-148C/T) was also not associated with risk of stroke (unpublished data).

We did not observe associations between individual SNPs in the FGG and FGA genes with ischemic or hemorrhagic stroke, although participants with variant SNPs were at slightly higher risk of ischemic stroke than participants with wild type SNPs. These SNPs have not been studied previously in relationship with stroke. In studies with other vascular endpoints no significant association was seen between FGG-H3 and myocardial infarction[16, 32] but FGG-H3 (named FGG-H2 in that study) was associated with increased risk of venous thrombosis.[26] Moreover, FGG-H2 was associated with the risk of myocardial infarction in one study but not in another[16, 32] and not associated with risk of deep vein thrombosis (FGG-H2 was named SNP2 or FGG-H3 in these studies).[26] All studies used the haplotype with common alleles for all SNPs as reference group. Also reported were associations of single SNPs in the FGA gene (-58G>A (rs2070011) and 4253A>G (rs6050)) or FGG gene (7792T>C (rs1049636)) that are associated with myocardial infarction, plasma fibrinogen concentration or fibrin clot characteristics,[7, 15-18] but these SNPs were not associated with stroke in our study. As reported previously, in the Rotterdam Study FGG and FGA haplotypes were not related with coronary events, nor with coronary and extracoronary atherosclerosis.[37] The differences of the observed associations between genetic variation in FGG and FGA with stroke, myocardial infarction and deep vein thrombosis may be explained by differences in etiology of these endpoints, medication and methods for statistical analysis.

With the extended haplotypes composed of SNPs in the fibrinogen FGG and FGA genes, we observed an association between FGG+FGA-H3 and ischemic stroke. In only one previous study, Mannila and colleagues combined SNPs in the FGG and FGA genes and they observed a decreased risk of myocardial infarction for a haplotype combining FGG 7792T>C (rs1049636) and FGA -58G>A (rs2070011).[16] This haplotype would cover our FGG+FGA-H5, and contains the rare alleles of these two SNPs, but we did not see an association of this

haplotype with stroke.

We were able to separate ischemic and hemorrhagic stroke in the majority of our stroke cases. Although the group of patients with a hemorrhagic stroke was small with only 62 cases, it was very interesting to observe that the associations between FGG+FGA-H3 with ischemic and hemorrhagic stroke were opposite. This is what one would expect if thrombosis and bleeding were important mechanisms in the two types of stroke. However, the results need to be considered with caution and this observation needs to be replicated in independent populations.

In our study, the individual SNPs were not associated with risk of ischemic stroke, but we observed associations with ischemic stroke when we combined the SNPs and constructed haplotypes. This illustrates that haplotype analysis is a more powerful analysis when it is uncertain if functional variants are being studied or in the presence of multiple susceptibility alleles, in the pattern or magnitude of their allelic associations.[33]

It has recently been reported that the 8486C>T SNP, tagged in haplotype FGG+FGA-H2, affects a cleavage stimulatory factor binding site near the gamma-A specific polyadenylation site and would therefore result in a relative decrease of the formation of fibrinogen gamma' specific mRNA, thus altering the proportion of the gamma' chains.[34] This would ultimately result in a changed fibrin clot formation since gamma' fibrinogen has an antithrombin effects because it binds thrombin, misses the platelet binding site and can bind factor XIII.[35] It has also been shown that this SNP is associated with elevated risk of deep venous thrombosis by reducing plasma fibrinogen gamma' levels.[26] However, we did not see an association between this SNP and risk of stroke in this study, and also not in another case-control study in ischemic stroke.[36]

In conclusion, we found a higher risk of ischemic stroke in subjects with haplotype H3 compared to H1 in the extended FGA-FGG haplotype but not in individual SNP analysis in this population-based cohort study. An opposite association may exist for hemorrhagic stroke. Our data illustrate that haplotype analysis may reveal associations with risk of stroke that may be missed by single SNP analysis.

References

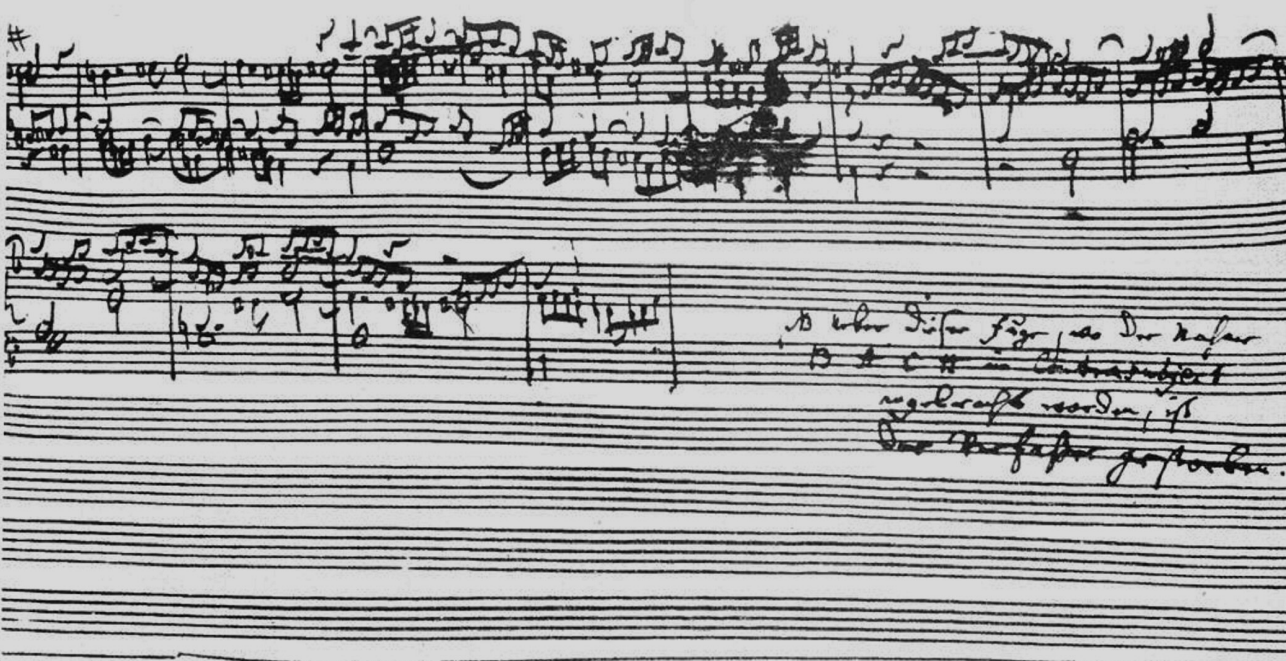
1. Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. Ann N Y Acad Sci. 2001;936:11-30.
2. Kant JA, Fornace AJJ, et al. Evolution and organization of the fibrinogen locus on chromosome 4: gene duplication accompanied by transposition and inversion. Proc Natl Acad Sci U S A. 1985 Apr; 82(8):2344-8.
3. Wilhelmsen L, Svardsudd K, et al. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med. 1984 Aug 23;311(8):501-5.
4. Maresca G, Di Blasio A, et al. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol. 1999 Jun;19(6):1368-77.
5. Danesh J, Lewington S, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. Jama. 2005 Oct 12;294(14):1799-809.

6. Koenig W. Fibrin(ogen) in cardiovascular disease: an update. Thromb Haemost. 2003 Apr;89(4):601-9.
7. Cooper AV, Standeven KF, Ariens RA. Fibrinogen gamma-chain splice variant gamma' alters fibrin formation and structure. Blood. 2003 Jul 15;102(2):535-40.
8. Fatah K, Hamsten A, et al. Fibrin gel network characteristics and coronary heart disease: Relations to plasma fibrinogen concentration, acute phaes protein, serum lipoproteins and coronary atherosclerosis. Thrombosis and Haemostasis. 1992 1992;68(2):130-5.
9. Davey Smith G, et al. Fibrinogen, C-reactive protein and coronary heart disease: does Mendelian randomization suggest the associations are non-causal? QJM. 2004 Mar;97(3):163-6.
10. Dunn EJ, Ariens RA, et al. Genetics of fibrin clot structure: a twin study. Blood. 2004 Mar 1;103(5):1735-40.
11. Mills JD, Ariens RA, et al. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. Circulation. 2002 Oct 8;106(15):1938-42.
12. Fowkes FG, Connor JM, et al. Fibrinogen genotype and risk of peripheral atherosclerosis. Lancet. 1992 Mar 21;339(8795):693-6.
13. Green FR. Fibrinogen polymorphisms and atherothrombotic disease. Ann N Y Acad Sci. 2001;936:549-59.
14. Lim BC, Ariens RA, et al. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. Lancet. 2003 Apr 26;361(9367):1424-31.
15. Mannila MN, Eriksson P, et al. Epistatic and pleiotropic effects of polymorphisms in the fibrinogen and coagulation factor XIII genes on plasma fibrinogen concentration, fibrin gel structure and risk of myocardial infarction. Thromb Haemost. 2006 Mar;95(3):420-7.
16. Mannila MN, Eriksson P, et al. Contribution of haplotypes across the fibrinogen gene cluster to variation in risk of myocardial infarction. Thromb Haemost. 2005 Mar;93(3):570-7.
17. Carter AM, Catto AJ, Grant PJ. Association of the alpha-fibrinogen Thr312Ala polymorphism with poststroke mortality in subjects with atrial fibrillation. Circulation. 1999 May 11;99(18):2423-6.
18. Carter AM, Catto AJ, et al. Alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism. Blood. 2000 Aug 1;96(3):1177-9.
19. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991 Jul;7(4):403-22.
20. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000;15(1):7-12.
21. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002 Apr 13;359(9314):1309-10.
22. Epstein MP, Satten GA. Inference on haplotype effects in case-control studies using unphased genotype data. Am J Hum Genet. 2003 Dec;73(6):1316-29.
23. Lake SL, Lyon H, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered. 2003;55(1):56-65.
24. Schaid DJ, Rowland CM, et al. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet. 2002 Feb;70(2):425-34.
25. Ajjan RA, Grant PJ. Role of clotting factors and fibrin structure in predisposition to atherothrombotic disease. Expert Rev Cardiovasc Ther. 2005 Nov;3(6):1047-59.
26. Uitte de Willige S, de Visser MC, et al. Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen gamma' levels. Blood. 2005 Dec 15;106(13):4176-83.
27. Yu S, Sher B, et al. Fibrinogen precursors. Order of assembly of fibrinogen chains. J Biol Chem. 1984 Aug 25;259(16):10574-81.
28. van 't Hooft FM, von Bahr SJ, et al. Two common, functional polymorphisms in the promoter region of the beta-fibrinogen gene contribute to regulation of plasma fibrinogen concentration. Arterioscler Thromb Vasc Biol. 1999 Dec;19(12):3063-70.
29. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood. 2000 2000;95(5):1517-32.
30. Martiskainen M, Pohjasvaara T, et al. Fibrinogen gene promoter -455 A allele as a risk factor for lacunar stroke. Stroke. 2003 Apr;34(4):886-91.
31. Kessler C, Spitzer C, et al. The apolipoprotein E and beta-fibrinogen G/A-455 gene polymorphisms are associated with ischemic stroke involving large-vessel disease. Arterioscler Thromb Vasc Biol. 1997 Nov;17(11):2880-4.
32. Uitte de Willige S, Doggen CJ, et al. Haplotypes of the fibrinogen gamma gene do not affect the risk of myocardial infarction. J Thromb Haemost. 2006 Feb;4(2):474-6.

33. Akey J, Jin L, Xiong M. Haplotypes vs single marker linkage disequilibrium tests: what do we gain? Eur J Hum Genet. 2001 Apr;9(4):291-300.
34. Uitte de Willige S, Rietveld IM, et al. Polymorphism 10034C>T Is Located in a Functional Cleav age Stimulation Factor (CstF) Binding Site of the Fibrinogen Gamma Gene and Influences the {gamma}'/{gamma}A mRNA Ratio. 2006:543.
35. Mosesson MW. Fibrinogen gamma chain functions. J Thromb Haemost. 2003 Feb;1(2):231-8.
36. Cheung E, Uitte de Willige S, et al. Fibrinogen in ischemic stroke: a case-control study. Stroke. 2007, In Press.
37. Kardys I, Uitterlinden AG, et al. Fibrinogen gene haplotypes in relation to risk of coronary events and coronary and extracoronary atherosclerosis: the Rotterdam Study. Thromb Haemost 2007 Feb; 97(2):288-95

Chapter 4.

Stroke risk factors: impact and prediction



4.1.
Impact of potentially modifiable etiologic factors on the burden of stroke

Background and purpose: *The population attributable risk (PAR) of an etiologic factor is the proportion of strokes for which the factor plays an indispensable role in the pathophysiological pathway. This is the proportion of strokes that would not have occurred if the etiologic factor had not been present in the population, or the impact of the factor on the occurrence of stroke. We assessed the impact of potentially modifiable etiologic factors, alone and in combination, on the occurrence of stroke in a large population-based cohort study.* ***Methods:*** *The study was based on 6844 participants of the Rotterdam Study who at baseline (1990-1993) were aged 55 years or over and free from stroke. We computed PARs and 95% confidence intervals (CIs) using the Interactive Risk Attributable Program (IRAP).* ***Results:*** *During 69,809 person years of follow-up, 742 strokes occurred. The age and sex adjusted combined PAR of the probable etiologic factors (pre)hypertension, smoking, diabetes mellitus and atrial fibrillation was 0.54 (95% CI 0.43-0.66). This number can probably be increased to about 0.68 if various possible etiologic factors (coronary disease, HDL cholesterol, obesity, silent myocardial infarction, C-reactive protein, alcohol intake, fruit and vegetable intake, carotid intima-madia thickness, and serum uric acid level) prove to be causal and modifiable. The proportion of strokes explained by established causal factors was higher in men than in women and comparable for ischemic and hemorrhagic stroke.* ***Conclusions:*** *The proportion of strokes attributable to currently known modifiable etiologic factors is considerable but may be increased by identifying new factors.*

Introduction

In the 1960s it was discovered that treating hypertension could reduce the incidence of stroke among persons with severe diastolic hypertension by more than 90 percent.[1] The finding that such a simple intervention can prevent such a devastating disease inspired many researchers to search for other potentially modifiable etiologic factors for stroke, a quest that has been ongoing up to the present day.

This search indeed led to the identification of many other risk factors for stroke, some of which are presumed not to play a role in the causal pathway (risk indicators), and some of which are presumed to be causal (etiologic factors).[2] The impact of an etiologic factor on the occurrence of stroke is determined by the proportion of strokes for which the etiologic factor played an indispensable role in the pathophysiological pathway. This proportion is called the population attributable risk (PAR).[3] Because a PAR shows in what proportion of strokes the etiologic factor played an indispensable role, it also shows the maximum proportion of strokes that may be prevented by complete elimination of the etiologic factor, which is of course only relevant for etiologic factors that could potentially be modified by medical intervention.

The question emerges which proportion of strokes is caused by currently known potentially modifiable etiologic factors: this tells us which proportion of strokes can theoretically be prevented by adequate treatment of etiologic factors. It also shows how closely we have approached our ultimate goal of finding a modifiable causal factor for every occurring stroke. PARs have been estimated for various etiologic factors in the past.[4] However, a commonly ignored fact is that, because strokes typically occur when a combination of etiologic factors is present, the PARs for the various etiologic factors cannot simply be summed up, not even when they are fully adjusted for confounding and interaction. Hence, to assess the impact of combinations of etiologic factors on stroke, special statistical techniques have to be applied.[5, 6] To our knowledge, no studies have investigated PARs of combinations of etiologic factors before with adequate methods.

We assessed the impact of potentially modifiable etiologic factors on the occurrence of stroke in a large population-based cohort study among persons aged 55 years and over, distinguishing between probable and possible etiologic factors.

Methods

Source population: The Rotterdam Study is a population-based cohort study on chronic and disabling diseases.[8] All inhabitants of Ommoord, a district in the city of Rotterdam in the Netherlands, aged 55 years and over were invited to participate. Participation rate of those invited for the study was 78%; in total, 7983 subjects participated in the first study survey

(1990-1993). The Medical Ethics Committee of Erasmus University Rotterdam approved of the study. Written informed consent to retrieve information from treating physicians was obtained from all participants.

Assessment of Stroke: History of stroke at baseline was positive if a stroke was reported during the baseline interview and confirmed by medical records. After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Also nursing home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes with an experienced stroke neurologist (P.J.K.) to verify all diagnoses.[9, 10] Subarachnoid hemorrhages were excluded. A stroke was subclassified as ischemic if a CT or MRI scan, made within 4 weeks after the stroke occurred, confirmed the diagnosis, or if indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature of the stroke. A stroke was subclassified as hemorrhagic if a relevant hemorrhage was shown on CT or MRI scan. If we could not retrieve enough information to subclassify a stroke as ischemic or hemorrhagic, it was called unspecified. Follow-up was complete until January 1, 2005, for 98.7 % of potential person years.[11]

Baseline measurements: Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these two measurements in the analyses, which was classified as prehypertension (systolic > 120 mmHg or diastolic > 80 mmHg), stage I hypertension (systolic > 140 mmHg or diastolic > 90 mmHg), or stage II hypertension (systolic > 160 mmHg or diastolic > 100 mmHg). Use of antihypertensive medication was assessed during a home interview. Treated hypertension was considered controlled if the blood pressure measurement did not fulfill criteria for stage I or II hypertension. Smoking habits were assessed during the home interview and classified as former and current. The number of pack years of smoking was calculated by multiplying the number of cigarette packs smoked per day by the number of years smoked. The threshold between light and heavy smoking was defined as the median number of pack years. History of angina pectoris was assessed with the Rose questionnaire.[12] History of percutaneous transluminal angioplasty (PTCA) or coronary artery bypass graft (CABG) and history of myocardial infarction were positive if reported by the participant and confirmed by ECG or medical records. Atrial fibrillation was considered present when seen on ECG during the center visit or when it was reported in medical records. We defined diabetes mellitus as a random or post-load glucose level of 11.1 mmol/l or higher, or use of antidiabetic medication. Left ventricular hypertrophy was assessed with a 12-lead resting ECG and the Modular ECG Analysis System (MEANS)[13]

implemented on an ACTA electrocardiograph (ESAOTE, Florence, Italy). Total cholesterol, high-density lipoprotein, uric acid, and C-reactive protein were measured in non-fasting baseline serum with automated enzymatic procedures.[9] Medication was assessed during a home interview. Carotid intima-media thickness (IMT) was measured by longitudinal 2-dimensional ultrasound of the carotid artery.[14] Alcohol intake and fruit and vegetable consumption were assessed by means of a food frequency questionnaire in nondemented participants.[15] Excessive alcohol intake was defined as more than 3 units (32 grams) of alcohol a day. One serving of fruit was 80 grams, one serving of vegetables was 77 grams.

Population for Analysis: Of all 7983 participants who were enrolled into the Rotterdam study, 7717 were free from stroke at study baseline. After exclusion of participants who refused informed consent for retrieval of stroke follow-up data (N=171) and of those who had incomplete data assessment since they had no baseline research center visit (N=702), 6844 could be included in the present analyses. C-reactive protein, alcohol intake, fruit and vegetable deficiency, carotid IMT, and uric acid were only assessed in random subgroups of the cohort; alcohol intake and fruit and vegetable consumption were not assessed in participants with dementia at baseline. Therefore, these factors were studied in combination in a subgroup of 3265 participants who had complete assessment of all covariates.

Statistical Analysis: We calculated hazard ratios (HRs) and PARs with 95% confidence intervals (CIs) using the cohort analysis function of the Interactive Risk Attributable Program (IRAP v2.2), which is provided by US National Institutes of Health, National Cancer Institute at http://dceg.cancer.gov/tools/analysis/irap. This package bases its survival estimates on a Poisson model and enables the calculation of PARs of combinations of presumed etiologic factors. We divided potential etiologic factors into probable etiologic factors (well-documented risk factors that can be treated and treatment results in a decreased stroke risk) and possible etiologic factors (factors of which the etiologic role in stroke is not entirely clear or which cannot easily be modified by medical intervention). We categorized the etiologic factors in as many categories as possible since this is presumed to result in more accurate PARs.[16] We tested for statistical interaction by examining the statistical significance of interaction terms with Cox regression models at an alpha level of 0.10 (SPSS 11.0, 2001, Chicago).

Results

The median age at start of follow-up was 68 years (interquartile range 62-76 years) and 60.1% of the participants were women (table 1). The participants in the subgroup with assessment of additional characteristics were slightly younger and more often female than the complete study cohort and differed slightly on several other characteristics.

During 69,809 person years of follow-up, 742 strokes occurred, of which 432 could be classified as ischemic, 73 as hemorrhagic, and 237 remained unspecified.

The most important etiologic factor for any stroke was hypertension, with a PAR of 0.40 (95% CI, 0.26-0.54; table 2). The second cause of stroke was smoking with a PAR of 0.17 (95% CI 0.08-0.26). Also diabetes mellitus (PAR 0.07, 95% CI 0.03-0.10) and atrial fibrillation (PAR 0.03, 95% CI 0.01-0.05) contributed significantly to the burden of stroke. The total proportion of strokes that was presumably caused by any combination of these four etiologic factors was 54%. Of the possible etiologic factors, coronary disease, low HDL cholesterol, obesity and silent myocardial infarction seemed to have a small and, with the exception of silent myocardial infarction, not statistically significant contribution to the burden of

Table 1. Baseline characteristics

Characteristic	Median (interquartile range) or percentage		P difference*
	Complete study cohort (N=6844)	Subgroup with additional risk factors (N=3265)	
Age, yrs	68.2 (62.0-75.6)	67.8 (62.2-74.0)	0.01
Female sex	60.1	60.5	0.01
Systolic blood pressure, mmHg	138 (123-153)	137 (123-152)	0.78
Diastolic blood pressure, mmHg	73 (66-81)	73 (66-81)	0.10
Antihypertensive medication, %	12.8	12.3	0.74
Current smoking, %	22.2	24.4	<0.001
Former smoking, %	40.1	42.1	0.004
Diabetes mellitus, %	10.3	9.3	0.002
Atrial fibrillation, %	5.0	3.9	0.04
Angina pectoris, %	3.6	4.0	0.11
PTCA/CABG, %	2.7	2.8	0.71
Symptomatic MI , %	6.1	6.9	0.005
Silent MI, %	5.2	5.1	0.66
LVH, %	4.5	3.5	0.03
Non-HDL cholesterol, mmol/l	5.2 (4.4-6.0)	5.2 (4.5-6.1)	0.005
HDL cholesterol, mmol/l	1.3 (1.1-1.6)	1.3 (1.1-1.6)	0.21
Body mass index, kg/m²	26.0 (23.8-28.4)	26.0 (23.9-28.5)	0.10
Serum C-reactive protein, mg/l	-	1.74 (0.87-3.35)	-
Alcohol intake, servings	-	0.29 (0.01-1.40)	-
Fruit and vegetable intake, servings	-	6.6 (5.1-8.2)	-
Carotid IMT, mm	-	0.77 (0.68-0.87)	-
Serum uric acid, µmol/l	-	309 (263-368)	-

*Difference between participants included and not included in the subgroup with additional risk factors. Logistic regression model adjusted for all other characteristics.

Table 2. Population attributable risks of presumed etiologic factors for any stroke (n/N=742/6844).

Probable etiologic factors	Classification	Prevalence (%)	HR (95% CI)*	PAR (95% CI)* per classification	PAR (95% CI)* combined
Hypertension	prehypertension	29.9	1.35 (1.02-1.79)	0.06 (0.01-0.11)	
	stage I	25.0	1.86 (1.41-2.44)	0.13 (0.08-0.19)	
	stage II	14.0	2.20 (1.65-2.95)	0.11 (0.07-0.15)	} 0.40 (0.26-0.54)
	treated controlled	5.5	1.92 (1.32-2.81)	0.03 (0.01-0.05)	
	treated uncontr.	7.3	2.18 (1.57-3.03)	0.06 (0.03-0.09)	
Smoking	former light	18.2	1.21 (0.97-1.51)	0.03 (0.00-0.06)	
	former heavy	22.6	1.27 (1.01-1.60)	0.05 (0.00-0.10)	} 0.17 (0.08-0.26)
	current light	9.9	1.64 (1.26-2.14)	0.04 (0.02-0.07)	
	current heavy	12.4	1.90 (1.30-2.79)	0.05 (0.02-0.08)	
Diabetes mellitus	present	10.3	1.64 (1.35-1.99)		0.07 (0.03-0.10)
Atrial fibrillation	present	5.0	1.51 (1.15-1.98)		0.03 (0.01-0.05)
Total					**0.54 (0.43-0.66)**
Possible etiologic factors					
Coronary disease	AP	2.5	1.27 (0.87-1.86)	0.01 (0.00-0.02)	
	PTCA/CABG	1.4	1.13 (0.62-2.07)	0.00 (0.00-0.01)	} 0.02 (0.00-0.05)
	MI	6.1	1.20 (0.89-1.60)	0.01 (0.00-0.03)	
LVH	present	4.5	0.98 (0.70-1.36)	0.00 (0.00-0.03)	0.00 (0.00-0.02)
Non-HDL chol.	quartile 2	23.7	0.91 (0.94 1.11)	0.00 (0.00-0.03)	
	quartile 3	27.7	0.95 (0.78-1.16)	0.00 (0.00-0.04)	} 0.00 (0.00-0.03)
	quartile 4	24.9	0.79 (0.64-0.98)	0.00 (0.00-0.01)	
	treated	2.3	0.69 (0.39-0.21)	0.00 (0.00-0.00)	
HDL cholesterol	quartile 3	23.3	1.03 (0.84-1.26)	0.01 (0.00-0.06)	
	quartile 2	24.4	0.85 (0.69-1.06)	0.00 (0.00-0.02)	} 0.06 (0.00-0.15)
	quartile 1	23.9	1.22 (1.00-1.50)	0.05 (0.00-0.10)	
Obesity	BMI 25-30	45.4	1.03 (0.47-1.21)	0.01 (0.00-0.09)	} 0.03 (0.00-0.13)
	BMI>30	16.6	1.11 (0.89-1.37)	0.02 (0.00-0.06)	
Silent MI	present	5.2	1.58 (1.22-2.05)		0.03 (0.01-0.05)
Grand total					**0.57 (0.44-0.69)**

* All analyses are adjusted for age, sex, hypertension, smoking, atrial fibrillation, and diabetes mellitus, if appropriate. All HRs are relative to those in whom the etiologic factor is not present.

stroke. Inclusion of these factors raised the total PAR slightly to 0.57 (95% CI, 0.44-0.69).

The PAR of hypertension was larger in men than in women (0.48, 95% CI 0.29-0.67 versus 0.32, 95% CI 0.13-0.51; tables 3a and 3b), although not statistically significantly at α=0.05. This was not because of differences in the prevalence of hypertension, but because the association between hypertension and stroke is stronger in men than in women. The PARs of smoking, diabetes mellitus and atrial fibrillation were similar for men and women.

Of the possible etiologic factors, HDL cholesterol, obesity, and silent myocardial infarction had PARs ranging from 0.06 to 0.09 in men (although only statistically significantly different (α=0.05) from the null value for silent myocardial infarction), whereas they played no role in women. The total proportion of strokes that was presumably caused by any combination of the probable etiologic factors hypertension, smoking, atrial fibrillation and diabetes mellitus was higher in men (0.59; 95% CI 0.36-0.82) than in women (0.48; 95% CI 0.32-0.64). Inclu-

Table 3a. Population attributable risks of presumed etiologic factors for any stroke: men (n/N=297/2732).

Probable etiologic factors	Classification	Prevalence (%)	HR (95% CI)*	PAR (95% CI)* per classification	PAR (95% CI)* combined
Hypertension	prehypertension	31.4	1.80 (1.16-2.80)	0.13 (0.04-0.22)	
	stage I	24.4	2.20 (1.42-3.42)	0.16 (0.08-0.24)	
	stage II	12.3	2.75 (1.72-4.41)	0.12 (0.06-0.17)	}0.48 (0.29-0.67)
	treated controlled	6.1	1.85 (0.98-3.47)	0.02 (0.00-0.05)	
	treated uncontr.	7.3	2.32 (1.34-4.02)	0.05 (0.01-0.09)	
Smoking	former light	19.7	1.08 (0.67-1.76)	0.01 (0.00-0.10)	
	former heavy	41.6	1.12 (0.72-1.75)	0.05 (0.00-0.22)	}0.15 (0.00-0.48)
	current light	9.8	1.25 (0.73-2.16)	0.02 (0.00-0.07)	
	current heavy	19.4	1.48 (0.92-2.38)	0.07 (0.00-0.14)	
Diabetes mellitus	present	10.1	1.59 (1.15-2.21)		0.06 (0.01-0.10)
Atrial fibrillation	present	5.6	1.17 (0.74-1.86)		0.01 (0.00-0.04)
Total					**0.59 (0.36-0.82)**
Possible etiologic factors					
Coronary disease	AP	2.0	1.44 (0.73-2.84)	0.01 (0.00-0.03)	
	PTCA/CABG	2.2	1.16 (0.54-2.48)	0.00 (0.00-0.02)	}0.04 (0.00-0.09)
	MI	10.7	1.25 (0.87-1.80)	0.03 (0.00-0.07)	
LVH	present	4.8	0.92 (0.54-1.59)		0.00 (0.00-0.02)
Non-HDL chol.	quartile 2	24.1	0.89 (0.65-1.22)	0.00 (0.00-0.05)	
	quartile 3	24.0	0.92 (0.67-1.26)	0.00 (0.00-0.06)	}0.00 (0.00-0.09)
	quartile 4	23.8	0.80 (0.57-1.11)	0.00 (0.00-0.03)	
	treated	2.6	0.61 (0.25-1.53)	0.00 (0.00-0.01)	
HDL cholesterol	quartile 3	21.9	1.16 (0.74-1.61)	0.03 (0.00-0.11)	
	quartile 2	30.3	0.90 (0.65-1.26)	0.00 (0.00-0.05)	}0.09 (0.00-0.27)
	quartile 1	23.3	1.49 (1.08-2.05)	0.09 (0.02-0.16)	
Obesity	BMI 25-30	50.0	1.15 (0.89-1.47)	0.07 (0.00-0.20)	}0.07 (0.00-0.22)
	BMI>30	8.3	1.06 (0.69-1.65)	0.01 (0.00-0.04)	
Silent MI	present	5.7	2.21 (1.55-3.17)		0.06 (0.03-0.10)
Grand total					**0.67 (0.48-0.87)**

* All analyses are adjusted for age, sex, hypertension, smoking, atrial fibrillation, and diabetes mellitus, if appropriate.

sion of the possible etiologic factors raised the PAR to 0.67 (95% CI 0.47-0.87) for men and did not change the PAR in women.

When we restricted the analyses to ischemic strokes, the PARs did not materially change for men (table 4); the largest observed increase was that of obesity which increased to 0.17 (95% CI 0.02-0.34). For women, the PAR of hypertension decreased to 0.22 (95% CI 0.00-0.49). The PARs of non-HDL cholesterol, HDL cholesterol, and obesity increased

Table 3b. Population attributable risks of presumed etiologic factors for any stroke: women. (n/N=445/4112).

Probable etiologic factors	Classification	Prevalence (%)	HR (95% CI)*	PAR (95% CI)* per classification	PAR (95% CI)* combined
Hypertension	prehypertension	28.9	1.06 (0.74-1.53)	0.01 (0.00-0.08)	
	stage I	25.3	1.58 (1.12-2.25)	0.11 (0.03-0.18)	
	stage II	15.2	1.88 (1.30-2.70)	0.10 (0.05-0.16)	}0.32 (0.13-0.51)
	treated controlled	5.0	1.99 (1.25-3.16)	0.03 (0.01-0.06)	
	treated uncontr.	7.3	2.02 (1.35-3.03)	0.06 (0.03-0.10)	
Smoking	former light	17.2	1.26 (0.96-1.64)	0.03 (0.00-0.06)	
	former heavy	10.0	1.33 (0.96-1.85)	0.05 (0.02-0.08)	}0.14 (0.06-0.21)
	current light	9.9	1.82 (1.34-2.49)	0.05 (0.02-0.08)	
	current heavy	7.7	1.70 (1.14-2.53)	0.03 (0.00-0.05)	
Diabetes mellitus	present	10.4	1.63 (1.28-2.09)		0.07 (0.03-0.11)
Atrial fibrillation	present	4.5	1.88 (1.35-2.63)		0.04 (0.01-0.07)
Total					**0.48 (0.32-0.64)**
Possible etiologic factors					
Coronary disease	AP	2.8	1.13 (0.72-1.79)	0.01 (0.00-0.03)	
	PTCA/CABG	0.9	0.87 (0.32-2.33)	0.00 (0.00-0.01)	}0.01 (0.00-0.04)
	MI	3.1	1.04 (0.63-1.71)	0.00 (0.00-0.02)	
LVH	present	4.3	1.04 (0.69-1.57)		0.00 (0.00-0.03)
Non-HDL chol.	quartile 2	22.8	0.90 (0.69-1.17)	0.00 (0.00-0.04)	
	quartile 3	24.7	0.97 (0.75-1.25)	0.00 (0.00-0.06)	}0.00 (0.00-0.06)
	quartile 4	24.0	0.77 (0.59-1.02)	0.00 (0.00-0.00)	
	treated	2.2	0.76 (0.37-1.56)	0.00 (0.00-0.01)	
HDL cholesterol	quartile 3	21.9	0.95 (0.73-1.23)	0.00 (0.00-0.06)	
	quartile 2	30.3	0.81 (0.60-1.08)	0.00 (0.00-0.02)	}0.00 (0.00-0.14)
	quartile 1	23.3	1.07 (0.82-1.40)	0.02 (0.00-0.09)	
Obesity	BMI 25-30	42.4	0.93 (0.74-1.15)	0.00 (0.00-0.06)	}0.00 (0.00-0.12)
	BMI>30	22.1	1.07 (0.83-1.36)	0.02 (0.00-0.08)	
Silent MI	present	4.8	1.20 (0.82-1.75)		0.01 (0.00-0.04)
Grand total					**0.48 (0.32-0.64)**

* All analyses are adjusted for age, sex, hypertension, smoking, atrial fibrillation, and diabetes mellitus, if appropriate.

Table 4. Population attributable risks of presumed etiologic factors for ischemic stroke.

Probable etiologic factors	PAR (95% CI)* combined		
	All (n/N=432/6844)	Men (n/N=193/2732)	Women (n/N=239/4112)
Hypertension	0.34 (0.16-0.52)	0.46 (0.22-0.70)	0.22 (0.00-0.49)
Smoking	0.19 (0.06-0.32)	0.19 (0.00-0.60)	0.15 (0.05-0.26)
Diabetes mellitus	0.06 (0.02-0.09)	0.04 (0.00-0.10)	0.06 (0.01-0.12)
Atrial fibrillation	0.01 (0.00-0.03)	0.00 (0.00-0.03)	0.02 (0.00-0.05)
Total	**0.50 (0.34-0.66)**	**0.58 0.27-0.89)**	**0.40 (0.17-0.63)**
Possible etiologic factors			
Coronary disease	0.03 (0.00-0.07)	0.07 (0.00-0.13)	0.00 (0.00-0.04)
LVH	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Non-HDL cholesterol	0.01 (0.00-0.18)	0.00 (0.00-0.14)	0.10 (0.00-0.33)
HDL cholesterol	0.09 (0.00-0.25)	0.05 (0.00-0.28)	0.12 (0.00-0.35)
Obesity	0.15 (0.03-0.28)	0.17 (0.02-0.34)	0.14 (0.00-0.32)
Silent MI	0.04 (0.01-0.07)	0.08 (0.03-0.13)	0.01 (0.00-0.05)
Grand total	**0.60 (0.44-0.76)**	**0.64 (0.34-0.93)**	**0.54 (0.31-0.77)**

* All analyses are adjusted for age, sex, hypertension, smoking, atrial fibrillation, and diabetes mellitus, if appropriate.

Table 5. Population attributable risks of presumed etiologic factors for hemorrhagic stroke.

Probable etiologic factors	PAR (95% CI)* combined (n/N=73/6844)
Hypertension	0.25 (0.00-0.70)
Smoking	0.39 (0.16-0.62)
Diabetes mellitus	0.04 (0.00-0.13)
Total	**0.51 (0.18-0.84)**
Possible etiologic factors	
Non-HDL cholesterol	0.00 (0.00-0.04)
HDL cholesterol	0.00 (0.00-0.27)
Obesity	0.00 (0.00-0.00)
Grand total	**0.51 (0.18-0.84)**

* All analyses are adjusted for age, sex, hypertension, smoking, and diabetes mellitus, if appropriate.

Table 6. Population attributable risks of presumed etiologic factors that were only available in subgroups for any stroke (n/N=352/3265).

Etiologic factors	Classification	Prevalence men/women (%)	HR (95% CI)*	PAR (95% CI)* per classification	PAR (95% CI)* combined	PAR (95% CI)* combined: Men	PAR (95% CI)* combined: Women
Previous total†					**0.60 (0.42-0.79)**	**0.65 (0.34-0.96)**	**0.56 (0.36-0.76)**
Serum C-reactive protein	quartile 2	25 / 25	1.07 (0.79-1.46)	0.02 (0.00-0.10)			
	quartile 3	25 / 25	1.12 (0.82-1.53)	0.03 (0.00-0.11)	} 0.08 (0.00-0.10)	} 0.23 (0.00-0.51)	} 0.00 (0.00-0.25)
	quartile 4	25 / 25	1.16 (0.85-1.59)	0.04 (0.00-0.11)			
Alcohol intake	0 units	13 / 26	1.02 (0.79-1.31)	0.00 (0.00-0.06)	} 0.00 (0.00-0.06)	} 0.00 (0.00-0.07)	} 0.00 (0.00-0.10)
	> 3 units	16 / 3	0.81 (0.52-1.25)	0.00 (0.00-0.01)			
Fruit and vegetable intake	3-5 servings	22 / 18	1.27 (0.99-1.62)	0.05 (0.00-0.11)	} 0.05 (0.00-0.11)	} 0.09 (0.00-0.20)	} 0.02 (0.00-0.10)
	<3 servings	4 / 3	0.92 (0.50-1.70)	0.00 (0.00-0.02)			
Carotid IMT	quartile 2	25 / 25	0.98 (0.68-1.52)	0.00 (0.00-0.07)			
	quartile 3	25 / 25	1.48 (1.05-2.08)	0.11 (0.02-0.20)	} 0.20 (0.00-0.40)	} 0.02 (0.00-0.36)	} 0.32 (0.06-0.58)
	quartile 4	25 / 25	1.39 (0.98-1.99)	0.09 (0.00-0.18)			
Serum uric acid	quartile 2	25 / 25	1.33 (1.00-1.78)	0.08 (0.00-0.16)			
	quartile 3	25 / 25	0.96 (0.70-1.32)	0.00 (0.00-0.06)	} 0.07 (0.00-0.25)	} 0.05 (0.00-0.34)	} 0.08 (0.00-0.32)
	quartile 4	25 / 25	1.00 (0.73-1.36)	0.00 (0.00-0.07)			
Grand total					**0.68 (0.50-0.86)**	**0.66 (0.32-0.99)**	**0.69 (0.50-0.88)**

* All analyses are adjusted for age, sex, hypertension, smoking, atrial fibrillation, and diabetes mellitus.

† Total PAR based on hypertension, smoking, diabetes, atrial fibrillation, coronary disease, and obesity, calculated in this subgroup of the study population.

considerably, but not statistically significantly. The total proportion of ischemic strokes that was presumably caused by any combination of the included etiologic factors was 0.64 (95% CI 0.34-0.93) in men and 0.54 (95% CI 0.31-0.77) in women.

When we studied hemorrhagic strokes (table 5), smoking was the most important etiologic factor (PAR 0.39; 95% CI 0.16-0.62), followed by hypertension (PAR 0.25; 95% CI 0.00-0.70). All etiologic factors together, excluding the clearly thrombotic etiologic factors, accounted for 51% of all hemorrhagic strokes (95% CI 18%-84%).

For some possible etiologic factors, we had only data in random subgroups of our study population (and alcohol intake and fruit and vegetable deficiency were not assessed in participants who were demented at baseline). In men, serum C-reactive protein had a PAR of 0.23 (95% CI 0.00-0.51), which however did not increase the total PAR of all risk factors combined (table 6). In women, the PAR for intima-media thickness was 0.32 (95% CI 0.06-0.58), which together with serum uric acid (PAR 0.08, 95% CI 0.00-0.32) increased the total PAR from 0.56 (95% CI 0.36-0.76) to 0.69 (95% CI 0.50-0.88).

We found no evidence for statistically significant interaction at an apha level of 0.10.

Discussion

We found that about 54% of strokes are attributable to hypertension, smoking, atrial fibrillation, and diabetes mellitus. This proportion was similar for ischemic and hemorrhagic strokes, but higher in men than in women. If several possible etiologic factors prove to be causal and modifiable, this number may increase to about 68%.

Several methodological issues need to be discussed. Strengths of our study are the meticulous stroke case finding and the nearly complete follow-up (loss of potential person-years, 1.3 %). An advantage of our stringent stroke monitoring procedures was that we could include also stroke patients who had not been referred to a neurologist (31% of all stroke cases). As in these cases neuroimaging had not been performed, we could subclassify only 16% of them into ischemic or hemorrhagic. In contrast, 92% of strokes that had been referred to a neurologist could be subclassified. As a result, we could not determine the subtype of stroke in 237 participants. Some hazard ratios may be lower in our study population than in other populations, since our study participants are all aware of their risk factors due to their participation in the study. For hypertension some misclassification may have occurred since the diagnosis was based on two measurements only, possibly leading to an underestimation of the true hazard ratios.

To our knowledge, our study is the first that assessed PARs of etiologic factors for stroke in combination with each other in a population-based cohort study using adequate statistical methods. Two earlier studies reported on the combined PAR of all etiologic fac-

tors for stroke: the first, by Hankey, reported PARs that were partly based on data from a case-control study and partly on estimations.[2] This study included TIAs, carotid stenosis, and mitral valve disease. We chose not to include TIA since we think it is not an etiologic factor but a risk indicator. We had no information on carotid stenosis and mitral valve disease but measured carotid IMT and left ventricular hypertrophy instead. A second study, by Ezzati and colleagues, did not directly calculate PARs but estimated them from worldwide prevalences and HRs reported elsewhere.[7] This study included physical inactivity, of which we had no data. We did include some additional possible etiologic factors: HDL cholesterol, silent myocardial infarction, C-reactive protein, and serum uric acid.

The PAR of smoking was higher in our study than reported previously, the PAR of hypertension was in between previous estimates, and the PAR of diabetes mellitus was similar to that reported previously. [2, 7] The PARs for hypercholesterolemia, obesity, fruit and vegetable deficiency, alcohol consumption, ischemic heart disease, and atrial fibrillation were somewhat lower than in previous studies. These differences may be explained by the different prevalences of etiologic factors in various populations; for example, poverty-related problems like malnourishment and alcoholism are rare in our study population. Also the estimates of the HRs for the relation between etiologic factors and risk of stroke differed slightly between studies, in part because we did not dichotomize the etiologic factors but categorized them, which is argued to result in more accurate estimates.[16]

Hankey reported a total PAR of 80%[2] and Ezzati and colleagues a total PAR of 67-74%.[7] We estimate the total PAR of probable modifiable etiologic factors (hypertension, smoking, atrial fibrillation, and diabetes mellitus) as a more modest 54%. The addition of the possible modifiable etiologic factors coronary heart disease, HDL cholesterol, obesity, silent myocardial infarction, serum C-reactive protein, alcohol intake, and fruit and vegetable deficiency, high carotid IMT, and high serum uric acid could increase this number to 68%. However, it should be kept in mind that the causal role and the modifiability of these factors has not been established.

An explanation why the PAR we calculated was lower than previously reported is that the median age at start of follow-up of our study participants was relatively high (median 68 years), and the relation between stroke and its etiologic factors weakens with increasing age.[17] Another explanation is that the method we used to combine the PARs of the individual etiologic factors yielded a much lower PAR than would be expected based on the estimates of the individual PARs. This illustrates the fact that PARs, even extensively adjusted ones, cannot be summed up; the total PAR has to be calculated with an appropriate method.

In conclusion, we think that a maximum of 54% of strokes in persons aged 55 years and over might be prevented by treatment of the currently known established modifiable etiologic factors hypertension, smoking, diabetes mellitus, and atrial fibrillation. This number may be increased to about 68% if various possible etiologic factors indeed prove to be causal and modifiable.

References

1. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. JAMA. 1967;202:1028-1034
2. Hankey GJ. Potential new risk factors for ischemic stroke: What is their potential? Stroke. 2006;37:2181-2188
3. Rothman KJ, Greenland S. Modern epidemiology. Philadelphia: Lippincott Williams & Wilkins; 1998.
4. Goldstein LB, Adams R, et al. Primary prevention of ischemic stroke: A guideline from the American Heart Association/American Stroke Association Stroke Council. Circulation. 2006;113:e873-923
5. Bruzzi P, Green SB, et al. Estimating the population attributable risk for multiple risk factors using case-control data. Am J Epidemiol. 1985;122:904-914
6. Benichou J, Gail MH. Variance calculations and confidence intervals for estimates of the attributable risk based on logistic models. Biometrics. 1990;46:991-1003
7. Ezzati M, Hoorn SV, et al. Estimates of global and regional potential health gains from reducing multiple major risk factors. Lancet. 2003;362:271-280
8. Hofman A BM, van Duijn CM, et al. Rotterdam Study: Design update. Eur J Epidemiol. 2007;(in press)
9. Bos MJ, Schipper CM, et al. High serum C-reactive protein level is not an independent predictor for stroke: The Rotterdam Study. Circulation. 2006;114:1591-1598
10. Bos MJ, Koudstaal PJ, et al. Uric acid is a risk factor for myocardial infarction and stroke: The Rotterdam Study. Stroke. 2006;37:1503-1507
11. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;359:1309-1310
12. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ. 1962;27:645-658
13. van Bemmel JH, Kors JA, van Herpen G. Methodology of the modular ECG analysis system means. Methods Inf Med. 1990;29:346-353
14. Bots ML, Hoes AW, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. Circulation. 1997;96:1432-1437
15. van den Brandt PA, Goldbohm RA, et al. A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol. 1990;43:285-295
16. Greenland S. Attributable fractions: Bias from broad definition of exposure. Epidemiology. 2001;12:518-520
17. Whisnant JP. Modeling of risk factors for ischemic stroke. The Willis lecture. Stroke. 1997;28:1840-1844

4.2.
Serum C-reactive protein level is not an independent predictor for stroke

Background and purpose: *Current guidelines recommend the assessment of C-reactive protein (CRP) levels with a high-sensitivity assay in cardiovascular risk prediction. Recent studies put forward that, although elevated CRP is a risk factor for cardiovascular disease, it is not helpful in the prediction of cardiovascular disease risk. We studied the importance of CRP as a risk factor and as a risk predictor of future stroke.* ***Methods:*** *The study was based on 6430 participants of the Rotterdam Study who at baseline (1990-1993) were aged 55 years or over, stroke-free and had blood taken. Strokes were classified as hemorrhagic, ischemic or unspecified. Ischemic strokes were further subclassified. Whether stroke risk varied with baseline CRP serum levels was assessed with Cox proportional hazards models. Whether CRP was helpful in the prediction of individual stroke risk was assessed with Receiver-Operating Characteristic curves and by comparing the distribution of strokes between predicted risk strata.* ***Results:*** *During on average 8.2 years of follow-up, 498 first-ever strokes occurred. High CRP levels were significantly associated with risk of any stroke (age and sex adjusted HR per SD 1.14; 95% CI 1.04-1.24) and risk of ischemic stroke (age and sex adjusted HR per SD 1.17; 95% CI 1.04-1.32). However, taking CRP levels into account did not improve the individual stroke risk prediction, regardless of whether this was based on the Framingham stroke risk score or on age and sex only.* ***Conclusions:*** *Although CRP levels are associated with stroke risk, their use in the assessment of individual stroke risk seems limited.*

Introduction

Small persistent increases in systemic inflammatory activity, commonly referred to as low-grade inflammation, are associated with an increased risk of cardiovascular disease. Elevated levels of acute phase proteins may mark this low-grade inflammation when transient fluctuations during acute inflammation are ignored.[1-3] The acute phase protein that is strongest associated with cardiovascular disease appears to be C-Reactive Protein (CRP);[3,4] indeed an association between CRP measured with a high-sensitivity assay and coronary heart disease has been shown by many studies.[5] The January 2003 statement of the Centers for Disease Control and Prevention and the American Heart Association therefore recommends the use of CRP in the assessment of cardiovascular risk.[6] These recommendations are inspired by the strong associations that have been found between CRP levels and the risk of cardiovascular disease. However, a large meta-analysis and more recent findings suggested that CRP is a weaker risk factor than initially thought.[3,7] More importantly, what has often been overlooked is the difference between risk factors and clinically useful risk predictors: even when a strong association exists between a risk factor and the occurrence of a disease, additional statistical methods are required to determine whether that risk factor adds to the accuracy of disease risk prediction.[8-10] The number of studies that assessed the predictive value of CRP with appropriate statistical methods is quite small but they seem to suggest that the predictive value of CRP for cardiovascular disease is limited.[11-13] Several studies suggest that the strength of the association between CRP and stroke is similar to that between CRP and coronary heart disease,[14-17] but no studies thus far have examined the role of CRP as a risk predictor for stroke as a unique endpoint.

In this study, we examined whether CRP is a risk factor for stroke and its subtypes. Moreover, we assessed whether assessment of CRP levels contributes to the identification of patients at increased risk of stroke, both in addition to age and sex and in addition to the 'classical' risk factors used in the 1991 Framingham Stroke Risk Function[18] that performed reasonably well in our study population.[19]

Methods

Population: The study is part of the Rotterdam Study, a population-based cohort study on chronic and disabling diseases in the elderly. All inhabitants of Ommoord, a district of Rotterdam, aged 55 years and over were invited. People living in homes for the elderly were included. Participation rate of those invited for the study was 78%; in total, 7983 subjects participated.[20] The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent to retrieve information from treating physicians was obtained from all participants. Baseline measurements were obtained from 1990 through 1993

and consisted of a home interview and 2 visits to the research center for physical examination. At the baseline visit to the research center, we sampled blood and performed carotid duplex ultrasonography and electrocardiography. After exclusion of participants who had a stroke before baseline (n=261),[21] who did not visit the research center due to death, refusal or physical inability (n=844), of whom no blood was available for the CRP assay (n=413), or who had a very high serum CRP level (logarithm of CRP more than 3 standard deviations above the mean; n=35), a total of 6430 persons were included in this study.

Assessment of Stroke: History of stroke or transient ischemic attack at baseline was assessed and verified as described previously.[21] Once subjects enter the Rotterdam Study, they are continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physicians' files are scrutinized. For reported events, additional information (including brain images) is obtained from hospital records. Stroke research physicians reviewed information on all possible strokes and transient ischemic attacks; an experienced stroke neurologist (P.J.K.) verified all diagnoses blinded for CRP status. Subarachnoid hemorrhages and retinal strokes were excluded. Follow-up was completed until January 1, 2002 for 97.1% of all potential person years.[22]

Definitions of stroke subtypes: Ischemic strokes were diagnosed when a patient had typical symptoms and a CT or MRI that was made within 4 weeks ruled out other diagnoses or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in the absence of anticoagulants) pointed at an ischemic nature of the stroke. Hemorrhagic stroke was diagnosed when a relevant hemorrhage was shown on CT or MRI scan, or when the subject permanently lost consciousness or died within hours after onset of focal signs. If a stroke did not match these criteria, it was classified as unspecified.

Ischemic strokes were further subdivided into clinical syndromes. A hemispheric lacunar ischemic stroke syndrome was diagnosed when a patient suffered from a pure motor stroke, pure sensory stroke, ataxic hemiparesis, or dysarthria and a clumsy hand or arm, in the absence of cortical symptoms or signs (dysphasia, hemineglect, apraxia, acalculia, dysgraphia, or visual field defects). A hemispheric cortical ischemic stroke syndrome was diagnosed when any cortical symptom or sign was present. Posterior fossa ischemic stroke syndromes were diagnosed when cerebellar or brainstem signs were the only clinical manifestation.

An alternative subdivision of hemispheric ischemic strokes was based on neuroimaging: lacunar infarctions were subcortical infarctions smaller than 15 mm in diameter and cortical infarctions were all infarctions in which the cerebral cortex was involved.

Measurement of CRP: At baseline, a venipuncture was performed by application of minimal

stasis with a 21-gauge Butterfly needle with tube (Surflo winged infusion set, Terumo). Non-fasting blood was collected, and all tubes were stored on ice before and after blood sampling. High-sensitivity CRP was determined in serum, which was stored at -20°C until performance of the CRP measurements in 2003-2004. CRP was measured using Rate Near Infrared Particle Immunoassay (Immage® Immunochemistry System, Beckman Coulter, USA). This system measures concentrations from 0.2 to 1440 mg/l, with a within-run precision < 5.0%, a total precision < 7.5% and a reliability coefficient of 0.995.

Possible Confounders: Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements. The intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery. We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far wall of both the right and left common carotid artery. Atrial fibrillation was considered present when ECG confirmed it during the center visit or when it was reported in medical records. We considered diabetes mellitus to be present if a random or post-load glucose level was 11.1 mmol/L or higher, or if a person used antidiabetic medication. Cardiovascular history was diagnosed if a participant had a myocardial infarction, coronary artery bypass graft, or percutaneous transluminal angioplasty that was confirmed by ECG or medical records. Left ventricular hypertrophy was assessed with a 12-lead resting ECG and the Modular ECG Analysis System (MEANS).[23] Smoking status was assessed during the home interview and classified as current, former or never.

Statistical analysis: To examine the association between CRP and various stroke subtypes we used Cox' proportional hazards models. No violations to the proportional hazards assumption were detected by inspection of log(-log) survival curves. We used only first-ever strokes. Lost subjects were censored at the date last known to be alive. We calculated hazard ratios with 95% confidence intervals (CIs), which were expressed per standard deviation (SD) increase in logarithmically transformed CRP level and in CRP quartiles (relative to the lowest quartile). Because extremely high CRP levels likely reflect an acute inflammation at the time of blood sampling, individuals of whom the logarithm of the CRP level was more than 3 standard deviations above the mean were excluded from the study sample.

We corrected all hazard ratios for age and sex (model 1), and additionally for the variables of the Framingham Risk Score (age, systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, left ventricular hypertrophy)[18] (model 2) and for these Framingham risk factors and intima-media thickness (model 3). Missing values in covariates were imputed using a linear regression model based on age and sex. To examine interactions with sex or intima media thickness,[14] we stratified analyses according to sex and according to strata of carotid intima-media thickness. Also, we tested for interaction of the association

between CRP and the risk of stroke by sex and by stroke risk factors by entering an interaction term into the models.

The area under the curve (AUC) of a Receiver-Operating Characteristic (ROC) reflects the sensitivity and specificity, and hence the overall accuracy of a model. To examine the accuracy of CRP in predicting stroke risk we assessed whether adding CRP to a logistic prediction model increased the AUC.[24] This was done for a model that used age and sex and for a model that used the variables of the Framingham Risk Score.[18] Finally, to assess whether the predictive utility of CRP varied with background risk, we compared a prediction model that used age, sex and CRP with a model that used age and sex alone in various strata of the Framingham Risk Score.[13,25,26] To examine whether CRP improved identification of high-risk subjects, we studied whether the aforementioned prediction models better allocated actual stroke patients to a highest quartile, octile or decile of predicted risk when CRP was included than when CRP was not included.

Results

The total follow-up time till study end, death or first-ever stroke was 52,781 person years (on average 8.2 years). During follow-up 498 first-ever strokes occurred of which 278 (56%) were subclassified as ischemic strokes, 51 (10%) as hemorrhagic strokes and 169 (34%) as unspecified. Median CRP level was 1.86 mg/l; the interquartile range was 0.90 mg/l to 3.59 mg/l.

Table 1. Baseline characteristics of eligible population (n=6430).

Baseline characteristics	Median (interquartile range) or percentage
Age (years)	68.2 (62.0-75.6)
Female sex	60.0 %
CRP (mg/l)	1.86 (0.90-3.59)
Systolic blood pressure (mmHg)	138 (123-153)
Diastolic blood pressure (mmHg)	73 (66-81)
Intima-media thickness (mm)	0.78 (0.68-0.88)
Waist/hip ratio	0.90 (0.84-0.97)
Left ventricular hypertrophy	4.6 %
Antihypertensive therapy	18.0 %
Atrial fibrillation	5.0 %
Diabetes mellitus	10.7 %
Cardiovascular history	16.8 %
Current smoking	22.8 %
Ever smoked	64.6 %

Table 2. Hazard ratios and 95% confidence intervals for the associations between serum CRP (per SD increase in logarithmically transformed CRP and in CRP quartiles) and stroke subtypes.

Stroke subtype	CRP	Hazard ratios (95% confidence intervals)		
(n/N)		Model 1*	Model 2*	Model 3*
All strokes	Per SD	1.14 (1.04-1.24)	1.05 (0.96-1.15)	1.04 (0.95-1.13)
(n=498/N=6430)	Quartile 1	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	1.11 (0.85-1.45)	1.04 (0.80-1.35)	1.04 (0.80-1.36)
	Quartile 3	1.19 (0.91-1.54)	1.03 (0.80-1.34)	1.00 (0.77-1.30)
	Quartile 4	1.36 (1.05-1.76)	1.11 (0.86-1.45)	1.09 (0.84-1.41)
Ischemic strokes	Per SD	1.17 (1.04-1.32)	1.08 (0.95-1.22)	1.06 (0.94-1.20)
(n=278/N=6430)	Quartile 1	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	1.08 (0.76-1.53)	1.00 (0.71-1.42)	1.00 (0.71-1.42)
	Quartile 3	1.21 (0.86-1.71)	1.04 (0.73-1.47)	0.99 (0.70-1.41)
	Quartile 4	1.38 (0.98-1.94)	1.12 (0.79-1.58)	1.08 (0.77-1.53)
Hemorrhagic strokes	Per SD	1.13 (0.86-1.50)	1.01 (0.76-1.34)	1.01 (0.76-1.34)
(n=51/N=6430)	Quartile 1	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	1.01 (0.44-2.29)	0.94 (0.41-2.13)	0.94 (0.41-2.13)
	Quartile 3	1.10 (0.49-2.46)	0.92 (0.41-2.07)	0.92 (0.41-2.07)
	Quartile 4	1.40 (0.64-3.06)	1.07 (0.48-2.36)	1.07 (0.48-2.36)

* Model 1: Adjusted for age and sex. Model 2: As model 1 and additionally adjusted for systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, and left ventricular hypertrophy. Model 3: As model 2 and additionally adjusted for intima-media thickness.

Table 1 describes baseline characteristics. The age and sex adjusted survival plot (figure 1) shows that stroke-free survival decreased with increasing levels of CRP. The age- and sex-adjusted hazard ratio for the association between CRP and stroke calculated with a Cox proportional hazards model was 1.14 (95% CI 1.04 to 1.24) per standard deviation increase in logarithmically transformed CRP; persons with CRP levels in the highest quartile had a 36% increased stroke risk (hazard ratio 1.36; 95% CI 1.05 to 1.76) compared with those with CRP levels in the lowest quartile (table 2). The hazard ratios for ischemic and hemorrhagic strokes were similar, although the latter association was not statistically significant, probably because of limited power. Addition of stroke risk factors into the models attenuated these associations whilst addition of intima-media thickness attenuated them slightly further (table 2).

Although associations seemed stronger in males than in females, interaction was not significant (P interaction 0.07 for stroke, 0.57 for ischemic strokes, and 0.14 for hemorrhagic

strokes; table 3). Analyses in strata of intima-media thickness showed stronger associations between CRP and stroke in persons with intima-media thickness in the middle and the upper tertile compared with the lower tertile of the population distribution, yet the continuous interaction term was not significant (P interaction=0.48): age and sex adjusted hazard ratios for stroke were 0.86 (95% CI 0.65 to 1.13) in the lower tertile, 1.14 (95% CI 0.95 to 1.38) in the middle tertile and 1.26 (95% CI 1.07 to 1.46) in the upper tertile. For ischemic stroke the figures were very similar (corresponding age and sex adjusted hazard ratios were 0.90 (95% CI 0.63 to 1.28), 1.15 (95% CI 0.91 to 1.50), and 1.28 (95% CI 1.05 to 1.56) (P interaction=0.34)).

We studied 3 clinical subtypes of ischemic stroke: hemispheric lacunar syndromes (n=70), hemispheric cortical syndromes (n=140), and posterior fossa syndromes (n=38). The age and sex adjusted hazard ratio (95% CI) per SD increase in logarithmically transformed

Table 3. Hazard ratios for the associations between serum CRP (per SD increase in logarithmically transformed CRP and in sex specific CRP quartiles) and stroke subtypes for men and women separately.

Stroke type (n for ♂, n for ♀)	CRP	Hazard ratios (95% confidence intervals)*			
		Males (N=2574)		Females (N=3856)	
		Model 1*	Model 2*	Model 1*	Model 2*
All strokes	Per SD	1.25 (1.09-1.43)	1.18 (1.03-1.36)	1.05 (0.93-1.18)	0.96 (0.85-1.08)
(n=200, 298)	Quartile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	1.29 (0.84-1.97)	1.17 (0.76-1.80)	1.01 (0.72-1.42)	0.96 (0.69-1.35)
	Quartile 3	1.35 (0.89-2.06)	1.14 (0.74-1.76)	1.14 (0.82-1.58)	0.97 (0.70-1.35)
	Quartile 4	1.72 (1.14-2.59)	1.40 (0.93-2.13)	1.10 (0.79-1.53)	0.94 (0.67-1.32)
Ischemic strokes	Per SD	1.22 (1.03-1.45)	1.13 (0.94-1.36)	1.12 (0.96-1.32)	1.03 (0.87-1.22)
(n=121, 157)	Quartile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	1.31 (0.77-2.23)	1.15 (0.67-1.96)	0.93 (0.58-1.48)	0.88 (0.55-1.41)
	Quartile 3	1.40 (0.83-2.37)	1.14 (0.67-1.97)	1.11 (0.71-1.74)	0.95 (0.61-1.49)
	Quartile 4	1.53 (0.90-2.61)	1.19 (0.70-2.03)	1.25 (0.80-1.96)	1.06 (0.67-1.66)
Hemorrhagic strokes	Per SD	1.46 (0.93-2.29)	1.36 (0.87-2.14)	0.95 (0.67-1.36)	0.81 (0.57-1.17)
(n=18, 33)	Quartile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Quartile 2	0.96 (0.19-4.77)	0.97 (0.19-4.84)	1.01 (0.39-2.62)	0.93 (0.36-2.42)
	Quartile 3	1.65 (0.39-6.95)	1.67 (0.39-7.18)	0.91 (0.34-2.43)	0.66 (0.24-1.78)
	Quartile 4	2.55 (0.65-9.97)	1.98 (0.50-7.85)	0.94 (0.35-2.53)	0.71 (0.26-1.94)

* Model 1: Adjusted for age. Model 2: As model 1 and additionally adjusted for systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, and left ventricular hypertrophy.

Table 4. Discriminant accuracy of CRP for 2.5-year stroke risk (n=72), 5-year stroke risk (n=149), and 7.5-year stroke risk (n=226) in women (N=3856). Area under Receiver-Operating Characteristic curve (AUC) and proportion of events within quartile, octile or decile of highest predicted risk for two prediction models with and without CRP.

Stroke risk	Age model			Framingham model		
	Age	Age, CRP	Effect of CRP	Framingham predictors†	Framingham predictors†, CRP	Effect of CRP
AUC (95% CI)						
2.5 y	0.755 (0.704-0.806)	0.757 (0.706-0.807)	0.51*	0.798 (0.751-0.846)	0.798 (0.750-0.846)	0.14*
5 y	0.730 (0.691-0.768)	0.730 (0.691-0.768)	0.99*	0.762 (0.723-0.800)	0.762 (0.724-0.801)	0.41*
7.5 y	0.716 (0.684-0.748)	0.716 (0.684-0.748)	0.81*	0.750 (0.719-0.781)	0.751 (0.721-0.782)	0.34*
events in highest risk quartile, %						
2.5 y	58%	58%	0%	64%	64%	0%
5 y	53%	53%	0%	58%	58%	0%
7.5 y	51%	51%	0%	55%	57%	2%
events in highest risk octile, %						
2.5 y	40%	38%	-2%	39%	39%	0%
5 y	37%	36%	-1%	41%	40%	-1%
7.5 y	31%	33%	2%	38%	38%	0%
events in highest risk decile, %						
2.5 y	32%	33%	1%	38%	38%	0%
5 y	31%	33%	2%	34%	34%	0%
7.5 y	26%	26%	0%	32%	33%	1%

* P-value calculated as described by DeLong et al.[24]

† Age, systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, left ventricular hypertrophy.

Table 5. Discriminant accuracy of CRP for 2.5-year stroke risk (n=47), 5-year stroke risk (n=103), and 7.5-year stroke risk (n=162) in men (N=2574). Area under Receiver-Operating Characteristic curve (AUC) and proportion of events within quartile, octile or decile of highest predicted risk for two prediction models with and without CRP.

Stroke risk	Age model			Framingham model		
	Age	Age, CRP	Effect of CRP	Framingham predictors†	Framingham predictors†, CRP	Effect of CRP
AUC (95% CI)						
2.5 y	0.684 (0.610-0.758)	0.702 (0.630-0.774)	0.24*	0.727 (0.652-0.803)	0.737 (0.667-0.808)	0.47*
5 y	0.653 (0.606-0.701)	0.670 (0.624-0.716)	0.16*	0.719 (0.671-0.768)	0.723 (0.675-0.771)	0.56*
7.5 y	0.658 (0.620-0.697)	0.667 (0.630-0.705)	0.22*	0.710 (0.671-0.749)	0.712 (0.673-0.751)	0.64*
events in highest risk quartile, %						
2.5 y	47%	49%	2%	62%	55%	-7%
5 y	41%	42%	1%	56%	54%	-2%
7.5 y	40%	40%	0%	49%	50%	1%
events in highest risk octile, %						
2.5 y	30%	36%	6%	40%	36%	-4%
5 y	19%	24%	5%	36%	36%	0%
7.5 y	20%	22%	2%	30%	30%	0%
events in highest risk decile, %						
2.5 y	30%	34%	4%	36%	30%	-6%
5 y	18%	21%	3%	30%	31%	1%
7.5 y	18%	19%	1%	25%	24%	-1%

* P-value calculated as described by DeLong et al.[24]

† Age, systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, smoking, coronary heart disease, atrial fibrillation, left ventricular hypertrophy.

CRP was 1.20 (0.95-1.53) for hemispheric lacunar syndromes; 1.12 (0.95-1.33) for hemispheric cortical syndromes; and 1.36 (0.99-1.86) for posterior fossa syndromes. We also studied 2 radiological subtypes of hemispheric ischemic stroke: lacunar infarctions (n=37) and cortical infarctions (n=57). The age and sex adjusted hazard ratio (95% CI) was 1.35 (0.98-1.87) for lacunar infarctions and 1.27 (0.97-1.65) for cortical infarctions.

When we used only age and sex to predict 5-year stroke risk, the AUC was 0.700 (95% CI 0.670 to 0.731; figure 2). When CRP was added to this model, the AUC increased only marginally to 0.704 (95% CI 0.674-0.734), which was not significantly different (p=0.18). Also for 2.5-year and 7.5-year stroke risk the AUC for the model with CRP was not significantly larger than for the model without CRP. Likewise, the addition of CRP to a model based on the Framingham stroke risk factors resulted in a non-significant increase in AUC for 5-year risk prediction from 0.741 (95% CI 0.711-0.772) to 0.742 (95% CI 0.712-0.772; figure 2). Results were similar for women (table 4) and men (table 5). When we compared the age, sex and CRP model with the age and sex model in various strata of the Framingham Risk Score, there was no statistically significant difference between the models (table 6). The prediction models with CRP did not allocate substantially more actual stroke patients to the highest risk quartile, octile or decile than the models without CRP: in women the largest observed increase was only 2% (table 4). In men, CRP increased the number of actual strokes within 2.5 years in the octile of highest estimated risk from 30% to 36%. However, with the Framingham Risk Score this percentage was 40%, which was decreased again to 36% when CRP was added to the prediction model. Similar effects were seen in the quartile and decile of highest estimated risk (table 5).

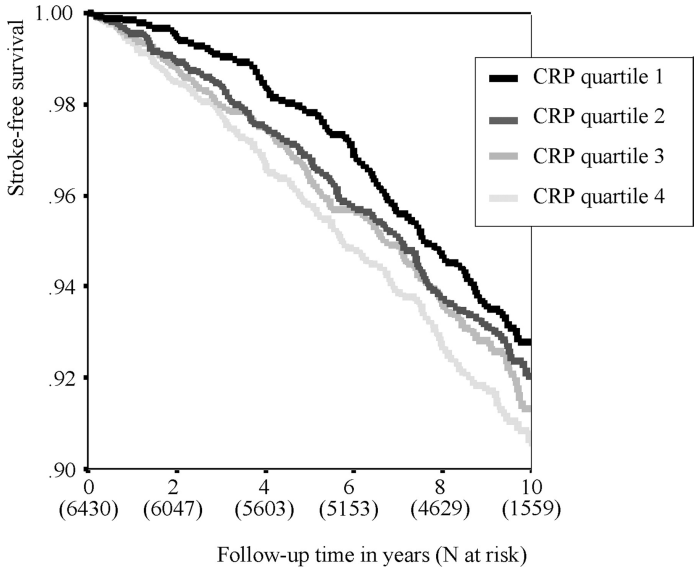


Figure 1. Survival plot for stroke-free survival by quartiles of CRP at mean of covariates age and sex.

Table 6. Discriminant accuracy of CRP for 2.5-year stroke risk (n=119), 5-year stroke risk (n=252), and 7.5-year stroke risk (n=388) in various risk strata that were calculated with the risk predictors from the Framingham Risk Score.

Risk quintile*	2.5-yr stroke risk			5-yr stroke risk			7.5-yr stroke risk		
	Age, sex AUC (95%CI)	Age, sex, CRP AUC (95%CI)	Effect of CRP†	Age, sex AUC (95%CI)	Age, sex, CRP AUC (95%CI)	Effect of CRP†	Age, sex AUC (95%CI)	Age, sex, CRP AUC (95%CI)	Effect of CRP†
1	0.898 (0.848-0.949)	0.899 (0.856-0.953)	0.83	0.599 (0.438-0.759)	0.600 (0.437-0.763)	0.88	0.502 (0.328-0.676)	0.512 (0.361-0.663)	0.74
2	0.784 (0.568-1.000)	0.796 (0.583-1.000)	0.53	0.680 (0.584-0.776)	0.683 (0.588-0.778)	0.83	0.609 (0.501-0.718)	0.621 (0.519-0.722)	0.45
3	0.539 (0.394-0.683)	0.636 (0.491-0.782)	0.16	0.572 (0.491-0.653)	0.636 (0.544-0.728)	0.17	0.512 (0.448-0.576)	0.524 (0.458-0.591)	0.61
4	0.531 (0.425-0.636)	0.522 (0.414-0.630)	0.62	0.549 (0.477-0.620)	0.545 (0.473-0.617)	0.86	0.517 (0.458-0.577)	0.532 (0.472-0.593)	0.43
5	0.585 (0.517-0.652)	0.597 (0.527-0.666)	0.48	0.556 (0.502-0.610)	0.564 (0.510-0.619)	0.46	0.534 (0.488-0.580)	0.535 (0.489-0.581)	0.85

* Calculated with Framingham risk score

† P-value calculated as described by DeLong et al.[24]

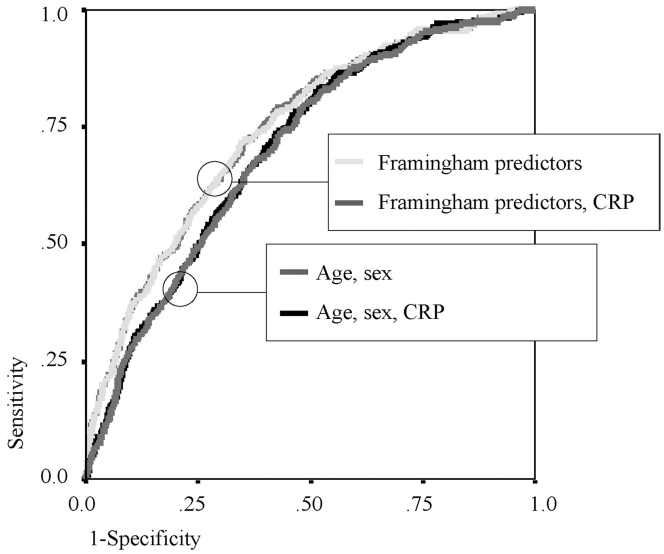


Figure 2. ROC-curves for 5-year stroke prediction models. Framingham predictors: sex, age, systolic blood pressure, antihypertensive therapy, systolic blood pressure x antihypertensive therapy, diabetes mellitus, current smoking, former smoking, coronary heart disease, atrial fibrillation, and left ventricular hypertrophy.

Discussion

In this population-based follow-up study of 6430 subjects aged 55 years and over who were free from stroke at baseline, CRP serum levels were significantly associated with incident stroke and incident ischemic stroke. However, adjustment for other cardiovascular risk factors attenuated these associations. Although CRP was a risk factor for stroke, it did not discriminate well between individuals at high and low stroke risk: it neither made stroke prediction more accurate when added to a model containing only age and sex nor when added to a model based on the stroke risk predictors of the Framingham Risk Score.

Before these results can be interpreted, some methodological issues need to be discussed. First, 1247 participants were excluded from the cohort at risk because they did not visit the research center due to death, refusal or physical inability or because we did not have baseline blood of them. Participants in this subgroup were more likely to be female (69%) and of older age (median age 75.7 years) than the study population. However, this selection could only have biased our results if the association between CRP levels and risk of subsequent stroke were different in those individuals as compared to the remainder of the cohort that was included in the analyses, which we consider highly unlikely. More important for selection bias in a prospective study is the completeness of follow-up. Since we had follow-up for 97.1% of all potential person years, we feel confident that selection bias will not have played a major role, if any, in our study. Second, blood samples had been stored for

almost 10 years at -20°C before the assays were carried out. We assessed the comparability of these CRP assays with assays in blood from the same participants that had been stored at -80ºC (n=29). Although the median CRP was lower in -20°C blood, Spearman's correlation coefficient for the association between CRP in -20°C blood and CRP in -80°C blood was highly significant (correlation coefficient 0.99; $p<0.001$) and associations therefore unaffected. Third, CRP measurements were performed only once per participant. It has been recommended to perform measurements twice[6] but as it has been shown that CRP levels are very constant over many years[1] and given the size of our cohort we do not think that this can explain our results. Fourth, 35 participants were excluded because of extremely high CRP levels. Results did not materially change when these subjects were included in the analyses. Fifth, our stringent stroke monitoring procedures allowed us to include also stroke patients who were not referred to a hospital. A restraint is that in these cases neuroimaging was often lacking (61% of our cases had neuroimaging) and examinations were not thorough enough to subclassify 36% of strokes. Finally, general strengths of our study are the large eligible population (n=6430), the intense stroke case finding and the nearly complete follow-up (loss of potential person-years 2.9%).[22]

Our finding that increased CRP serum levels are associated with stroke risk is in accordance with other population-based follow-up studies[14-16] and with studies that reported significant associations between CRP and fatal stroke,[27] CRP and stroke and TIA,[28] and CRP and stroke univariately.[17] In previous studies adjustment for other cardiovascular risk factors generally attenuated the association between CRP and stroke, although often the association did remain significant.[14,15] In our study CRP was not an independent risk factor after adjustment for other vascular risk factors and risk markers.

This is the first study to report on subtypes of ischemic stroke. CRP appeared to be a risk factor for both large artery hemispheric strokes and small vessel lacunar strokes.

The mechanisms that may underlie the association between CRP and cardiovascular disease are as yet not fully elucidated. Increased levels of CRP reflect inflammation, in this context probably the inflammatory condition of the vascular wall. This is now generally accepted as the main mechanism underlying the relationship between CRP and cardiovascular disease. However, there are also indications that CRP is directly involved in the occurrence of cardiovascular events.[29] Recent studies show that lower CRP levels upon statin therapy go indeed hand in hand with a reduced risk of recurrent myocardial infarction and coronary artery disease, supporting a causal role of inflammation in cardiovascular disease.[30,31]

Although our results confirm a possible causal relationship between CRP and stroke, the ROC analyses imply that CRP measurement does not contribute to individual stroke risk estimation, similar to what has been found for coronary disease.[3,12] Also in the subset of persons in the quartile, octile or decile with the highest estimated risk, CRP assessment did not prove helpful. Our study underscores the importance of distinguishing between risk factors and risk predictors of disease. Association of a risk factor with the occurrence of disease may

suggest important causal mechanisms, but it does not necessarily imply clinical utility of assessment of that same factor for risk prediction.[10]

It has recently been suggested that CRP might be less useful in the assessment of coronary disease risk than initially thought.[3,12] Our study suggests that CRP measurement does not contribute much to the assessment of stroke risk either.

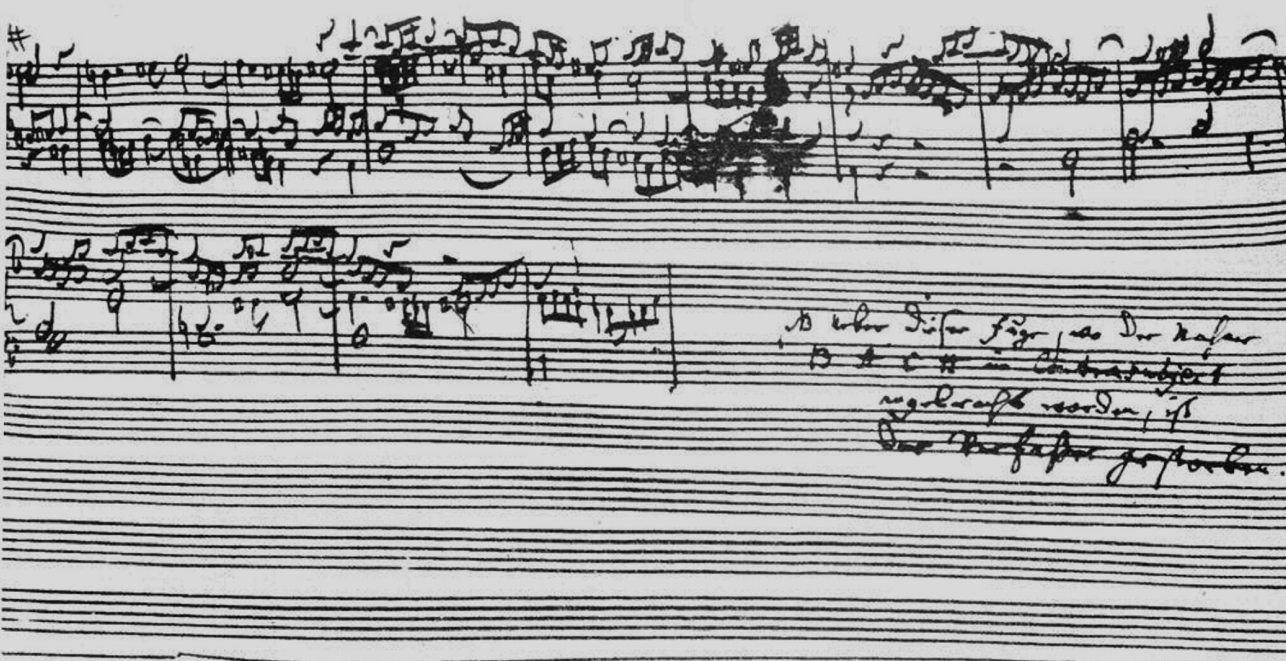
References

1. Ockene IS, Matthews CE, et al. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem. 2001; 47:444-50.
2. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol. 2001; 38:189-97.
3. Danesh J, Wheeler JG, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004; 350:1387-97.
4. Ridker PM, Hennekens CH, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000; 342:836-43.
5. Danesh J, Whincup P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ. 2000; 321:199-204.
6. Pearson TA, Mensah GA, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499-511.
7. Pai JK, Pischon T, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004; 351:2599-610.
8. Greenland P, O'Malley PG. When is a new prediction marker useful? A consideration of lipoprotein-associated phospholipase A2 and C-reactive protein for stroke risk. Arch Intern Med. 2005; 165:2454-6.
9. Pepe MS, Janes H, et al. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol. 2004; 159:882-90.
10. Lloyd-Jones DM, Liu K, et al. Narrative Review: Assessment of C-Reactive Protein in Risk Prediction for Cardiovascular Disease. Ann Intern Med. 2006; 145:35-42.
11. Wilson PW, Nam BH, et al. C-reactive protein and risk of cardiovascular disease in men and women from the Framingham Heart Study. Arch Intern Med. 2005; 165:2473-8.
12. van der Meer IM, de Maat MP, et al. The value of C-reactive protein in cardiovascular risk prediction: the Rotterdam Study. Arch Intern Med. 2003; 163:1323-8.
13. Ridker PM, Rifai N, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002; 347:1557-65.
14. Cao JJ, Thach C, et al. C-reactive protein, carotid intima-media thickness, and incidence of ischemic stroke in the elderly: the Cardiovascular Health Study. Circulation. 2003; 108:166-70.
15. Curb JD, Abbott RD, et al. C-reactive protein and the future risk of thromboembolic stroke in healthy men. Circulation. 2003; 107:2016-20.
16. Cesari M, Penninx BW, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC Study. Circulation. 2003; 108:2317-22.
17. Ridker PM, Cushman M, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997; 336:973-9.
18. Wolf PA, D'Agostino RB, et al. Probability of stroke: a risk profile from the Framingham Study. Stroke. 1991; 22:312-8.
19. Voko Z, Hollander M, et al. How do American stroke risk functions perform in a Western European population? Neuroepidemiology. 2004; 23:247-53.
20. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991; 7:403-22.
21. Hollander M, Bots ML, et al. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam Study. Circulation. 2002; 105:2872-7.
22. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;

359:1309-10.
23. van Bemmel JH, Kors JA, van Herpen G. Methodology of the modular ECG analysis system MEANS. Methods Inf Med. 1990; 29:346-53.
24. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988; 44:837-45.
25. Von Eckardstein A, Schulte H, et al. Lipoprotein(a) further increases the risk of coronary events in men with high global cardiovascular risk. J Am Coll Cardiol. 2001; 37:434-9.
26. Koenig W, Lowel H, et al. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. Circulation. 2004; 109:1349-53.
27. Gussekloo J, Schaap MC, et al. C-reactive protein is a strong but nonspecific risk factor of fatal stroke in elderly persons. Arterioscler Thromb Vasc Biol. 2000; 20:1047-51.
28. Rost NS, Wolf PA, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham Study. Stroke. 2001; 32:2575-9.
29. de Maat MP, Trion A. C-reactive protein as a risk factor versus risk marker. Curr Opin Lipidol. 2004; 15:651-7.
30. Nissen SE, Tuzcu EM, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med. 2005; 352:29-38.
31. Ridker PM, Cannon CP, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005; 352:20-8.

Chapter 5.

Transient neurological attacks



5.1.
Prognosis of transient neurological attacks

Background and purpose: *Transient neurological attacks (TNAs) are attacks with temporary (< 24 hours) neurological symptoms. These symptoms can be focal, nonfocal, or a mixture of both. The prognostic significance of TNAs with focal symptoms (better known as transient ischaemic attack (TIAs)) is well understood. Conversely, hardly anything is known about the prognostic significance of TNAs with nonfocal or mixed symptoms. We studied incidence and prognosis of focal TNAs (or TIAs), nonfocal TNAs, and mixed TNAs.* ***Methods:*** *Our study population comprised 6,062 community-dwelling Rotterdam Study participants who were aged ≥ 55 years and free from stroke, myocardial infarction, and dementia at baseline (1990-1993). They were followed for events until January 1, 2005. We analysed the associations between incident TNAs and subsequent adverse events with age and sex adjusted Cox regression models.* ***Results:*** *During 60,535 person years, 548 participants developed TNA (282 focal, 228 nonfocal, and 38 mixed), 619 stroke, 609 dementia, and 848 cardiovascular disease. Participants with focal TNA, nonfocal TNA or mixed TNA were all at higher risk of subsequent stroke than participants without TNA. Furthermore, nonfocal TNA patients were at higher risk of dementia (HR 1.59, 95%CI 1.11-2.26) and mixed TNA patients were at higher risk of ischaemic heart disease (HR 2.26, 95%CI 1.07-4.78), vascular death (n=662; HR 2.54, 95%CI 1.31-4.91), and dementia (HR 3.46, 95%CI 1.72-6.98) than participants without TNA.* ***Conclusions:*** *Patients who suffer from nonfocal TNAs have an unfavourable overall prognosis, almost equal to that of TIA patients. Patients with mixed TNAs have a particularly bad prognosis.*

Introduction

The first internationally accepted clinical classification for cerebrovascular disorders was formulated in 1975.[1] It included diagnostic criteria for transient attacks of neurological dysfunction, which in the present paper we will call transient neurological attacks (TNAs). The classification attempted to create a formal distinction between TNAs with an unfavourable clinical course (which were presumed to be of vaso-occlusive origin and were therefore called transient ischaemic attacks (TIAs)) and more benign attacks. TIAs were defined as temporary attacks (commonly 2 to 15 minutes, maximum 24 hours) with focal symptoms, which are attributable to dysfunction of one arterial territory of the brain. The remaining TNAs, with diffuse, non-localizing cerebral symptoms, were considered more benign. We will call these TNAs either nonfocal TNAs, if they present with only nonfocal symptoms, or mixed TNAs, if they present with a mix of focal and nonfocal symptoms.

Although the assumption that TIA patients are at high risk of major vascular disease has repeatedly been confirmed since 1975,[2-6] hardly any studies have challenged and verified the assumption that nonfocal TNAs have a benign clinical course. Those that did, focused only on small subgroups of TNAs and on a limited number of adverse events, and included mostly a selected subgroup of patients referred to a neurologist.[7-11] Only one previous study, with participants of the Dutch TIA trial, assessed the prognosis of mixed TNAs, and suggested a higher risk of cardiac events in patients with mixed attacks.[12]

We studied incidence and prognosis of focal TNAs, nonfocal TNAs, and mixed TNAs in a population-based cohort.

Methods

Source population: The present study is part of the Rotterdam Study, a prospective population-based cohort study of chronic and disabling diseases.[13] Invitations were sent to all inhabitants of Ommoord, a district in the city of Rotterdam in the Netherlands, aged 55 years and over. People living in homes for the elderly were included. The participation rate of those invited for the study was 78%; in total, 7983 subjects participated. The study was approved by the Medical Ethics Committee of Erasmus University Rotterdam. Written informed consent to retrieve information from treating physicians was obtained from all included participants. Baseline assessments were obtained from 1990 through 1993 and consisted of a home interview and 2 visits to the research centre for physical examination.

Baseline assessments: History of stroke at study baseline was positive if a stroke was self-reported during the baseline interview and confirmed by medical records.[14,15] History of myocardial infarction was positive if it was reported during the baseline interview and confirmed

by baseline ECG or medical records. History of coronary artery bypass graft (CABG) or percutaneous transluminal coronary angioplasty (PTCA) was positive if it was reported during the baseline interview and confirmed by medical records. Angina pectoris was assessed using the World Health Organization (WHO) angina pectoris questionnaire.[16] History of dementia was assessed using a three-step protocol:[17] two brief tests of cognition, the mini-mental state examination (MMSE) and the geriatric mental state schedule (GMS) organic level, were used to screen all participants. Patients with positive screens (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Those who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDSADRDA),[18] and vascular dementia (NINDS-AIREN)[19] by a panel consisting of a neurologist, a neuropsychologist, and a research physician. We had no adequate information on TNAs at baseline.

Blood pressure was measured twice in the right arm using a random-zero sphygmomanometer, with the subject in sitting position. We used the average of these 2 measurements. Hypertension was defined as stage II hypertension according to the seventh Joint National Committee on Detection, Evaluation, and Diagnosis of High Blood Pressure criteria (diastolic blood pressure of at least 100 mm Hg and/or a systolic blood pressure of at least 160 mm Hg) and/or use of antihypertensive medication indicated to treat high blood pressure. C-reactive protein, total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), and uric acid were measured in non-fasting baseline blood with automated enzymatic procedures. We considered diabetes mellitus to be present if a random or post-load glucose level was 11.1 mmol/L or higher, or if a person used antidiabetic medication. Atrial fibrillation at baseline was considered to be present if it was seen on ECG during the centre visit or if reported in medical records. The intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery.[20] During the home interview, smoking status and medication use were assessed. Genotyping for APOE was done on coded DNA specimens. Two groups were formed on the basis of presence or absence of an APOE ε4 allele. Education was dichotomised into high (higher vocational or university education) and low.

Population for analysis: We excluded 1858 participants because they had myocardial infarction (n=871), stroke (n=261), or dementia (n=482) at baseline, or because they did not undergo cognitive screening at baseline (n=455). Some participants had more than one of these conditions. Participants who refused informed consent to the retrieval of information from general practitioners were also excluded (n=63). This left 6062 participants eligible for the analyses.

Monitoring of events during follow-up: After enrolment into the Rotterdam Study, par-

ticipants were continuously monitored for strokes, TIA, ischaemic heart disease, dementia, and death through automated linkage of the study database with files held by general practitioners, the Regional Institute for Outpatient Mental Health Care, and the municipality. If available, additional information (including brain imaging) was obtained from hospitals. In addition, during three follow-up surveys (1993-1995, 1997-1999 and 2002-2004), a research physician screened all eligible participants in person for occurrence of TIA by asking for transient neurological symptoms, and carefully registered all mentioned symptoms and attack characteristics. During these surveys we also assessed presence of dementia as described below. All information thus collected was verified as described below. Follow-up for all events was completed until January 1, 2005 for 96.2 % of potential person years.[21]

Verification of events: The information collected on potential strokes and TNAs during follow-up was reviewed and structured by a research physician. An experienced stroke neurologist (PJK) verified all diagnoses. A stroke was diagnosed if a patient had typical symptoms that lasted longer than 24 hours. Strokes were subclassified as ischaemic if a CT or MRI scan made within 4 weeks after onset of symptoms ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, or atrial fibrillation in absence of anticoagulant therapy) indicated the ischaemic nature of the stroke. TNAs were defined as attacks of sudden neurological symptoms that completely resolved within 24 hours, with no clear evidence for the diagnosis of migraine, epilepsy, Ménière's disease, hyperventilation, cardiac syncope, hypoglycaemia, or orthostatic hypotension. If only focal brain symptoms were reported, the event was classified as a focal TNA. If only nonfocal brain symptoms were reported, the event was classified as nonfocal TNA. If focal and nonfocal symptoms were reported for one and the same attack, a mixed TNA was diagnosed.

The information collected on potential ischaemic heart disease during follow-up was independently coded by two research physicians. Diagnoses on which the research physicians disagreed were discussed in order to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events. Ischaemic heart disease was coded according to the International Classification of Diseases, 10th edition (ICD-10). Incident ischaemic heart disease during follow-up was defined as the occurrence of a fatal or non-fatal myocardial infarction (ICD-10 code I21), a revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft), other forms of acute (I24) or chronic ischaemic (I25) heart disease, sudden (cardiac) death (I46 and R96), and death due to ventricular fibrillation (I49) and death due to congestive heart failure (I50).[22]

Vascular death was diagnosed when a participant died and the most probable underlying cause of death was stroke or ischemic heart disease.

Presence of dementia was assessed during the three follow-up surveys with the same protocol that was used at baseline (see above), in combination with information from general

practitioners, the Regional Institute for Outpatient Mental Health Care, the municipality, and the hospitals.[17]

Statistical Analysis: First, we assessed the age and sex specific incidence rates of all types of first TNAs. Then we assessed the association between occurrence of TNA (entered into the model as a time-dependent variable) and risk of adverse events with Cox proportional hazards models, comparing the risk of an adverse event developing after TNA to the risk of an adverse event developing in TNA-free participants. We adjusted for age and sex (model 1) and additionally for a propensity score[23] that was based on C-reactive protein, systolic blood pressure, carotid intima-media thickness, total cholesterol, HDL cholesterol, uric acid, waist/hip ratio, mini mental state examination, current smoking, ever smoking, atrial fibrillation, diabetes mellitus, hypertension, PTCA or CABG, angina pectoris, APO ε4 carriership, and education; model 2), and present results as hazard ratios with 95% confidence intervals (CIs). Only first TNAs during follow-up were used in the analyses. Follow-up ended at time of the outcome event, end of study, loss to follow-up, or death, whichever occurred first. Participants were censored at time of stroke, ischaemic heart disease, or dementia if it occurred before TNA. All analyses were performed with SPSS for Windows, Rel. 11.0.1 (SPSS Inc, Chicago, IL, 2001).

Results

The median age at baseline was 67.7 years and 62% of participants were women (table 1). During 60,535 person years of follow-up, a TNA occurred in 548 participants; 282 of these were classified as focal, 228 as nonfocal, and 38 as mixed, on the basis of the symptoms with which they presented (table 2). Twelve participants experienced an attack that did not fit into either category as it consisted of a single isolated vertebrobasilar symptom (isolated diplopia, vertigo, or dysphagia). Of the patients with focal TNA, 35% consulted a neurologist, 50% consulted a general practitioner only, and 15% did not consult any physician; 33% reported the event at the subsequent visit to the research center. Of the patients with mixed TNA, 61% consulted a neurologist, 37% consulted another physician only, and 3% did not consult any physician; 18% reported the event at the subsequent visit to the research center. Of the patients with nonfocal TNA, 30% consulted a neurologist, 46% a general practitioner only, and 24% did not consult any physician; 26% reported the event at the subsequent visit to the research center. Twenty-one percent of reported possible TNAs were left out of the analyses; this was mainly because in these cases the symptoms lasted longer than 24 hours, or because a clear alternative diagnosis could be made (most notably migraine, epilepsy, Ménière's disease, hyperventilation, cardiac syncope, hypoglycaemia, or orthostatic hypotension).

Other events that were observed during follow-up were stroke (619 participants),

Table 1. Baseline characteristics of the study population (N=6062).

Characteristic	Median (interquartile range) or N (percentage)
Age (yrs)	67.7 (61.7-75.0)
Female	3758 (62 %)
Systolic blood pressure (mmHg)	137 (123-153)
Intima-media thickness (mm)	0.77 (0.68-0.87)
C-Reactive Protein (mg/l)	1.79 (0.87-3.43)
Total cholesterol (mmol/l)	6.6 (5.8-7.4)
HDL cholesterol (mmol/l)	1.3 (1.1-1.6)
Uric acid (μmol/l)	309 (263-364)
Waist/hip ratio	0.90 (0.83-0.96)
Body mass index (kg/m²)	25.9 (23.8-28.4)
Mini Mental State examination (/30)	28 (27-29)
Current smoking	1334 (22 %)
Ever smoking	3819 (63 %)
Atrial fibrillation	242 (4 %)
Diabetes mellitus	546 (9 %)
Hypertension	2000 (33 %)
PTCA or CABG	121 (2 %)
Angina pectoris	182 (3 %)
APOε4 carrier	1758 (29 %)
High vocational / university education	485 (8 %)

ischaemic heart disease (848 participants), vascular death (n=662; these participants were also classified as having stroke (n=192) or ischemic heart disease (n=430)), and dementia (609 participants).

Both in men and in women, nonfocal TNAs were almost as frequent as focal TNAs, and for both types of events the incidence rates strongly increased with increasing age (figure 1). Mixed TNAs were less frequent and their relation with age was less clear.

The Kaplan-Meier event-free survival time after TNA is shown in figure 2. The Cox regression analysis showed that, compared to participants without TNA, participants with focal TNA had a higher risk of stroke (HR 2.14, 95% CI 1.57-2.91) and ischaemic stroke (HR 2.61, 95% CI 1.78-3.84; tables 3 and 4); the risk of stroke within 90 days after focal TNA (TIA) was 3.5%. Nonfocal TNA patients had a higher risk of stroke (HR 1.56, 95% CI 1.08-2.28) and dementia (HR 1.59, 95% CI 1.11-2.26, table 5), especially vascular dementia (HR 5.05, 95% CI 2.21-11.6), than participants without TNA. Mixed TNA patients were at increased risk of stroke, and especially ischaemic stroke (HR 2.99, 95% CI 1.11-8.03); ischaemic heart disease, and especially myocardial infarction (HR 3.34, 95% CI 1.24-8.99); vascular death (HR 2.54, 95% CI 1.31-4.91); and dementia, especially vascular dementia

Table 2. Symptoms of TNAs that occurred during follow-up. Symptoms are based on all available information (general practitioners records, discharge letters, nursing home records, hospital discharge letters, self-reported information at research center).

	Focal TNA (n = 282)	Nonfocal TNA (n = 228)	Mixed TNA (n = 38)
Focal symptoms:			
Hemiparesis	49%	-	34%
Hemihypaesthesia	17%	-	3%
Dysphasia/dysartria	40%	-	55%
Amaurosis fugax / hemianopia	18%	-	29%
Diplopia	3%	-	8%
Vertigo	6%	-	8%
Hemiataxia	6%	-	3%
Nonfocal symptoms:			
Decreased consciousness	-	7%	0%
Unconsciousness	-	18%	21%
Confusion	-	6%	26%
Amnesia	-	12%	8%
Unsteadiness	-	15%	0%
Non-rotatory dizziness	-	19%	3%
Positive visual phenomena	-	8%	29%
Cardiac or vegetative signs	-	0%	11%
Paraesthesias	-	4%	0%
Bilateral weakness	-	3%	0%
Unwell	-	3%	3%
Other	-	6%	0%

(HR 21.5, 95% CI 6.48-71.3) compared to participants without TNA. Adjustment for confounding did not materially change these associations. Of the 12 patients with an aspecific vertebrobasilar TNA, during follow-up one experienced an unspecified stroke and two were diagnosed with Alzheimer's disease.

Discussion

In this large, prospective population-based study, TNAs with nonfocal symptoms were almost as frequent as focal TNAs, and had an equally unfavourable overall subsequent clinical course with a slightly higher risk of stroke and a higher risk of vascular dementia than persons without TNA. TNAs with combined focal and nonfocal symptoms had a particularly

Table 3. Hazard ratios for the association between incident TNA and risk of subsequent stroke.

Model	Type of TNA	Hazard ratio (95% CI)			
		Stroke		Ischaemic stroke	
		n		n	
Model 1*	No TNA (N=5714)	540	1 (ref)	316	1 (ref)
	Focal TNA (N=282)	46	2.14 (1.57-2.91)	31	2.61 (1.78-3.84)
	Nonfocal TNA (N=228)	27	1.56 (1.08-2.28)	10	1.16 (0.65-2.08)
	Mixed TNA (N=38)	6	2.48 (1.11-5.56)	4	2.99 (1.11-8.03)
Model 2 *	No TNA (N=5714)	540	1 (ref)	316	1 (ref)
	Focal TNA (N=282)	46	2.03 (1.50-2.76)	31	2.46 (1.67-3.63)
	Nonfocal TNA (N=228)	27	1.45 (1.00-2.10)	10	1.07 (0.60-1.91)
	Mixed TNA (N=38)	6	2.87 (1.28-6.44)	4	3.41 (1.26-9.21)

* Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and propensity score of C-reactive protein, systolic blood pressure, carotid intima-media thickness, total cholesterol, HDL cholesterol, uric acid, waist/hip ratio, mini mental state examination, current smoking, ever smoking, atrial fibrillation, diabetes mellitus, hypertension, PTCA or CABG, angina pectoris, APO ε4 carriership, education.

Table 4. Hazard ratios for the association between incident TNA and risk of subsequent ischaemic heart disease and vascular death.

	Type of TNA	Hazard ratio (95% CI)					
		Ischaemic heart disease		Myocardial infarction		Vascular death	
		n		n		n	
Model 1*	No TNA (N=5714)	779	1 (ref)	342	1 (ref)	594	1 (ref)
	Focal TNA (N=282)	32	1.07 (0.75-1.52)	10	0.74 (0.38-1.44)	35	1.21 (0.88-1.67)
	Nonfocal TNA (N=228)	29	1.19 (0.82-1.73)	10	1.05 (0.55-1.97)	25	1.14 (0.79-1.64)
	Mixed TNA (N=38)	8	2.26 (1.07-4.78)	5	3.34 (1.24-8.99)	8	2.54 (1.31-4.91)
Model 2*	No TNA (N=5714)	779	1 (ref)	342	1 (ref)	594	1 (ref)
	Focal TNA (N=282)	32	0.99 (0.70-1.42)	10	0.69 (0.35-1.34)	35	1.13 (0.82-1.57)
	Nonfocal TNA (N=228)	29	1.13 (0.77-1.64)	10	0.96 (0.51-1.81)	25	1.11 (0.77-1.60)
	Mixed TNA (N=38)	8	3.11 (1.47-6.57)	5	4.43 (1.64-11.9)	8	3.45 (1.71-6.95)

* Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and propensity score of C-reactive protein, systolic blood pressure, carotid intima-media thickness, total cholesterol, HDL cholesterol, uric acid, waist/hip ratio, mini mental state examination, current smoking, ever smoking, atrial fibrillation, diabetes mellitus, hypertension, PTCA or CABG, angina pectoris, APO ε4 carriership, education.

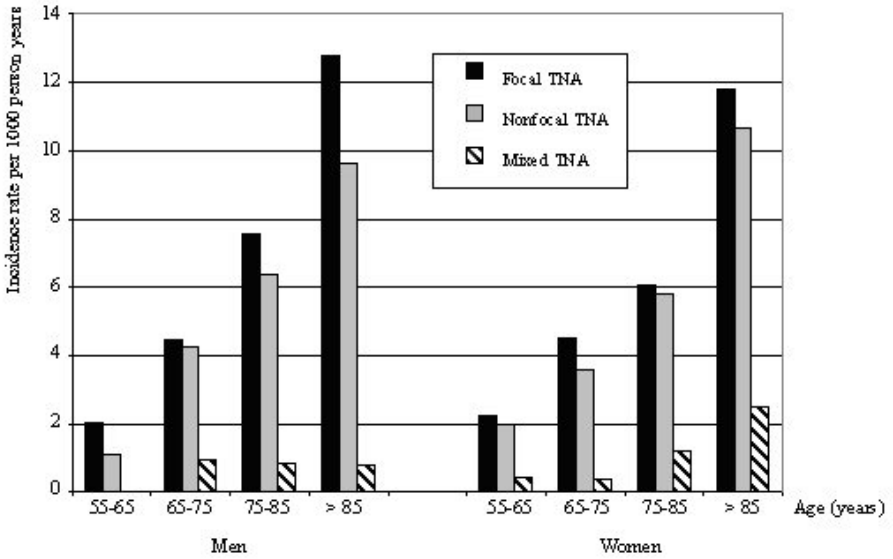


Figure 1. Age and sex specific incidence rates of TNAs.

bad prognosis, indicating that patients are at high risk of stroke, ischaemic heart disease, vascular dementia, and vascular death.

Before these results can be interpreted, some methodological issues need to be discussed. The strengths of our study are the large study population (N=6062), the intense monitoring of occurrence of diseases, the nearly complete follow-up (loss of potential person-years 3.8%), and the close collaboration with general practitioners, who are the gatekeepers of the Dutch medical system. We had full access to the general practitioners' medical records and verified each event for which the treating physician considered TIA, stroke, ischaemic heart disease, or dementia as a differential diagnosis. In addition, we also interviewed the participants in person at regular intervals. These procedures enabled us to ascertain cases that had not been referred to a neurologist, but that had only been seen by a general practitioner, nursing home physician, Rotterdam Study physician, or other physician, which was the case with 32% of all strokes, 39% of all mixed TNAs, 65% of all focal TNAs, and nearly all non-focal TNAs. We think that this makes our database unique. However, due to lack of neuroimaging and thorough examinations, 34% of strokes could not be subclassified as ischaemic or haemorrhagic. In addition, 129 possible TNAs had to be left out of the analyses because the symptoms had been described inadequately.

Regarding the allocation of TNAs to the various subgroups, a possible source of error is that, since most physicians (including those of the Rotterdam Study research center) are conditioned to look for focal symptoms, nonfocal symptoms may have been underreported, which may have led to the inclusion of mixed TNAs in the focal TNA group.

Participants with TNA before baseline were not excluded from our present study:

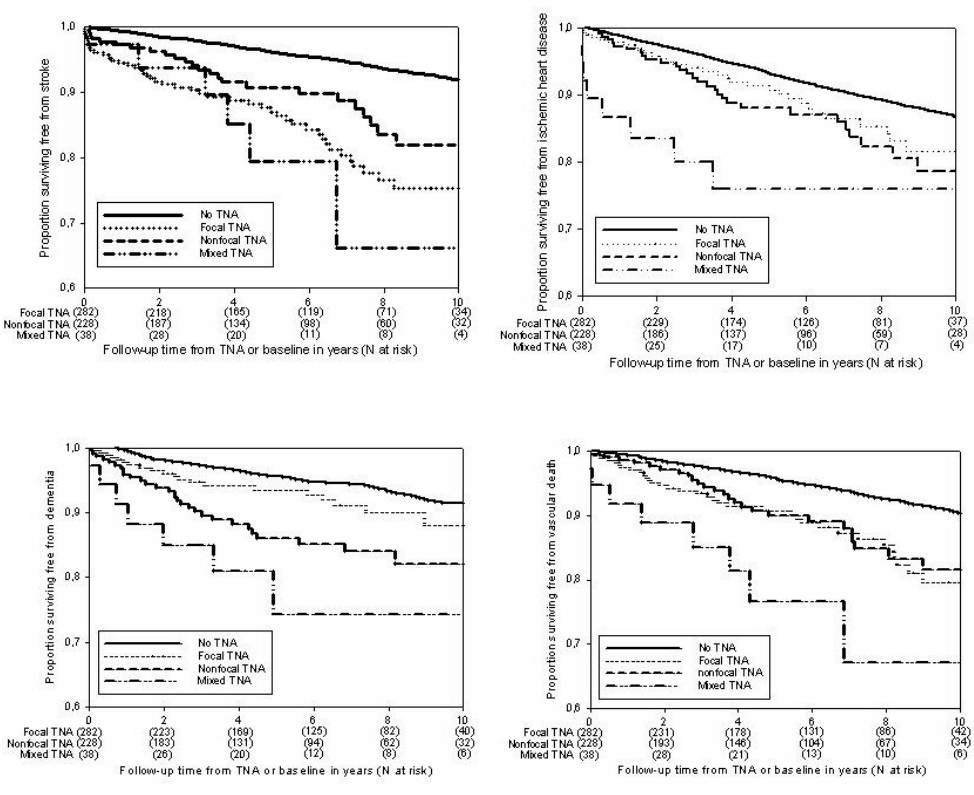


Figure 2. Kaplan-Meier event-free survival time after various types of TNAs.

no medical records on TNA before baseline were available, and we feared that participants would not be able to reliably recall TNA symptoms years after the event.

In our study, the risk of stroke within 90 days after focal TNA (TIA) was lower than in previous reports: 3.5% in our study compared to 10.5%[4] and 17.3%[5] reported previously. We think this may be because most other studies recruited patients from hospitals or asked the general practitioners to call a stroke consultant, whereas we had direct access to general practitioners' files and could also question the participants in person. Our study may therefore have included more focal TNAs with mild symptoms of short duration, which would have remained unnoticed by other studies, and which have a better prognosis.[24]

Because nonfocal TNAs present with a wide variety of symptoms, which are often ascribed to an equally wide variety of relatively harmless nonvascular conditions (although this is not supported by scientific evidence), they have not been studied as a group before. It is likely that our nonfocal TNAs are a heterogeneous group with respect to aetiology and prognosis. While further subdivision of nonfocal TNAs may be tempting, this should be done with great caution, not only because the subdivision has to be based on unreliable anamnestic data (with the patient often being confused, anxious, or even unconscious), but also because

Table 5. Hazard ratios for the association between incident TNA and risk of subsequent dementia.

	Type of TNA	Hazard ratio (95% CI)					
			Dementia		Alzheimer's disease		Vascular dementia
		n		n		n	
Model 1*	No TNA (N=5714)	552	1 (ref)	440	1 (ref)	49	1 (ref)
	Focal TNA (N=282)	20	0.94 (0.63-1.41)	19	0.96 (0.62-1.49)	1	1.16 (0.28-4.91)
	Nonfocal TNA (N=228)	30	1.59 (1.11-2.26)	22	1.30 (0.85-2.00)	5	5.05 (2.21-11.6)
	Mixed TNA (N=38)	7	3.46 (1.72-6.98)	3	2.05 (0.76-5.49)	3	21.5 (6.48-71.3)
Model 2*	No TNA (N=5714)	552	1 (ref)	440	1 (ref)	49	1 (ref)
	Focal TNA (N=282)	20	0.95 (0.63-1.43)	19	0.98 (0.63-1.51)	1	1.12 (0.27-4.77)
	Nonfocal TNA (N=228)	30	1.54 (1.08-2.20)	22	1.26 (0.83-1.93)	5	4.87 (2.12-11.2)
	Mixed TNA (N=38)	7	3.80 (1.79-8.04)	3	2.03 (0.65-6.33)	3	18.8 (5.67-62.6)

* Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and propensity score of C-reactive protein, systolic blood pressure, carotid intima-media thickness, total cholesterol, HDL cholesterol, uric acid, waist/hip ratio, mini mental state examination, current smoking, ever smoking, atrial fibrillation, diabetes mellitus, hypertension, PTCA or CABG, angina pectoris, APO ε4 carriership, education.

there is no empirical basis for the subdivision of nonfocal TNAs.

Our findings challenge the strong but unfounded conviction that nonfocal TNAs are harmless. On the contrary, our findings suggest that nonfocal TNAs are not only a risk factor for stroke, but also for dementia. For two small subgroups of nonfocal TNAs - transient global amnesia[9,10] and dizziness[8,25] - it has been reported that the clinical course is usually more benign. Unfortunately, our data do not allow us to determine the prognosis of various subgroups of nonfocal TNAs because of small sample size.

Only one previous study reported on the prognosis of mixed TNAs.[12] Although the definition of mixed TNAs differed slightly, in this study those with mixed TNA had a significantly higher risk of ischemic heart disease than those with focal TNA, which matches our findings very well. In addition, we found that mixed TNA patients are at increased risk of dementia, and especially of vascular dementia. Of course our findings regarding mixed TNAs have to be interpreted cautiously since the number of cases and events are relatively small.

Whereas the mechanisms leading to focal TNA have been well investigated,[26] we can only speculate on the mechanisms underlying nonfocal and mixed TNA. The strong association with cardiac events has led other researchers to hypothesise that mixed TNAs may be caused by cardiac arrhythmia[12] leading to transient cerebral hypoperfusion, which becomes most apparent in territories with pre-existing compromised arteries or brain tissue. The presumed (intermittent) poor cardiac performance may lead to dementia and stroke, and may deteriorate into clinically overt cardiac disease and death.

For nonfocal TNAs, it is imaginable that some subtypes in fact are atypical mani-

festations of vaso-occlusive disease or misinterpreted focal TNAs, which is supported by the association that we found between nonfocal TNA and stroke. This would mean that we are not looking at a distinct pathophysiological mechanism, but it does suggest that the diagnostic criteria that we use for TIA in clinical practice are too strict. However, the strong association with dementia supports the hypothesis that most nonfocal TNAs represent global or multifocal brain dysfunction (and not focal brain dysfunction) which can be attributed to two possible mechanisms: first, nonfocal TNAs could be caused by more global cerebral hypoperfusion, similar to mixed TNAs, but possibly with a smaller etiologic role of the heart. Second, nonfocal TNAs could represent a short and partly reversible exacerbation of a neurodegenerative process.

In conclusion, our findings confirm that focal TNAs (TIAs) indicate a high risk of stroke. Nonfocal TNAs and mixed TNAs do not have a benign clinical course: not only the risk of stroke, but also the risk of dementia is higher after a nonfocal TNA. Mixed TNAs may be harbingers of stroke, dementia, heart disease, and vascular death.

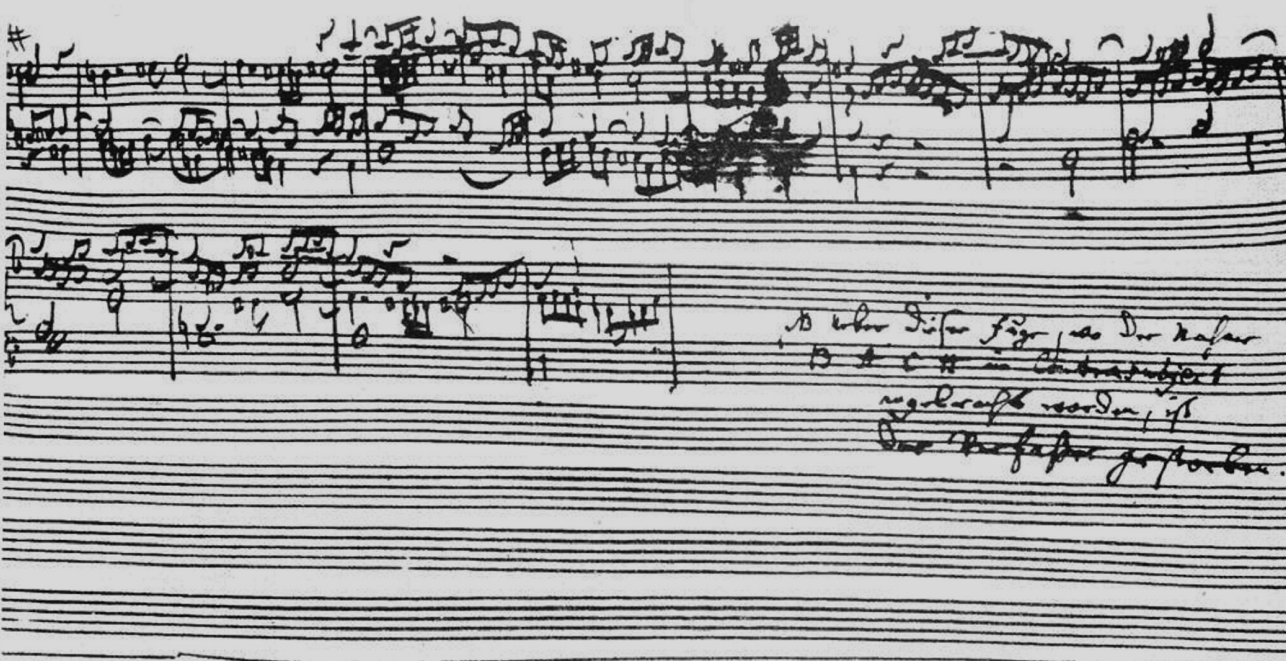
References

1. A classification and outline of cerebrovascular diseases. II. Stroke 1975;6(5):564-616.
2. Johnston SC. Editorial comment--Transient ischemic attacks are emergencies. Stroke 2005;36(4):724.
3. Daffertshofer M, Mielke O, et al. Transient ischemic attacks are more than “ministrokes”. Stroke 2004;35(11):2453-8.
4. Johnston SC, Gress DR, et al. Short-term prognosis after emergency department diagnosis of TIA. JAMA 2000;284(22):2901-6.
5. Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. BMJ 2004;328(7435):326.
6. Kleindorfer D, Panagos P, et al. Incidence and short-term prognosis of transient ischemic attack in a population-based study. Stroke 2005;36(4):720-3.
7. Strickberger SA, Benson DW, et al. AHA/ACCF Scientific Statement on the evaluation of syncope. Circulation 2006;113(2):316-27.
8. Evans JG. Transient neurological dysfunction and risk of stroke in an elderly English population: the different significance of vertigo and non-rotatory dizziness. Age Ageing 1990;19(1):43-9.
9. Gandolfo C, Caponnetto C, et al. Prognosis of transient global amnesia: a long-term follow-up study. Eur Neurol 1992;32(1):52-7.
10. Pantoni L, Bertini E, et al. Clinical features, risk factors, and prognosis in transient global amnesia: a follow-up study. Eur J Neurol 2005;12(5):350-6.
11. Bots ML, van der Wilk EC, et al. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. Stroke 1997;28(4):768-73.
12. Koudstaal PJ, Algra A, et al. Risk of cardiac events in atypical transient ischaemic attack or minor stroke. The Dutch TIA Study Group. Lancet 1992;340(8820):630-3.
13. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
14. Bos MJ, Koudstaal PJ, et al. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke 2006;37(6):1503-7.
15. Bos MJ, Schipper CM, et al. High serum C-reactive protein level is not an independent predictor for stroke: the Rotterdam Study. Circulation 2006;114(15):1591-8.
16. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 1962;27:645-58.

17. van Oijen M, Hofman A, et al. Plasma Abeta(1-40) and Abeta(1-42) and the risk of dementia: a prospective case-cohort study. Lancet Neurol 2006;5(8):655-60.
18. McKhann G, Drachman D, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34(7):939-44.
19. Roman GC, Tatemichi TK, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43(2):250-60.
20. Bots ML, Hoes AW, et al. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96(5):1432-7.
21. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet 2002;359(9314):1309-10.
22. Kardys I, Kors JA, et al. Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J 2003;24(14):1357-64.
23. Joffe MM, Rosenbaum PR. Invited commentary: propensity scores. Am J Epidemiol 1999;150(4):327-33.
24. Rothwell PM, Giles MF, et al. A simple score (ABCD) to identify individuals at high early risk of stroke after transient ischaemic attack. Lancet 2005;366(9479):29-36.
25. Tinetti ME, Williams CS, Gill TM. Dizziness among older adults: a possible geriatric syndrome. Ann Intern Med 2000;132(5):337-44.
26. Kimura K, Minematsu K, et al. The duration of symptoms in transient ischemic attack. Neurology 1999;52(5):976-80.

Chapter 6.

Stroke, depression, and cognitive function



6.1.
Depressive symptoms, depressive disorder, and risk of stroke

Background and purpose: *Previous studies that assessed whether presence of depressive symptoms predisposes to stroke in the general elderly population are contradictive. Moreover, they did not distinguish between men and women, nor did they perform psychiatric workups in those with depressive symptoms. We examined the association between depressive symptoms, depressive disorder, and risk of stroke in the general population.* ***Methods:*** *This prospective population-based cohort study was based on 4424 participants of the third Rotterdam Study survey (1997-1999), who at that time were ≥ 61 years of age and free from stroke. Depressive symptoms were assessed with the Center for Epidemiological Studies Depression Scale (CESD) and considered present if CESD score was ≥ 16. Participants with depressive symptoms had a diagnostic interview for depressive disorder. Follow-up was complete until January 1, 2005. Data were analyzed with Cox proportional hazards models with adjustment for relevant confounders.* ***Results:*** *Men with depressive symptoms (N=73) were at increased risk of stroke (adjusted HR 2.15; 95% CI 1.10-4.22) and ischemic stroke (adjusted HR 3.25; 95% CI 1.62-6.50). These associations were at least partly attributable to men who reported depressive symptoms but who did not fulfil DSM-IV diagnostic criteria for depressive disorder (N=32): they had a very high risk of stroke (adjusted HR 2.70, 95% CI 1.15-6.33) and ischemic stroke (adjusted HR 4.01, 95% CI 1.68-9.57). In women there was no association between presence of depressive symptoms and risk of stroke.* ***Conclusions:*** *Presence of depressive symptoms is a strong risk factor for stroke in men but not in women.*

Introduction

It was noted in the early 1970s that elderly patients with depressive disorder had a higher vascular disease burden than persons without depressive disorder.[1] This observation evolved into the *vascular depression hypothesis*, which assumes that depressive disorder can be caused by otherwise subclinical cerebrovascular disease.[2] The vascular depression hypothesis is supported by the observation that persons with depressive disorder have more white matter lesions on MRI.[3, 4] However, the directionality of the association between vascular disease and depressive disorder may also be the reverse, with depressive disorder not being a consequence but a cause of vascular disease. Depressive disorder may induce or enhance vascular disease by several mechanisms: for example, depressive disorder has been found to be associated with raised cortisol levels,[5] increased platelet reactivity,[6] and reduced heart rate variability.[7] In addition, depressive disorder is associated with unhealthy lifestyle choices, including choices regarding smoking[8] and medication adherence.[9] However, it seems that the prevalence of classic vascular risk factors is similar among persons with and without depressive disorder[10] and does not predict incident depression.[11]

It has indeed been reported that persons with depressive symptoms are at increased risk of vascular disease.[10] Salaycik and colleagues recently found that presence of depressive symptoms was a risk factor for stroke in persons younger than 65 years of age, but not in the elderly.[12] This contradicts earlier studies, that reported an increased stroke risk in elderly persons with depressive symptoms.[13, 14] More controversies remain regarding the association between depressive disorder and stroke. First, most previous studies assessed presence of depressive symptoms with a short questionnaire,[3, 13-18] thus ignoring the nature of depressive symptoms, which are often not part of a depressive disorder.[19] Although most commentators acknowledge this, they still opt for taking the line that depressive symptoms in general represent depressive disorders.[20] Second, depressive symptoms have much higher prevalence in women than in men,[21] and it would be helpful to know whether gender differences in the association between depressive disorder and risk of stroke exist.

We assessed the association between depressive symptoms, depressive disorder and risk of subsequent stroke in men and women in a population-based cohort study.

Methods

Source population: The present study is embedded within the Rotterdam Study, a population-based cohort study on chronic and disabling diseases.[22] Invitations to participate in the first study survey (1990-1993) were sent to all inhabitants of Ommoord, a district in the city of Rotterdam in the Netherlands, aged 55 years and over. The participation rate of those invited for the study was 78%; in total, 7,983 subjects participated. Participants have been

followed since and screening for depressive symptoms was added to the core protocol of the Rotterdam Study at the third survey (1997-1999), which constitutes the baseline survey for the present study. At this time participants were 61 years of age and over. The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus University Rotterdam. After complete description of the study to the subjects, written informed consent was obtained. Follow-up for incident stroke was complete until January 1, 2005.

Assessment of depressive symptoms and depressive disorder: Depressive disorders were assessed by a 2-step procedure. First, participants completed the Dutch version of the original Center for Epidemiological Studies Depression Scale (CESD) during the home interview. The CESD is a 20-item self-reported measure of symptoms scored on a scale of 0 to 3 points.[23] We used a score of 16 as a cutoff to indicate depressive symptoms. This cutoff had a very high sensitivity for major depressive disorder in a random sample of older subjects in the Netherlands.[19] Previous studies have verified that a score of 16 and above on the CESD indicates clinically significant depressive symptoms.[15] In a second step, screen-positive subjects had a psychiatric workup. They were studied with the Dutch version of the Present State Examination, a semistructured psychiatric interview included in the schedules for Clinical Assessment in Neuropsychiatry.[24] All interviews were conducted by one of 2 experienced clinicians, a psychiatrist and a clinical psychologist. Psychiatric disorders were classified according to the DSM-IV criteria with an algorithm based on the Present State Examination scores. The diagnostic criteria included major depression, dysthymia, and minor depressive disorder as defined in the DSM-IV and its appendix.

Other Measurements: Blood pressure was measured twice in the right arm using a random-zero sphygmomanometer, with the participant in sitting position. We used the average of these 2 measurements. The carotid intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery. We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far walls of both the right and left common carotid artery. Cognitive performance was assessed with a Mini Mental State Examination (MMSE).[25] We considered diabetes mellitus to be present if a fasting glucose level was 7.0 mmol/L or higher, or if a person used antidiabetic medication. History of transient ischemic attack (TIA) was positive if an attack of presumed vascular origin with focal symptoms that completely resolved within 24 hours in absence of signs of nonfocal (global) brain dysfunction was reported during the interview or in general practitioners' files. TIAs that occurred more than 3 years before the first study survey were not considered. History of myocardial infarction, percutaneous transluminal angioplasty (PTCA), and coronary artery bypass graft (CABG) was assessed during the first survey home interview and considered positive if a positive answer was confirmed by medical records or ECG; after enrollment into the study medical records were monitored continuously. Smoking and medication use were

assessed during the home interview.

Assessment of Stroke: History of stroke at the first Rotterdam Study survey (1990-1993) was positive if a stroke was reported during the baseline interview and confirmed by medical records. After enrollment into the Rotterdam Study, participants were continuously monitored for stroke and TIA through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all reported events with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. Strokes were coded according to the International Classification of Diseases, 10th edition (ICD-10). Strokes were ischemic strokes (I63), primary intracerebral hemorrhages (I61), and unspecified strokes (I64). A stroke was subclassified as ischemic (I63) if a CT or MRI scan made within 4 weeks after onset of symptoms ruled out other diagnoses, or if indirect evidence (deficit limited to 1 limb or complete recovery within 72 hours, or atrial fibrillation in absence of anticoagulant therapy) indicated the ischemic nature of the stroke. Follow-up was complete until January 1, 2005, for 99.0% of potential person years.[26]

Population for Analysis: A total of 5685 Rotterdam Study participants were free from stroke and eligible to participate in the third study survey. Of these, 1045 refused the interview and 140 were physically unable to participate. Consequently, 4500 participants were subjected to the CESD interview. Incomplete information on CESD led to the exclusion of 76 participants, leaving a study population of 4424 participants for the analyses of the association between depressive symptoms and risk of stroke. Thirty participants with depressive symptoms did not undergo psychiatric workup, leaving 4394 participants eligible for analyses of the association between depressive disorder and risk of stroke.

Statistical Analysis: We compared the risk of stroke and ischemic stroke in participants with depressive symptoms (CESD score ≥ 16) with the risk of stroke and ischemic stroke in participants without depressive symptoms (CESD score < 16) with Cox proportional hazards models. Subsequently, we repeated these analyses distinguishing between participants who had depressive symptoms with and without depressive disorder according to the DSM-IV. Participants were censored at time of first-ever stroke, death, study end or loss to follow-up, whichever occurred first. We performed the analyses for men and women combined and separately. We adjusted for confounding by age and sex (model 1), and additionally for confounding by other putative confounders (systolic blood pressure, diabetes mellitus, cigarette smoking (ever), cigarette smoking (current), intima-media thickness, history of myocardial infarction, history of PTCA or CABG, history of TIA, antithrombotic drug use, antihyper-

tensive drug use, cholesterol lowering drug use, psycholeptic drug use, and psychoanaleptic drug use; model 2). We replaced missing values in putative confounders by the geometric mean. We had missing values for intima-media thickness (20%), systolic blood pressure (12%), MMSE score (12%), and medication use (8%). Analyses were performed with SPSS® 11.0 for Windows and results expressed as hazard ratios (HRs) with 95% confidence intervals (CIs).

Results

During 24,657 person years of follow-up, 291 strokes (190 ischemic, 31 hemorrhagic, and 70 unspecified) occurred. At baseline the median age was 71.9 years and 60% of participants were women (baseline characteristics: table 1). Participants with depressive symptoms at baseline had a non-significantly higher risk of stroke (age and sex adjusted HR 1.20, 95% CI 0.81-1.80) and of ischemic stroke (age and sex adjusted HR 1.43, 95% CI 0.89-2.31) than participants without depressive symptoms (table 2). Associations between depressive symptoms and risk of stroke were stronger in men than in women (P for interaction 0.05 for stroke and 0.008 for ischemic stroke): men with depressive symptoms were at strongly increased risk of stroke and ischemic stroke compared to men without depressive symptoms: the age and sex adjusted HR was 2.11 (95%CI 1.11-4.04) for stroke and 3.09 (95%CI 1.60-

Table 1. Baseline characteristics of study population at third Rotterdam Study survey.

Characteristic	Men (N=1759)*	Women (N=2665)*
Age	71.2 (66.6-76.4)	72.5 (67.1-78.7)
Systolic blood pressure (mmHg)	142 (129-157)	142 (128-156)
Intima-media thickness (mm)	0.89 (0.80-0.99)	0.83 (0.75-0.93)
MMSE score	28 (27-29)	28 (27-29)
Diabetes mellitus	266 (15 %)	346 (13 %)
Cigarette smoking (ever)	1546 (88 %)	1314 (49 %)
Cigarette smoking (current)	314 (18 %)	414 (16 %)
History of myocardial infarction	304 (17 %)	189 (7 %)
History of PTCA or CABG	167 (9 %)	83 (3 %)
History of TIA	107 (6 %)	156 (6 %)
Antithrombotic drug use	476 (27 %)	535 (20 %)
Antihypertensive drug use	654 (37 %)	1051 (39 %)
Cholesterol lowering drug use	224 (13 %)	294 (11 %)
Psycholeptic drug use	143 (8 %)	526 (20 %)
Psychoanaleptic drug use	35 (2 %)	102 (4 %)

* Values are presented as median (interquartile range) or percentage

Table 2. Depressive symptoms at third Rotterdam Study survey and risk of subsequent first-ever stroke.

Included participants	CESD score (N)	Hazard ratio (95% CI)			
		All strokes*		Ischemic strokes*	
		Model 1†	Model 2†	Model 1†	Model 2†
All participants	CESD < 16 (4100)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(N=4424)	CESD ≥ 16 (324)	1.20 (0.81-1.80)	1.21 (0.80-1.83)	1.43 (0.89-2.31)	1.43 (0.87-2.35)
Men	CESD < 16 (1686)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(N=1759)	CESD ≥ 16 (73)	2.11 (1.11-4.04)	2.17 (1.11-4.23)	3.09 (1.60-5.98)	3.21 (1.62-6.38)
Women	CESD < 16 (2414)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(N=2665)	CESD ≥ 16 (251)	0.94 (0.57-1.56)	0.91 (0.55-1.53)	0.86 (0.43-1.71)	0.78 (0.39-1.59)

* We observed 124 first-ever strokes of any type in men and 167 in women. Of these, 91 were ischemic in men and 99 in women.

† Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diabetes mellitus, cigarette smoking (ever), cigarette smoking (current), intima-media thickness, history of myocardial infarction, history of PTCA or CABG, history of TIA, antithrombotic drug use, antihypertensive drug use, cholesterol lowering drug use, psycholeptic drug use, and psychoanaleptic drug use.

5.98) for ischemic stroke. In women there was no association between presence of depressive symptoms and risk of (ischemic) stroke. HRs were not attenuated by further adjustment for confounding.

Of all participants with depressive symptoms on CESD, the subsequent clinical examination showed that only 46% had a DSM-IV depressive disorder; the remainder had anxiety disorder (N=21), other axis 1 psychiatric diagnosis (N=14), or no axis 1 psychiatric diagnosis (N=123).

Men who met DSM-IV criteria for depressive disorder were at increased stroke risk

(age and sex adjusted HR 1.75, 95% CI 0.56-5.51; table 3), although not statistically significantly at α=0.05. Men with depressive symptoms who did not meet DSM-IV criteria were at significantly increased risk of stroke (age and sex adjusted HR 2.45, 95% CI 1.07-5.58). When only ischemic strokes were included, both associations became stronger. Like men, women with depressive symptoms in absence of depressive disorder were at higher risk of stroke than women without depressive symptoms or women with depressive disorder. However, this was far from statistically significant.

Discussion

In this population-based study, we found that men with depressive symptoms were at increased risk of subsequent stroke and ischemic stroke compared to men without depressive symptoms. The increased risk of stoke in men with depressive symptoms was at least partly attributable to men who had depressive symptoms in absence of depressive disorder. In women there was no association between presence of depressive symptoms and risk of stroke.

Some methodological issues need to be discussed before these results can be interpreted. Strengths of our study are the large study population (n=4424), the thorough stroke case finding, the diagnostic workup for depressive disorder, and the nearly complete follow-up (loss of potential person years was 1%). Stringent stroke monitoring procedures made it possible to also include stroke patients who were not referred to a neurologist (31% of all stroke cases). As neuroimaging had not been performed in these non-referred cases, we could subclassify only 16% of them into ischemic or hemorrhagic. In contrast, 92% of strokes that had been diagnosed by a neurologist could be subclassified. For some putative confounding variables we had incomplete information. This is because participants were visited at home for the assessment of depressive symptoms and depressive disorder, whereas for most other measurements they had to attend the research center.

Several previous studies reported on the association between depressive symptoms and stroke: a positive association has been reported between presence of depressive symptoms and risk of fatal stroke,[16, 27] and between presence of depressive symptoms and stroke in hypertensives[18] and in young persons.[17, 28] Three studies were performed in the general elderly population; one of these found that risk of stroke increased with increasing CESD score (adjusted HR 1.23, 95% CI 1.05-1.44 per unit increase in logged CESD score),[13] another found an adjusted HR of 1.41 (95% CI 1.01-1.96) for stroke risk for highest versus lowest tertile of CESD score,[14] and the most recent one found no association between CESD score $\geq$ 16 and risk of stroke in subjects of 65 years of age and over (adjusted HR 0.78, 95% CI 0.46-1.32). However, in the last-mentioned study there was an association between presence of depressive symptoms and stroke in participants younger than 65 years of age. We found an association between presence of depressive symptoms and risk of stroke in elderly persons,

Table 3. Depressive disorder at third Rotterdam Study survey and risk of subsequent first-ever stroke.

Included participants	Diagnostic classification (N)	Hazard ratio (95% CI)			
		All strokes†		Ischemic strokes†	
		Model 1‡	Model 2‡	Model 1‡	Model 2‡
Men	CESD < 16 (1686)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(N=1759)	CESD ≥ 16 without depressive disorder (32)*	2.45 (1.07-5.58)	2.70 (1.15-6.33)	3.61 (1.57-8.30)	4.01 (1.68-9.57)
	CESD ≥ 16 with depressive disorder (32)*	1.75 (0.56-5.51)	1.63 (0.51-5.26)	2.52 (0.80-7.97)	2.43 (0.74-7.92)
Women	CESD < 16 (2414)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
(N=2665)	CESD ≥ 16 without depressive disorder (126)*	1.27 (0.70-2.28)	1.30 (0.72-2.35)	1.44 (0.70-2.98)	1.41 (0.68-2.92)
	CESD ≥ 16 with depressive disorder (104)*	0.69 (0.28-1.68)	0.62 (0.25-1.54)	0.24 (0.03-1.72)	0.19 (0.03-1.41)

* Diagnotic workup missing in 9 CESD positive men and 21 CESD positive women. Depressive disorders were major depression (13 men, 46 women), dysthymia (5 men, 16 women), and minor depresion (14 men, 42 women). Participants with CESD ≥ 16 without depressive disorder had the following diagnoses: subclinical depressive symptoms and no underlying psychiatric diagnosis (20 men, 103 women), anxiety disorder (6 men, 15 women), other axis 1 psychiatric diagnosis (6 men, 8 women).

† We observed 123 first-ever strokes of any type in men and 167 in women. Of these, 90 were ischemic in men and 99 in women.

‡ Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, diabetes mellitus, cigarette smoking (ever), cigarette smoking (current), intima-media thickness, history of myocardial infarction, history of PTCA or CABG, history of TIA, anti-thrombotic drug use, antihypertensive drug use, cholesterol lowering drug use, psycholeptic drug use, and psychoanaleptic drug use.

but only in men and not in women. Nearly all previous studies used self-reported depressive symptom scales to assess presence of depressive symptoms at baseline and did not study clinical diagnosis of depressive disorder. In our study more than half of participants who scored positive for depressive symptoms on CESD did not have depressive disorder, and we found that the observed association between depressive symptoms and stroke in men was at least partly driven by men with depressive symptoms in absence of depressive disorder. One previous study described the association between depressive disorder and stroke,[28] but this was a small study with younger participants which only studied fatal or self-reported stroke. Many mechanisms have been proposed that could explain the association between depressive disorder and vascular disease: for example, depressive disorder has been found to be associated with smoking,[8] medication non-adherence,[9] more cerebral white matter lesions,[3, 4] increased platelet reactivity,[6] raised cortisol levels,[5] reduced heart rate variability,[7] hypertension, and glucose intolerance,[29] which can be either causes or consequences of depressive disorder. According to our analyses, classical vascular risk factors could not explain the associations we found. As mentioned, we found a strong association between depressive symptoms in the absence of depressive disorder and stroke. Similar mechanisms may play a role here.

Our finding that the association between depressive symptoms in absence of depressive disorder and risk of stroke seemed stronger than the association between depressive disorder and risk of stroke could suggest that hypoxic brain damage (caused by irreversibly damaged cerebral arteries) is capable of causing depressive symptoms, that often do not fulfill psychiatric criteria of a depressive disorder. This may be because the development of depressive disorder is driven by genetic factors and not by vascular damage alone, whereas vascular disease could lead to depressive symptoms. This hypothesis is supported by the observation that the prevalence of depressive disorder decreases with age[30] whereas the prevalence of depressive symptoms increases with age.[31] However, the subdivision of men and women with depressive symptoms into those with depressive disorder and those without depressive disorder requires very much power, therefore these results have to be interpreted carefully.

The absence of an association between depressive symptoms and stroke in women might be attributable to the different, and probably more heterogeneous, etiology of depressive symptoms among women compared to men,[21] which is illustrated by the 2.5 fold excess in prevalence of depressive symptoms in women (9.4 %) compared to men (4.2 %): non-biological or hormonal factors without somatic consequences may play a larger role in women than in men. These prevalences are similar to those reported by others.[32]

In conclusion, presence of depressive symptoms is an important risk factor for stroke in men, perhaps more so if depressive symptoms cannot be attributed to depressive disorder. Presence of depressive disorder is not a risk factor for stroke in women.

References

1. Post F. The management and nature of depressive illnesses in late life: A follow-through study. Br J Psychiatry. 1972;121:393-404
2. Alexopoulos GS, Meyers BS, et al. 'vascular depression' hypothesis. Arch Gen Psychiatry. 1997;54:915-922
3. Steffens DC, Helms MJ, et al. Cerebrovascular disease and depression symptoms in the Cardiovascular Health Atudy. Stroke. 1999;30:2159-2166
4. de Groot JC, de Leeuw FE, et al. Cerebral white matter lesions and depressive symptoms in elderly adults. Arch Gen Psychiatry. 2000;57:1071-1076
5. O'Brien JT, Ames D, et al. Clinical and magnetic resonance imaging correlates of hypothalamic-pituitary-adrenal axis function in depression and Alzheimer's disease. Br J Psychiatry. 1996;168:679-687
6. Musselman DL, Tomer A, et al. Exaggerated platelet reactivity in major depression. Am J Psychiatry. 1996;153:1313-1317
7. Carney RM, Saunders RD, et al. Association of depression with reduced heart rate variability in coronary artery disease. Am J Cardiol. 1995;76:562-564
8. Breslau N, Peterson EL, et al. Major depression and stages of smoking. A longitudinal investigation. Arch Gen Psychiatry. 1998;55:161-166
9. Carney RM, Freedland KE, et al. Major depression and medication adherence in elderly patients with coronary artery disease. Health Psychol. 1995;14:88-90
10. Thomas AJ, Kalaria RN, O'Brien JT. Depression and vascular disease: What is the relationship? J Affect Disord. 2004;79:81-95
11. Kim JM, Stewart R, et al. Vascular risk factors and incident late-life depression in a korean population. Br J Psychiatry. 2006;189:26-30
12. Salaycik KJ, Kelly-Hayes M, et al. Depressive symptoms and risk of stroke: The Framingham study. Stroke. 2007;38:16-21
13. Colantonio A, Kasi SV, Ostfeld AM. Depressive symptoms and other psychosocial factors as predictors of stroke in the elderly. Am J Epidemiol. 1992;136:884-894
14. Simons LA, McCallum J, et al. Risk factors for ischemic stroke: Dubbo study of the elderly. Stroke. 1998;29:1341-1346
15. Blazer D, Burchett B, et al. The association of age and depression among the elderly: An epidemiologic exploration. J Gerontol. 1991;46:M210-215
16. Everson SA, Roberts RE, et al. Depressive symptoms and increased risk of stroke mortality over a 29-year period. Arch Intern Med. 1998;158:1133-1138
17. Jonas BS, Mussolino ME. Symptoms of depression as a prospective risk factor for stroke. Psychosom Med. 2000;62:463-471
18. Simonsick EM, Wallace RB, et al. Depressive symptomatology and hypertension-associated morbidity and mortality in older adults. Psychosom Med. 1995;57:427-435
19. Beekman AT, Deeg DJ, et al. Criterion validity of the center for epidemiologic studies depression scale (CES-D): Results from a community-based sample of older subjects in The Netherlands. Psychol Med. 1997;27:231-235
20. Carod-Artal FJ. Are mood disorders a stroke risk factor? Stroke. 2007;38:1-3
21. Prince MJ, Beekman AT, et al. Depression symptoms in late life assessed using the EURO-D scale. Effect of age, gender and marital status in 14 European centres. Br J Psychiatry. 1999;174:339-345
22. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The rotterdam elderly study. Eur J Epidemiol. 1991;7:403-422
23. Sawyer-Radloff L. The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Meas. 1977;1:385-401
24. Health WHODoM. Scan schedules for clinical assessment in neuropsychiatry, version 2.1: Distribution from training centers. 1997
25. Folstein MF, Folstein SE, McHugh PR. "mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189-198
26. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;359:1309-1310
27. Gump BB, Matthews KA, et al. Depressive symptoms and mortality in men: Results from the multiple risk factor intervention trial. Stroke. 2005;36:98-102

28. Larson SL, Owens PL, et al. Depressive disorder, dysthymia, and risk of stroke: Thirteen-year follow-up from the baltimore epidemiologic catchment area study. Stroke. 2001;32:1979-1983
29. May M, McCarron P, et al. Does psychological distress predict the risk of ischemic stroke and transient ischemic attack? The Caerphilly Study. Stroke. 2002;33:7-12
30. Eaton WW, Kalaydjian A, et al. Prevalence and incidence of depressive disorder: The Baltimore ECA follow-up, 1981-2004. Acta Psychiatr Scand. 2007;116:182-188
31. Cervilla J, Prince M, Rabe-Hesketh S. Vascular disease risk factors as determinants of incident depressive symptoms: A prospective community-based study. Psychol Med. 2004;34:635-641
32. Beekman AT, Copeland JR, Prince MJ. Review of community prevalence of depression in later life. Br J Psychiatry. 1999;174:307-311

6.2.
Pre-stroke cognitive performance, incident stroke and risk of dementia

Background and Purpose: *Several studies indicate that stroke increases the risk of dementia. Most of these studies lacked the ability to take accurately assessed pre-stroke cognitive function into account. Whether the effects of stroke merely unravel an ongoing underlying dementing process or in fact cause the dementia has implications for the prevention of dementia in persons with cerebrovascular disease. We explored in a prospective cohort study whether stroke occurrence was related to a higher risk of subsequent dementia, and whether this association was dependent on pre-stroke slope of cognitive function.* ***Methods:*** *Cox proportional hazard models were used to relate incident stroke as a time-varying exposure with the risk of dementia in 6,724 participants of the Rotterdam Study without dementia or stroke at baseline (49,361 person-years of follow-up). Subsequently Cox-proportional hazard models were performed to assess whether this association was dependent on slope of pre-stroke cognitive performance and other risk factors for cognitive decline.* ***Results:*** *Independent of both level and the rate of change of pre-stroke cognitive performance and other risk factors for cognitive decline, incident stroke was associated with a more than doubled risk of subsequent dementia (HR 2.1, 95% CI 1.55-2.81).* ***Conclusions:*** *Pre-stroke cognitive function is not a major determinant of the effect of stroke on the risk of post-stroke dementia.*

Introduction

Cerebrovascular disease and dementia are among the most common diseases in aging societies. Cerebrovascular disease is the second leading cause of mortality in western societies and the major cause of long-term disability, leaving 30% disabled.[1] About 1 percent of people aged 65-69 years have dementia, and this proportion increases with age to approximately 60% for people over the age of 95.[2]

Epidemiologic evidence is accumulating that both disorders are linked. In their recently published review, Leys et al.[3] summarized the previous studies that explored the impact of stroke on the risk of post-stroke dementia (PSD). According to these studies, stroke considerably increases the risk of dementia, with prevalence rates ranging from 13.6 to 32% within 3 months to 1 year after stroke, and incidence rates of new onset dementia after stroke ranging from 24% within 3 years to 33.3% within 5 years.[3-13]

Of essential clinical implication for the prevention of dementia in persons with cerebrovascular disease is the clarification of whether the effects of stroke merely unravel an ongoing underlying dementing process or whether they in fact cause the dementia. If stroke itself would cause the dementia syndrome, neuroprotective intervention after occurrence of stroke would be of major importance. If stroke would merely unravel a masked ongoing dementing process, the expected effect of such intervention would be much smaller and the underlying process should be targeted.

To have the ability to accurately interpret the impact of stroke on the risk of PSD, pre-stroke level of cognitive function must be taken into account. This in turn, demands several methodological features from the study design: it requires assessment of pre-stroke cognitive status using an adequate neuropsychogical test battery, a long enough-follow-up time between pre-stroke cognitive assessment and occurrence of stroke, and subsequently a long enough follow-up time between the incident stroke and subsequent dementia or censoring. Ideally, the impact of pre-stroke cognitive status should be assessed using the slope of pre-stroke cognitive performance over time, since the effect of stroke on risk of cognitive impairment may depend on the rate of decline in cognitive function before stroke.

As stated by Leys et al,[3] the studies that related pre-stroke cognitive performance with PSD reported a higher risk of PSD after 3 months[4-7] and 3 years[8, 9] in persons with pre-stroke cognitive decline compared with persons without cognitive impairment before stroke. These studies, however, had been obtained from stroke cohorts assessing pre-stroke cognitive function either by measuring cognitive performance at time of hospital admission, or by using dementia diagnoses based on pre-stroke medical records.[4, 5, 9-11] Thus, they in fact lacked the essential ability to take cognitive function before stroke into account, leaving the true impact of stroke on dementia risk unclear.

The objective of the present study was to elucidate the impact of stroke on the risk of PSD as a function of pre-stroke cognitive performance by assessing the impact of pre-stroke

cognitive performance on the association between incident stroke and risk of subsequent dementia in the large prospective population-based Rotterdam Study. We also sought to assess the effect of pre-stroke measures of other common risk factors for cognitive decline on the risk of dementia after stroke. Since the clinical distinction between dementia subtypes AD and VaD is difficult, in particular when a stroke has occurred, and persons with a diagnosis of stroke are by definition more likely to receive a diagnosis of VaD rather than AD, we focused on the risk of dementia, rather than on the dementia subtypes AD or VaD.

Methods

Participants: The Rotterdam Study is a population-based prospective cohort study that has been described elsewhere.[12] From 1990 to 1993, all 10 275 residents aged ≥55 years of Ommoord, a district of the city of Rotterdam, were invited to participate, and 7,983 (78%) men and women agreed. During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994, 1997-1999 and 2002-2004. Through linkage with records of general practitioners, the entire cohort was continuously monitored for morbidity and mortality. This follow-up information was available for all participants until January 1, 2005.
From the 7,983 participants who underwent baseline examination, 7,528 were screened for dementia (94.3%). From these, 482 persons (6.4%) were diagnosed with prevalent dementia, 175 persons (2.2%) had at baseline a history of stroke, and 147 persons (2.0%) did not agree to give informed consent for collecting stroke information. The final analytic sample included in this study comprised 6,724 persons without dementia or stroke at baseline. Follow-up with the respect to dementia and stroke was nearly complete (96.7%).

Diagnosis of Dementia: Diagnostic procedures for dementia have been described in detail.[13] At baseline and both follow-up examinations, a three-stage protocol was used to screen all participants cognitively with the Mini-Mental State Examination (MMSE)[14] and the Geriatric Mental State schedule (GMS) organic level.[15] If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)[16] was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, underwent magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health

Care.[13] The diagnoses of dementia and Alzheimer disease were based on Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition (DSM-III-R) criteria[17] and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria,[18] respectively, and were made by a panel of a neurologist, neuropsychologist, and research physicians who reviewed all existing information.[13] The diagnosis of vascular dementia was based on NINDS-AIREN criteria.[19]

Assessment of stroke: History of stroke at time of enrollment into the Rotterdam Study was assessed by the question 'did you ever suffer from a stroke, diagnosed by a physician?' Positive answers to this question were verified by review of medical records. After baseline assessment participants were continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. In addition, nursery home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information including brain imaging was obtained from hospital records. Research physicians discussed information on all potential strokes and transient ischemic attacks with an experienced stroke neurologist to verify all diagnoses. Subarachnoid hemorrhages and retinal strokes were excluded from the stroke diagnosis. Strokes were subclassified into hemorrhagic or ischemic stroke based on neuroimaging. Strokes which could not be subclassified as ischemic or hemorrhagic, were called unspecified.

Assessment of other covariates: Level of education was categorized into 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Smoking habits were categorized as ever smoking and non-smoking. Body mass index was calculated using the formula [weight (kg)/length^2 (m^2)]. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Diabetes mellitus was defined as a random or postload glucose level $\geq$11.1 mmol/L or a history of diabetes or the use of blood glucose-lowering medication.

Nonfasting blood samples were drawn and immediately frozen. Total cholesterol, high-density lipoprotein cholesterol, and glucose were measured within 2 weeks, as described previously.[20] Levels of serum C-reactive protein (CRP) were determined by the rate near infrared particle immunoassay method (Immage high-sensitivity CRP, Beckman Coulter).

Ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries, we used intima media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[21] Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without

knowledge of the dementia diagnosis.[22] We dichotomized APOE genotype into presence or absence of the apolipoprotein Eε4 (APOEε4) allele. APOEε2ε4 carriers were excluded from the analyses.

Statistical Methods: First we evaluated the demographic and clinical characteristics of the study sample at baseline. Then we performed Kaplan-Meier analyses to determine the proportion of participants surviving free of dementia among persons without incident stroke, persons with incident stroke with normal pre-stroke cognitive function (last MMSE score before stroke ≥ 26), and persons with incident stroke with low pre-stroke cognitive function (last MMSE score before stroke <26). In these analyses, the date of onset of dementia was considered to be the date of the visit at which dementia was diagnosed.

We explored the individual effects of incident stroke and pre-stroke cognitive function on the risk of PSD with Cox proportional hazards analyses. For the analysis regarding the effect of stroke on subsequent dementia, incident stroke was entered as a time-varying exposure. For the analysis regarding the effect of pre-stroke cognitive function on the risk of dementia after stroke, we first used baseline measures of MMSE, and in a subsequent analysis rate of decline in MMSE over time before occurrence of stroke or censoring.

To explore the impact of pre-stroke cognitive function on the association between incident stroke and subsequent dementia, we performed Cox proportional hazards analyses in which we added an interaction term to the model that contained both main effects described above, ie. the term relating incident stroke to PSD and the term relating pre-stroke cognitive impairment to PSD. Pre-stroke cognitive function was again assessed first using baseline measures of MMSE, and then using rate of decline in MMSE over time before occurrence of stroke or censoring.

To explore the effect modification of the association between incident stroke and subsequent incident dementia by other putative risk factors for cognitive decline, we finally repeated all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and the individual risk factor. Risk factors for cognitive decline assessed in these analyses were age, diabetes mellitus, APOEε4 genotype, systolic and diastolic blood pressure, serum CRP levels, body mass index, and IMT.

We initially adjusted all models for sex and age, and subsequently additionally for APOEε4 genotype and education. The time-to-event variable in all models was age at onset of dementia, death or end of follow-up. Individuals who developed dementia before incident stroke were censored at time of dementia diagnosis. Persons who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of dementia were censored at the time of their last evaluation. Because the distribution of serum CRP levels was skewed, logarithmic transformation of this variable was carried out before analyses were performed. All data analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, Ill).

Results

There were 6, 724 persons without dementia or stroke at baseline, with 49,361 person-years of follow-up (mean 7.3 person-years, SD 4.3 person-years). From these 6,724 individuals, 713 persons (10.6%) had a stroke during follow-up, and 55 persons subsequently developed dementia after stroke (8.3% of persons with incident stroke). Out of those 55 persons with dementia, 32 (58.2%) were diagnosed with VaD, and 18 (32.7%) were diagnosed with AD. During follow-up 627 persons (9.7%) developed dementia without previously having a stroke. Out of these, 514 persons (81.9%) developed AD, 42 persons (6.7%) VaD, and 71 persons developed dementia other than AD or VaD (11.3%).

The baseline demographic and clinical characteristics of the study sample are shown in table 1. In Kaplan-Meier-analyses, the cumulative proportion of survivors without dementia at the end of the follow-up period was 96.6%. The cumulative proportion of survivors without dementia at the end of the follow-up period was 87.8% in the group with a MMSE score of < 26 at last follow-up before incident stroke, and 92.5% in the group with a MMSE score of >= 26 at last follow-up before stroke (p=0.6). The cumulative proportion in the group without incident stroke was 97.6% (Figure 1).

In the Cox models relating incident stroke as a time-varying exposure with the risk

Table 1. Baseline characteristics of the study sample in 6724 persons followed prospectively.

Characteristic	Mean (SD) or percentage
Women	60.0 %
Age, year	69.2 (8.9)
Educational level	
Low	37.1 %
Intermediate	26.7 %
High	34.5 %
APOEε 4/- or 4/4 genotype	25.6 %
MMSE score	27.7 (1.9)
Diabetes mellitus	10.0 %
Systolic blood pressure, mmHg	139.1 (22.3)
Diastolic blood pressure, mmHg	73.8 (11.4)
Body mass index, kg/m²	26.3 (3.9)
CRP, mg/l	3.3 (6.7)
Total cholesterol, mg/dl	256.4 (47.0)
HDL, mg/dl	52.1 (13.9)
Smoking	63.7 %
Intima media thickness, mm	0.8 (0.2)

MMSE: Mini Mental State Examination, CRP: C-reactive protein, HDL: high-density lipoprotein cholesterol, IMT: intima media thickness.

of PSD, persons with incident stroke had a significantly higher risk of subsequent dementia than persons remaining free of stroke during follow-up (age and sex adjusted HR 2.1, 95% CI 1.55-2.81, p<0.0001; table 2). This association remained stable in models in which we additionally adjusted for APOE genotype and education. The magnitude of this association also did not change in models to which the pre-stroke measures of cognitive function or other potential risk factors for cognitive decline were added (table 2). In the analyses assessing the individual impact of pre-stroke cognitive performance on the risk of dementia, a better cognitive performance before stroke was associated with a significantly lower risk of developing dementia (age and sex adjusted HR 0.82, 95% CI 0.79-0.85). This risk estimate remained unchanged when incident stroke as a time-varying exposure was added (HR 0.81, 95% CI 0.79-0.84).

We then explored the impact of pre-stroke cognitive function on the association between incident stroke and subsequent dementia by repeating all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and pre-stroke

Table 2. Hazard ratios and 95% confidence intervals, relating incident stroke, and clusters of incident stroke with baseline measures of risk factors for cognitive decline, with the risk of incident dementia.

Variable	Dementia		
	Model 1 HR (95% CI)	Model 2 HR (95% CI)	p-value for interaction between incident stroke and risk factor ††
Incident stroke	2.1 (1.55-2.81)	2.1 (1.54-2.93)	--
+ MMSE	2.0 (1.45-2.69)	1.9 (1.36-2.68)	0.7
+ rate of decline in MMSE over time †	2.0 (1.41-2.87)	2.0 (1.34-2.87)	0.5
+ APOEε4 genotype	2.1 (1.53-2.84)	2.1 (1.54-2.93)	0.3
+ diabetes	2.1 (1.55-2.81)	2.1 (1.54-2.93)	0.9
+ systolic blood pressure	2.1 (1.52-2.82)	2.0 (1.43-2.78)	0.4
+ diastolic blood pressure	2.1 (1.51-2.81)	2.0 (1.44-2.81)	0.7
+ serum CRP *	2.0 (1.47-2.81)	1.9 (1.37-2.77)	0.1
+ total cholesterol	2.2 (1.59-2.91)	2.2 (1.57-2.99)	0.7
+ HDL	2.2 (1.61-2.92)	2.1 (1.54-2.93)	0.4
+ smoking	2.1 (1.54-2.81)	2.1 (1.55-2.95)	0.8
+ IMT	2.2 (1.54-3.99)	2.1 (1.46-4.19)	0.09

Model 1: adjusted for sex and age, Model 2: adjusted for sex, age, education and APOEε4 genotype.

* serum CRP was used as a logarithmic transformed continuous variable.

† beta coefficient for rate of decline in MMSE score over time before occurrence of incident stroke, derived by linear regression.

†† adjusted for sex, age, education and APOEε4 genotype.

cognitive performance. In these analyses, there was no interactive effect of incident stroke and measures of pre-stroke cognitive function on the risk of subsequent dementia (table 2). When we added an interaction term to the model that contained variables for incident stroke (yes/no) and age or baseline measures of other common risk factors for cognitive decline, there was no interactive effect of incident stroke and any of the assessed risk factors on the risk of PSD.

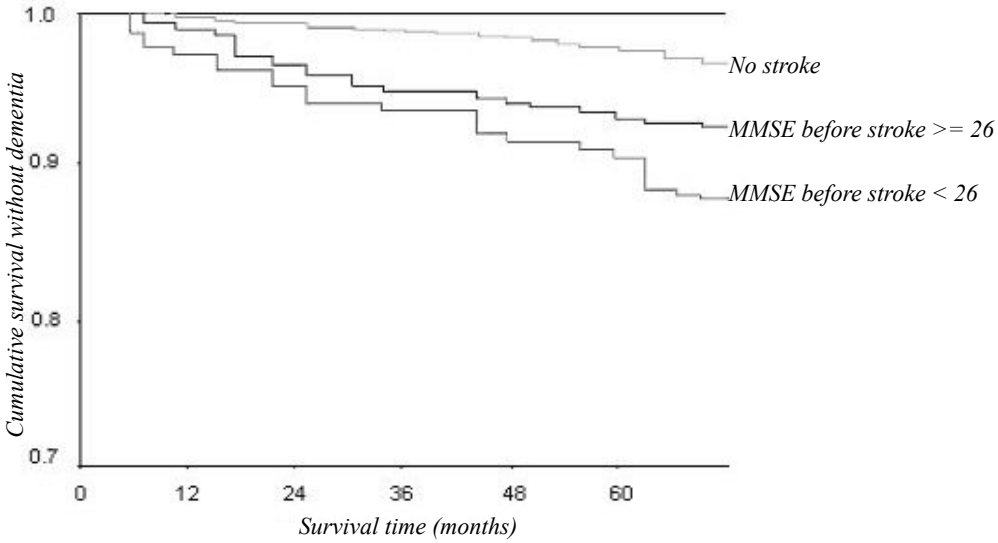


Figure 1. Age and sex adjusted cumulative proportion of persons surviving without developing dementia, as a function of the last MMSE score before incident stroke.

Discussion

We found that an incident stroke doubled the risk of subsequent dementia independent of pre-stroke level of cognitive function and pre-stroke rate of cognitive decline.

This study has limitations. We restricted our analyses to persons with complete information on the occurrence of incident stroke. Individuals which were excluded due to incomplete information were older and had a higher frequency of vascular risk factors. However, this will only have biased our results if the association between incident stroke, pre-stroke cognitive function and dementia is completely different in the people with incomplete data as compared with people with complete data, which we consider highly unlikely. We only had data on symptomatic stroke. Given that the frequency of asympotmatic infarcts is much higher than that of symptomatic stroke,[23] we may have underestimated the impact of subclinical cerebrovascular disease. Information on imaging from hospital records was available on approximately 92 % of all hospitalized stroke patients, but in only 65% of all persons with stroke. The lack of brain imaging in approximately one third of stroke patients implies

the possibility of misclassification of stroke subtype but we consider it unlikely that it led to a misclassification of stroke itself. We used as measure of pre-stroke cognitive function the MMSE test, which can be insensitive to early deficits due to cerebrovascular disease and prone to practice effects. Thus, there remains the possibility that the estimation of slope of pre-stroke cognitive decline was rather rough.

Our study has important strengths. It is a prospective population-based study with a large total number of participants, a large number of persons with incident stroke during follow-up, and nearly complete follow-up with respect to incident stroke and subsequent dementia. Previous studies relating stroke with the risk of dementia were mostly observational studies using prevalent information of stroke, or studies assessing cognitive deterioration after acute stroke in clinical settings.[4-6, 8, 19, 24, 25] To our knowledge, this is the first large population-based study relating incident stroke with the long-term risk of subsequent dementia in persons without dementia or stroke at baseline. This design provides the ability to explore the impact of stroke and other risk factors on the risk of dementia explicitly taking pre-stroke cognitive performance into account.

We observed an association between incident stroke and the risk of subsequent dementia, which was independent of level of pre-stroke cognitive performance. This finding contradicts previous studies reporting a higher risk of PSD in persons with pre-stroke cognitive impairment compared with persons with normal cognition before stroke.[4, 8, 10, 24, 26] However, as mentioned before, these studies either used prevalent information of stroke,[4, 10, 25, 27] or were conducted in stroke cohorts with pre-stroke cognitive function being measured after the stroke through informant questionnaires or by checking pre-stroke medical records for a diagnosis of dementia.[5, 8, 19, 24, 28] These studies thus lacked the ability to accurately assess pre-stroke cognitive function.[5, 6, 9, 19, 24, 25, 28, 29] Also, due to the difficulties in applying a comprehensive, formal neuropsychological assessment to patients who are physically and neurologically impaired, many of the studies in clinical settings examined only a subsample of the total patients registered,[5, 6, 28, 29] and thus may have been biased due to selective attrition.[30] Our study also contradicts the findings of the recently published study by Gamaldo et al.[26] This study reported an increased risk of post-stroke dementia in persons with pre-stroke mild cognitive impairment (MCI). In persons without MCI before stroke, the risk of dementia was similar to persons without stroke.[26] However, these findings were derived from a highly selected cohort without neuroimaging data, were derived from analyses that were solely adjusted for age and sex, and were based on 32 persons in total, leading to very imprecise risk estimates.[26] Also, none of the previous studies took change in cognitive function before occurrence of stroke into account. Our findings are in line with observations in a prospective population based neuroimaging study in which the occurrence of novel brain infarcts during follow-up was associated with cognitive decline, regardless of presence of baseline infarcts and regardless of baseline cognitive status.[31] The present study with a mean follow-up time of 6.3 years between first assessment of cognitive function at baseline and time of incident

first stroke, in which also the slope of cognitive performance before stroke was taken into account, and which had a nearly complete follow-up with respect to dementia, does not suggest that the pre-stroke level of cognitive function is a major determinant of the effect of stroke on the risk of PSD. Both prestroke cognitive function and the stroke itself have an effect on the risk of dementia after stroke, but these effects are largely independent.

The association between incident stroke and the risk of subsequent dementia was also independent from all other assessed risk factors for cognitive decline, including diabetes mellitus, APOEε4 genotype, blood pressure levels, body mass index, and IMT. This observation supports the notion that the effects of stroke result in dementia through mechanisms other than mechanisms of APOEε4 or other potential risk factors, and that stroke increases the risk of dementia independently of these risk factors.

There are alternative explanations for our findings. It is possible that incident stroke is not a risk factor but merely part of a pre-clinical syndrome of dementia, meaning that persons with pre-clinical dementia may have a higher frequency of stroke than persons without dementia. However, the mean follow-up time between incident stroke and subsequent dementia in persons developing PSD was relatively long (3.9 years), making this an unlikely explanation for our findings.[32] Also, the association between incident stroke and subsequent dementia was independent from pre-stroke cognitive function, regardless of length of follow-up time from incident stroke to subsequent dementia.

An alternative explanation for the missing interaction between incident stroke and risk factors for cognitive decline might be that elderly cohorts are too homogeneous to show differences in outcomes related to these risk factors. The measurement of these risk factors in our cohort did not take into account duration. Thus, it is possible that our results tend to underestimate the association between incident stroke, risk factors for cognitive decline and incident dementia, which could bias the results to the level of no interaction. However, this seems unlikely given the robustness of our findings across all assessed risk factors for cognitive decline.

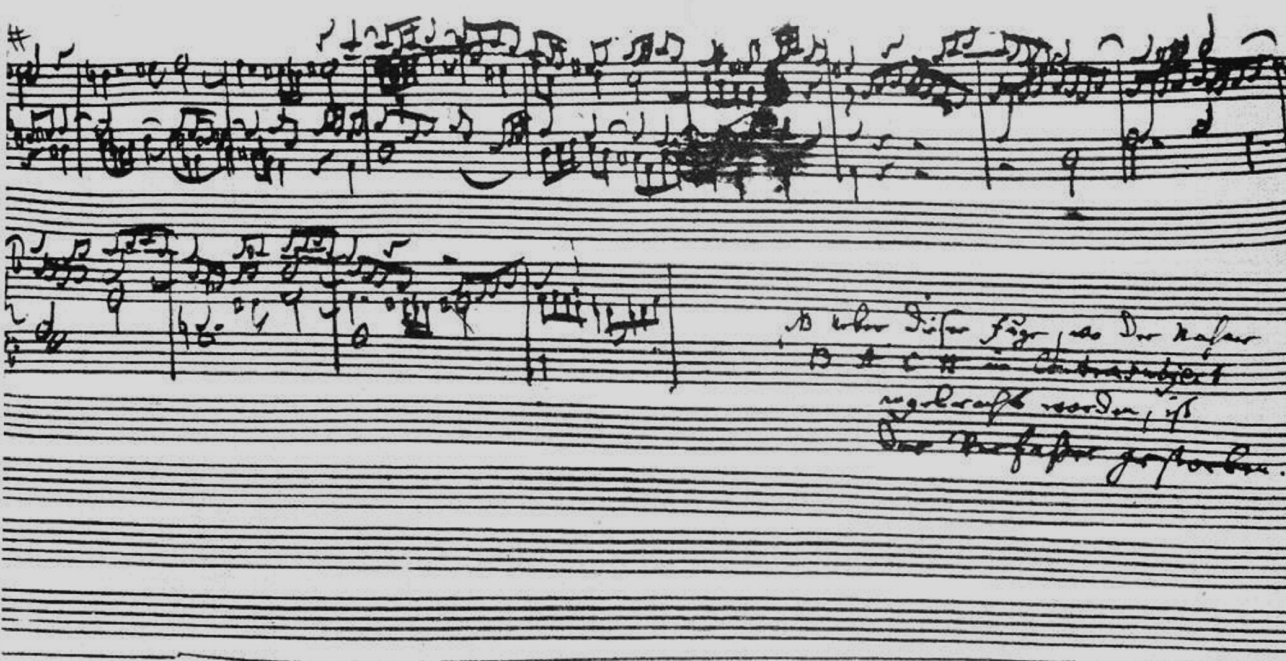
References

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global burden of disease study. Lancet. 1997;349:1498-1504
2. Fratiglioni L, De Ronchi D, Aguero-Torres H. Worldwide prevalence and incidence of dementia. Drugs Aging. 1999;15:365-375
3. Leys D, Henon H, et al. Poststroke dementia. Lancet Neurol. 2005;4:752-759
4. Desmond DW, Moroney JT, et al. Frequency and clinical determinants of dementia after ischemic stroke. Neurology. 2000;54:1124-1131
5. Censori B, Manara O, et al. Dementia after first stroke. Stroke. 1996;27:1205-1210
6. Pohjasvaara T, Erkinjuntti T, et al. Dementia three months after stroke. Baseline frequency and effect of different definitions of dementia in the Helsinki Stroke Aging Memory Study (SAM) cohort. Stroke. 1997;28:785-792
7. Tatemichi TK, Desmond DW, et al. Dementia after stroke: Baseline frequency, risks, and clinical features in a hospitalized cohort. Neurology. 1992;42:1185-1193

8. Henon H, Durieu I, et al. Poststroke dementia: Incidence and relationship to prestroke cognitive decline. Neurology. 2001;57:1216-1222
9. Tatemichi TK, Paik M, et al. Risk of dementia after stroke in a hospitalized cohort: Results of a longitudinal study. Neurology. 1994;44:1885-1891
10. Pohjasvaara T, Erkinjuntti T, et al. Clinical determinants of poststroke dementia. Stroke. 1998;29:75-81
11. Tatemichi TK, Desmond DW, et al. Clinical determinants of dementia related to stroke. Ann Neurol. 1993;33:568-575
12. Hofman A, Grobbee DE, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422
13. Ott A, Breteler MM, et al. Incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol. 1998;147:574-580
14. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189-198
15. Copeland JR, Kelleher MJ, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly: The Geriatric Mental State Schedule. I. Development and reliability. Psychol Med. 1976;6:439-449
16. Roth M, Tym E, et al. Camdex. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry. 1986;149:698-709
17. Diagnostic and Statistical Manual of Mental Disorders, revised third edition. Washington, DC: American Psychiatric Association; 1987.
18. McKhann G, Drachman D, et al. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. Neurology. 1984;34:939-944
19. Inzitari D, Di Carlo A, et al. Incidence and determinants of poststroke dementia as defined by an informant interview method in a hospital-based stroke registry. Stroke. 1998;29:2087-2093
20. Meijer WT, Hoes AW, et al. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol. 1998;18:185-192
21. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. Arterioscler Thromb. 1994;14:1885-1891
22. Slooter AJ, Cruts M, et al. Risk estimates of dementia by apolipoprotein e genotypes from a population-based incidence study: The Rotterdam Study. Arch Neurol. 1998;55:964-968
23. Vermeer SE, Koudstaal PJ, et al. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. 2002;33:21-25
24. Mok VC, Wong A, et al. Cognitive impairment and functional outcome after stroke associated with small vessel disease. J Neurol Neurosurg Psychiatry. 2004;75:560-566
25. Sachdev PS, Brodaty H, et al. Progression of cognitive impairment in stroke patients. Neurology. 2004;63:1618-1623
26. Gamaldo A, Moghekar A, et al. Effect of a clinical stroke on the risk of dementia in a prospective cohort. Neurology. 2006;67:1363-1369
27. Barba R, Martinez-Espinosa S, et al. Poststroke dementia : Clinical features and risk factors. Stroke. 2000;31:1494-1501
28. Kokmen E, Whisnant JP, et al. Dementia after ischemic stroke: A population-based study in Rochester, Minnesota (1960-1984). Neurology. 1996;46:154-159
29. Tatemichi TK, Foulkes MA, et al. Dementia in stroke survivors in the Stroke Data Bank Cohort. Prevalence, incidence, risk factors, and computed tomographic findings. Stroke. 1990;21:858-866
30. Desmond DW, Bagiella E, et al. The effect of patient attrition on estimates of the frequency of dementia following stroke. Arch Neurol. 1998;55:390-394
31. Vermeer SE, Prins ND, et al. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003;348:1215-1222
32. Barendregt JJ, Ott A. Consistency of epidemiologic estimates. Eur J Epidemiol. 2005;20:827-832

Chapter 7.

General discussion



In this thesis we have aimed to provide more insight in the pathophysiological mechanisms that lead to stroke and in the possibilities to distinguish persons who are at high risk of stroke from those at low risk of stroke.

During the last decades it has been shown that several factors that vary between individuals can be used to predict the risk of future stroke in individuals; other factors can even be modified to influence the risk of stroke. Nevertheless, the pathophysiological mechanisms that lead to stroke are fully elucidated for only a modest proportion of strokes, and the inadequacy of preventive measures is illustrated by the high incidence of stroke: for example, the lifetime risk of stroke is about 16% for a Dutch newborn.

The pathophysiological mechanisms studied in this thesis were those involved in the homeostasis of blood pressure and blood volume, as well as in atherosclerosis in various arterial territories. In addition, because a transient ischemic attack (TIA) is one of the best predictors of future stroke, we investigated whether attacks with neurological symptoms that do not fulfill the typical diagnostic criteria for TIA are also related to the risk of future diseases. Finally, because cerebrovascular damage does not only lead to stroke and TIA but also to disturbances in mood and cognition, we assessed the relation between stroke, depression, and cognitive function.

In the following paragraphs I will review our findings, place them in context, comment on possible clinical implications and suggest directions for future research.

Main findings of this thesis

Novel nongenetic risk factors: Most etiologic cardiovascular research nowadays focuses on inflammatory processes that stimulate atherosclerotic degeneration. However, the notion is growing that the relation between inflammation and stroke may have been overestimated in the recent past,[1] which is supported by our own finding in *chapter 4.2* that the relation between C-reactive protein, probably the best known marker of inflammatory activity, and risk of stroke was only weak. We therefore proceeded with a different approach, which was based on the observation that high blood pressure is the most important determinant of stroke. The main explanation for this observation is that atherosclerosis impairs the Windkessel effect, thereby increasing blood pressure, which in its turn stimulates the development of atherosclerosis by increasing sheer stress. However, besides the passive elasticity of the arterial wall, also the active elasticity of the arterial wall, the inotropy of the heart, and the circulating fluid volume are important determinants of blood pressure. We aimed to investigate whether factors involved in the homeostasis of the circulating fluid volume influence the risk of stroke. First we investigated the relation between serum uric acid level and risk of stroke in *chapter 2.1*, and found that high serum uric acid levels were associated with an increased risk of stroke and ischemic heart disease, especially in persons without hypertension. Sev-

eral hypotheses may explain this relationship. The most intriguing one is that uric acid may cause renal vasoconstriction, which leads to renal hypoperfusion, inducing the kidneys to reduce fluid excretion, thereby causing hypertension.[2] This leads to higher sheer stress on the arterial wall, which induces atherosclerosis. When high uric acid levels persist, anatomical alterations of the vasculature in the the kidney lead to an irreversible unequal distribution of the perfusion of the kidney, independent of serum uric acid.[2] An alternative explanation may that high uric acid levels may be a sign of chronic kidney disease: although uric acid levels are known to correlate poorly with glomerular filtration rate, they are invariably increased in chronic kidney disease.

This finding awoke our interest in glomerular filtration rate (GFR). Decreased GFR has been found to be a risk factor for ischemic heart disease.[3] Previous studies have found a slight increase in risk of stroke with decreasing GFR,[4] but no previous studies differentiated between ischemic and hemorrhagic stroke. We assessed the relation between GFR and stroke in *chapter 2.2* and found that decreased GFR was a strong risk factor for hemorrhagic stroke but not for ischemic stroke. We hypothesized that this may be because platelet function worsens with decreasing GFR.[5] A second hypothesis is that low GFR may be a sign of renal small vessel disease, which is correlated with cerebral small vessel disease, and cerebral small vessel disease plays a larger role in ischemic stroke than in hemorrhagic stroke.

As mentioned above, three main sources for the emboli that cause ischemic stroke are generally recognized: the heart, the large extracranial arteries, and the small penetrating arterioles deep within the brain. The large intracranial arteries would logically fit into this series, but are generally ignored because they were until recently hard to visualize noninvasely. However, CT angiography (CTA), and to al lesser extent MR angiography (MRA), visualize the large intracranial vessels with sufficient accuracy and have become widely available radiological techniques. Furthermore, several flow velocity parameters in the large intracranial arteries can be measured by means of transcranial Doppler ultrasonography, which is what we did for the middle cerebral artery in *chapter 2.3*. We found that there was no relation between vasomotor reactivity (VMR: the change in blood flow velocity in response to a vasodilatatory stimulus, in our case inhalation of carbon dioxide) and stroke, but the risk of stroke increased with increasing flow velocity. The side of highest flow velocity was not associated with the side of stroke. These findings suggest that lesions in the large intracranial arteries are important in the etiology of stroke, independent of pathology elsewhere in the arterial tree. Our results suggest that this is not attributable to high-grade focal stenoses but to low-grade generalized narrowing of the intracerebral arteries. The nature of the latter process unfortunately remains unknown; the homogeneity of the flow velocity and the absence of a relation between VMR and stroke suggest that it reflects active vasoconstriction such as we see with hypertension; the independence of the relation with hypertension, however, suggests that we are looking at passive low-grade diffuse narrowing such as we see with atherosclerosis.

Vioxx® (rofecoxib), a non-steroid anti-inflammatory drug (NSAID), has been with-

drawn from the market in 2004 because it increases the risk of cardiovascular diseases.[6] In *chapter 2.4* is described that Vioxx is not the only NSAID that increases the risk of cardiovascular diseases; in fact, our data suggest that all cyclooxygenase (COX) 2-selective NSAIDs as well as all COX non-selective NSAIDs increase the risk of stroke.

Genetic risk factors: At present the main focus of research into determinants of disease is shifting away from physiological measurements and serum levels towards genetic research. The main reasons for this development are that associations found between genes and diseases are less prone to confounding,[7] and an important practical consideration is that during the past 5 years genetic assays have become cheap, easy, and extremely fast.

Although more than 99% of the human genomic sequence is the same across the population, a frequent (>1% of the population) variation in the genomic sequence occurs in every 100 to 300 bases, which is called a single nucleotide polymorphism (SNP). SNPs, which in two thirds of cases concern the replacement of cytosine with thymine, may influence the production and structure of proteins and therefore a person's liability for disease. Nowadays, 1.8 million SNPs have been described in public databases.[8]

In *chapter 3.1*, we showed that the The Gly460Trp polymorphism of the alpha-adducin gene was associated with atherosclerosis, cardiovascular disease, and stroke, especially in hypertensive subjects. In *chapter 3.2* we studied the association between the M235T polymorphism of the angiotensinogen gene (AGT) and the C573T polymorphism of the angiotensin II type 1 receptor (AT1R) in relation to blood pressure, carotid atherosclerosis and cerebrovascular disease. We found that the variant polymorphism of AGT was associated with higher systolic and diastolic blood pressure, and with more white matter lesions on MRI scan. There was, however, no relation with stroke. In *chapter 3.3* we showed that there was no relation between genetic variation in the estrogen receptor alpha (ESR1) gene and risk of stroke. In *chapter 3.4* we found indications that variation in the fibrinogen alpha and beta genes was associated with risk of ischemic and hemorrhagic stroke.

The difficulty of SNP research is the combination of the huge number of available SNPs whereas associations with disease are expected to be weak. Classical statistical test methods cannot account for this, because high (alpha) significance levels will lead to a huge number of false-positive results and low alpha levels will fail to detect weak associations. Therefore, results have to be interpreted with great caution. With respect to alpha-adducin, the relations found were very consistent for all outcome measures (stroke, myocardial infarction, hypertension, intima-media thickness, white matter lesions, and silent brain infarctions). This remarkable consistency makes it, despite the relatively weak individual relations, quite likely that alpha-adducin is indeed involved in cardiovascular disease. Although our results suggest that AGT and AT1R are involved in cardiovascular disease, they were not –or only weakly– associated with stroke.

Our results show that it is unlikely that the difference in age-specific stroke inci-

dence between men and women is explained by genetic variation in the estrogen receptor alpha, negating previous reports that suggested otherwise.

Finally, we investigated the fibrinogen alpha and beta genes, which influence the structure of the fibrinogen clot. Our findings suggested a possible relation between these genes and the risk of stroke, but at best only a very weak one. This suggests that fibrin plasma levels are more important determinants of stroke[9] than fibrin clot structure.

Stroke risk factors: impact and prediction: In *chapter 4.1* we investigated what proportion of strokes is explained by known modifiable etiologic factors, since this is the proportion of strokes that might be prevented by complete elimination of these factors from the population. It appeared that the well-established modifiable causal factors hypertension, smoking, and diabetes together accounted for up to 54% of all strokes, which means that only half of strokes would be prevented by complete elimination of these risk factors from the population. Several less-well established risk factors had the potential to increase this proportion. From this we concluded that for adequate prevention of strokes, the search for new modifiable etiologic factors is necessary and feasible.

The question that we addressed in *chapter 4.2* was whether an exemplary emerging risk factor, C-reactive protein (CRP), could improve the prediction of stroke risk in individual persons. We found that, although CRP levels were associated with the risk of stroke, the assessment of CRP did not improve the prediction of stroke in individual patients. This contrasts with current recommendations to use patients' CRP levels to estimate their future risk of cardiovascular disease.[10] These recommendations were inspired on the strong associations that had been found between CRP levels and cardiovascular disease. Our results suggest that a risk factor, especially a highly prevalent one, has to be fairly strongly associated with a disease in order to ameliorate the prediction of the disease in a population with a low a priori risk of disease (the general population). The mere finding of an association between a risk factor and a disease is not enough evidence to support the recommendation to use the risk factor in disease prediction. A more generalized depiction of how a risk factor influences the estimated absolute risk of disease based on the odds ratio, the prevalence of the disease, the prevalence of the exposure, and the a priori risk of disease can be found in figure 1.

Transient neurological attacks: Transient ischemic attacks (TIAs) are important warning signs for stroke, and patients who experience TIAs need urgent clinical evaluation because they are at very high risk of stroke. Whereas we are well aware of the prognosis of TIAs, many patients suffer from attacks with neurological symptoms that do not fulfill diagnostic criteria for TIA. These attacks are generally presumed to have a benign subsequent clinical course, but data to support this assumption were lacking. We investigated in *chapter 5.1* whether the assumption is correct. We classified all transient neurological attacks (TNAs) in 3 groups: focal TNAs (better known as TIAs) with only focal symptoms, nonfocal TNAs

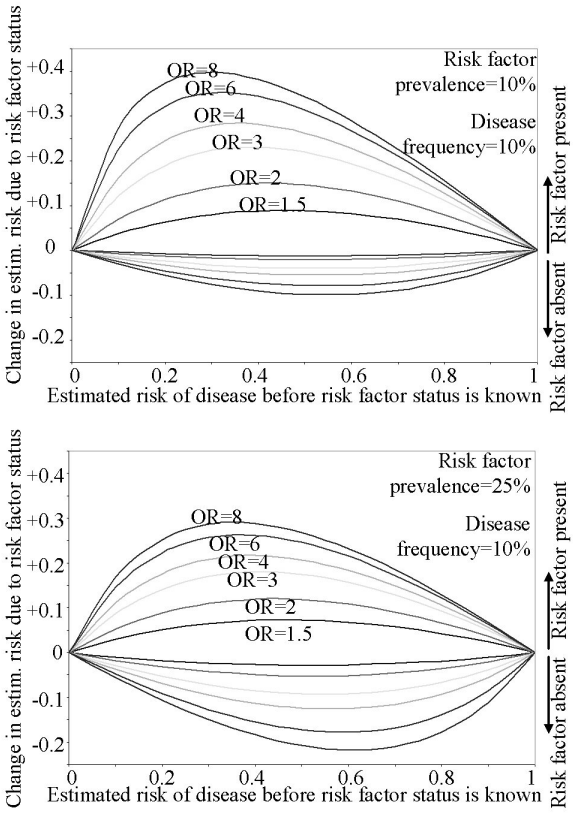


Figure 1. Change in estimated risk of disease due to knowledge of risk factor status based on the odds ratio (OR) of the association between the risk factor and the risk of disease; for a risk factor prevalence of 10% and 25%, with a disease that affects 10% of the population.

with only nonfocal symptoms, and mixed TNAs with a mixture of both focal and nonfocal symptoms during the same episode. As expected, we found that patients with focal TNAs had a higher risk of subsequent stroke than persons without TNA. Surprisingly, nonfocal TNAs indicated an increased risk of subsequent stroke and vascular dementia. The prognosis was particularly bad for mixed TNAs: these were associated with an increased risk of subsequent stroke, ischemic heart disease, dementia, and vascular death, which is in line with a previous study.[11] Therefore, it appears recommendable for physicians to suspect possible underlying heart disease if a patient presents with a mixed TNA. Nonfocal TNAs appear not to be as innocent as generally thought.

Stroke, depression, and cognitive function: As described in *chapter 5*, the scope of clinical manifestations of cerebrovascular disease is much broader than stroke and TIA alone. Cerebrovascular disease may also influence, or be influenced by, mood and cognition.[12] Since the 1970s it has been appreciated that depressive disorder is related to coronary and cerebrovascular disease.[13] In *chapter 6.1* we assessed whether presence of depressive symptoms was a risk factor for stroke. It appeared to be so only for men, and our results suggested that it

were not the men with depressive disorder but those with depressive symptoms in absence of depressive disorder who were at the highest risk of stroke. This is in line with previous observations that depressive disorder, of which the incidence decreases with aging, has an important genetic component and ischemic brain damage plays no more than a minor role. The incidence of depressive symptoms do increase with age, and this may be because they are caused by ischemic brain damage. As such, they are a sign of the bad condition of cerebral arteries and a risk factor for stroke in men.

It has been well established that the prevalence of dementia is much higher in stroke patients than in persons without stroke.[14] It was unclear whether this is because stroke patients already have a worse cognitive function before the stroke occurs or whether it is attributable to the ischemic brain damage caused by the stroke itself. We assessed in *chapter 6.2* whether pre-stroke cognitive function influenced the relation between stroke and dementia, and found that it did not influence this relation. We therefore concluded that ischemic brain damage caused by stroke is responsible for a worse cognitive function after stroke.

Methodological considerations related to the studies in this thesis

The Rotterdam Study has several unique strengths: first, participants were recruited before the onset of stroke, which not only makes it possible to assess determinants that are not influenced by the disease, but also reduces selection bias. Second, the large study population enables us to make precise estimates and to detect small effects. Third, the meticulous monitoring of participants during follow-up results in a nearly complete follow-up and detailed disease phenotyping.
Specific methodological issues are discussed in the relevant chapters; in this chapter I will only discuss potential sources of error and bias in the selection of the population and the diagnosis of stroke.

Selection of the population: The participants of the Rotterdam Study were recruited at a median age of 69.5 years. The population distribution of the Rotterdam Study at baseline and the age specific incidence rate of cardiovascular mortality[15] suggest that about 14% of the source population died from cardiovascular disease before baseline (ignoring time trends in incidence rate). In addition, 22% of those invited to participate declined the invitation. The reasons to decline are not known but might be related to cardiovascular disease.

Diagnosis of stroke: Our meticulous stroke case finding resulted in a nearly complete follow-up (loss of potential person-years, 1.3 %). Our stringent stroke monitoring procedures allowed us to include also stroke patients who had not been referred to a neurologist (31% of all stroke cases). As in these cases neuroimaging had not been performed, we could subclas-

sify only 16% of them into ischemic or hemorrhagic. In contrast, 92% of strokes that had been referred to a neurologist could be subclassified. As a result, we could not determine the subtype of stroke in 35% of participants. This may be a problem when looking at subtypes of stroke separately, but by using survival models and by censoring participants at the time of unspecified stroke we think this has not materially biased our results.

Clinical implications

Whereas the general aim of this thesis was to improve our understanding of the pathophysiologal mechanisms that lead to stroke, clinically relevant findings have one of these two characteristics:

- They identify potential targets for preventive treatment.
- They ameliorate the distinction of persons at high risk from those at low risk of stroke.

I will now discuss the clinical relevance of the findings in this thesis.

Potential targets for preventive treatment: There are two forms of preventive treatment: treatment that changes a characteristic from 'abnormal' to 'normal' (e.g., antidiabetic treatment) and treatment that changes a characteristic from 'normal' to 'better fitting a specific patient' (e.g. anticoagulation). The first form is in principle preferred over the second since it has the potential to be more effective with less side effects, especially in the general population. However, we do not have enough knowledge on the etiology of stroke and treatment of risk factors to prevent all strokes with an intervention of the first type: our results suggest that complete elimination of (pre)hypertension, diabetes, atrial fibrillation and smoking might result in the prevention of about half of strokes. Other established etiologic factors had no large impact on the occurrence of stroke. This thesis suggests several novel targets for preventive treatment: several of our findings suggest that determinants of renal perfusion pressure (uric acid levels, alpha-adducin polymorphisms, glomerular filtration rate), the response of the renin-angiotensin pathway to this pressure, and the resulting circulating fluid volume, are implicated in the etiology of stroke (and myocardial infarction). It may prove beneficial to influence these factors, which may be done in several ways; for example, uric acid levels can be lowered, but it is not unlikely that this results in serious side effects since uric acid is an important antioxidant. Another approach worth considering is to prevent the adverse effects that high uric acid levels or chronic kidney disease may have by blocking the renin-angiotensin signalling pathway, for example with angiotensin converting enzyme inhibitors. If our hypothesis that the link between chronic kidney disease and hemorrhagic stroke is explained by the functioning of thrombocytes is correct, it may be useful to monitor the function of thrombocytes in patients with chronic kidney disease who are at high risk of hemorrhagic stroke.

We were able to confirm earlier observations that use of COX2-selective[6] and non-selective NSAIDs is associated with an increased risk of stroke. It seems recommendable to restrict the use these NSAIDs as much as possible and not to use them when only an analgesic effect is required. When NSAIDs are deemed necessary, low dosages are preferred and perhaps concurrent use of platelet inhibitors may reduce the risk of cardiovascular diseases. It is unclear whether the gastrointestinal risk of COX1-selective drugs is worse than the cardiovascular risk of COX2-selective drugs.

The distinction of persons at high risk from those at low risk of stroke: If strong and consistent associations between a risk factor and a disease are found, many people are easily convinced that the risk factor may be helpful in estimating an individual patient's risk of disease. We showed for an exemplary risk factor, C-reactive protein, that this is not necessarily true. Especially in the general population, where the a priori risk of disease is relatively low,

Table 1. Hypothetical results of preventive stroke treatment with varying selection criteria for receiving treatment.

Treatment assignment either based on the estimated stroke risk (assessed with Framingham stroke risk score (FRS)), or based on age alone. Two hypothetical treatment regimens are implemented: one which prevents 100% of strokes and one which prevents 50% of strokes in those who receive treatment. Participants older than 83 years of age are excluded because the FRS is not validated in that age range.

Criterion for receiving treatment		Hypothetical efficacy of treatment	% of population that need treatment		% of strokes that are prevented		Number needed to treat†	
Estimated stroke risk*	Age (men / women), yrs							
> 0 %	≥ 55	100%	7131/7131=	100%	603/603=	100%	7131/603=	11.8
> 5 %		100%	4147/7131=	58%	453/603=	75%	4147/453=	9.1
> 10 %		100%	2115/7131=	30%	293/603=	49%	2115/293=	7.2
> 15 %		100%	1273/7131=	18%	192/603=	32%	1273/192=	6.6
	≥ 67 / ≥ 69	100%	3582/7131=	50%	451/603=	75%	3582/451	7.9
	≥ 74 / ≥ 75	100%	1772/7131=	25%	248/603=	41%	1772/248	7.1
> 0 %	≥ 55	50%	7131/7131=	100%	302/603=	50%	7131/302=	23.6
> 5 %		50%	4147/7131=	58%	227/603=	38%	4147/227=	18.3
> 10 %		50%	2115/7131=	30%	147/603=	24%	2115/147=	14.4
> 15 %		50%	1273/7131=	18%	96/603=	16%	1273/96=	13.3
	≥ 67 / ≥ 69	50%	3582/7131=	50%	226/603=	37%	3582/226	15.8
	≥ 74 / ≥ 75	50%	1772/7131=	25%	124/603=	21%	1772/124	14.3

* Based on the FRS. † Number needed to treat to prevent one stroke.

the result of a diagnostic test does often not materially change a patient's estimated risk of disease, especially if the risk factor is highly prevalent (as mentioned, this is illustrated in figure 1). It is not recommendable to rely on single weak risk factors, and indeed in practice more and more physicians start to base their treatment decisions on risk scores that combine various risk factors together. It remains questionable whether this is a good way to proceed since even risk scores do not predict risk particularly reliably; in table 1, I illustrate that treatment allocation based on the estimated stroke risk using the Framingham stroke risk score[16] is not much better than treatment allocation based on age alone (in terms of the number of people that require treatment, the proportion of strokes that are prevented, and the number needed to treat to prevent one stroke), whereas the latter method saves enormous amounts of money and is much easier to implement on a large scale; also it provokes much less unnecessary anxiety among the population. The question of course remains how to treat apparently healthy persons; primary prevention trials have shown a modest effect of aspirin on risk of stroke in healthy persons, but only in women (although in men the risk of myocardial infarction was reduced). It is not unlikely that HMG Co-A reductase inhibitors or antihypertensives are of additional value, also in persons without evidently increased blood pressure or blood cholesterol.

Directions for future research

Because in a considerable proportion of strokes no modifiable etiologic factor can be found, identification of new potential causes remains of great importance. Our results suggest that high serum uric acid levels might directly or indirectly account for a considerable proportion of strokes, and more research is needed to confirm the causal nature of the relation between uric acid level and stroke, for example by genetic studies (many SNPs are present in urate transporter genes). More research is also needed to confirm the hypothesis that the kidneys are involved in the etiology of stroke. A first step is to verify the relation between decreased kidney function and stroke with more accurate measures for kidney function, such as albuminuria. The ideal measures for glomerular filtration and renal perfusion are unfortunately too invasive to be performed at a large scale. A second step is to find out how this relation can be explained: whether it is volume overload that causes atherosclerosis (a more detailed study of the activity of the renin-angiotensin signaling pathway may be helpful), or whether the relation is attributable to metabolic disturbances that accompany decreased renal function.

As mentioned, the unraveling of the genetics of stroke at older age is precarious due to the enormous amount of genes that may be involved and the weakness of the relations between genes and stroke. Nobody knows how this challenge can be met, but a genome-wide association study to identify the genes that are statistically strongest associated with stroke

may be a starting point.

Our results for mixed TNAs have to be confirmed by other studies. It would be interesting to re-examine the nonfocal TNAs in new studies with more detailed phenotyping and subclassification.

The finding that depressive symptoms but not depressive disorders are associated with risk of stroke has to be replicated in a study with more power.

As mentioned, stroke prediction in the general population remains unreliable. Although yet unknown biomarkers with strong predictive values may exist, it is not very likely that they will differentiate so well that individual risk prediction becomes really reliable. It is possible that to reliably predict stroke risk, we may have to shift our attention away from systemic measures and start looking directly at locoregional lesions: the plaques and the heart chambers where thrombi originate. Direct visualization of plaques and the thrombi that grow on them may ameliorate stroke prediction. It is not possible to reach an appropriate level of detail with current technology, but novel imaging techniques and higher resolution scanners (7 Tesla to 11.4 Tesla MRI scanners already exist) may make this a realistic option in the not too distant future.

In conclusion, the accumulation of insights in stroke pathophysiology since the 1960s has changed the character of stroke from a sheer disaster towards a medical challenge. Tiny steps are taken every day that help us to reduce the incidence of stroke and to limit its damage. It is to be hoped that the suggestions made in this thesis may help smoothen the way towards another tiny but important step.

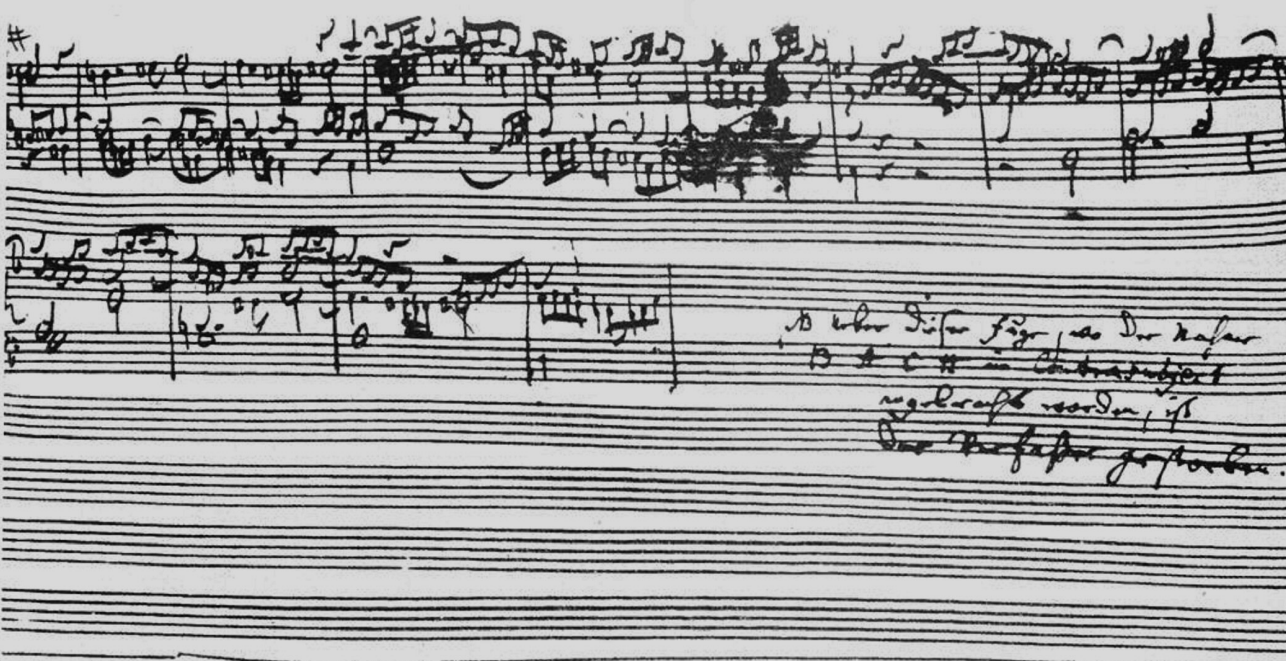
References

1. Danesh J, Wheeler JG, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350:1387-1397
2. Johnson RJ, Segal MS, et al. Essential hypertension, progressive renal disease, and uric acid: A pathogenetic link? J Am Soc Nephrol. 2005;16:1909-1919
3. Brugts JJ, Knetsch AM, et al. Renal function and risk of myocardial infarction in an elderly population: The Rotterdam Study. Arch Intern Med. 2005;165:2659-2665
4. Weiner DE, Tighiouart H, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: A pooled analysis of community-based studies. J Am Soc Nephrol. 2004;15:1307-1315
5. Noris M, Remuzzi G. Uremic bleeding: Closing the circle after 30 years of controversies? Blood. 1999;94:2569-2574
6. Bresalier RS, Sandler RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352:1092-1102
7. Smith GD, Ebrahim S. Mendelian randomization: Prospects, potentials, and limitations. Int J Epidemiol. 2004;33:30-42
8. Human genome project information fact sheet. U.S. Department of Energy Office of Science, http://genomics.energy.gov/.
9. Danesh J, Lewington S, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: An individual participant meta-analysis. JAMA. 2005;294:1799-1809
10. Pearson TA, Mensah GA, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for

Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499-511
11. Koudstaal PJ, Algra A, et al. Risk of cardiac events in atypical transient ischaemic attack or minor stroke. The Dutch TIA Study Group. Lancet. 1992;340:630-633
12. Alexopoulos GS, Meyers BS, et al. 'vascular depression' hypothesis. Arch Gen Psychiatry. 1997;54:915-922
13. Thomas AJ, Kalaria RN, O'Brien JT. Depression and vascular disease: What is the relationship? J Affect Disord. 2004;79:81-95
14. Leys D, Henon H, et al. Poststroke dementia. Lancet Neurol. 2005;4:752-759
15. Thom T, Haase N, et al. Heart disease and stroke statistics--2006 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2006;113:e85-151
16. Wolf PA, D'Agostino RB, et al. Probability of stroke: A risk profile from the Framingham Study. Stroke. 1991;22:312-318

Chapter 8.

Epilogue



8.1.
English summary

Background and purpose: During the last decades many factors have been identified that vary between individuals and that are related to the rate of arterial degeneration (atherosclerosis and lipohyalinosis), embolus formation, and bleeding tendency. Some of these factors can be used to predict the risk of future stroke in individuals, and other factors can even be modified to influence the risk of stroke. Nevertheless, the pathophysiological mechanisms that lead to stroke are only fully elucidated for a small proportion of strokes, and the preventive measures taken today do not prevent the 41 thousand strokes that occur annually in the Netherlands, making stroke the third cause of death and the most important cause of disability in our country. The studies in this thesis were performed to gain more insight in the pathophysiological mechanisms that lead to stroke and in the possibilities to identify persons who are at high risk of stroke. The pathophysiological mechanisms that this thesis focused on were those involved in the homeostasis of blood pressure and blood volume, as well as in atherosclerosis in various arterial territories. In addition we assessed, since the occurrence of transient ischemic attack (TIA) is one of the best predictors of future stroke, whether attacks with neurological symptoms that do not fulfill the typical diagnostic criteria for TIA are also related to the risk of future diseases. Because cerebrovascular damage does not only lead to stroke and TIA but also to disturbances in mood and cognition, we lastly described the relation between stroke, depression, and cognitive function.

Methods: The studies described in this thesis are based on the Rotterdam Study, which is a prospective population-based cohort study of chronic and disabling diseases in persons aged 55 years and over. The 7,721 participants who were eligible for these studies were all free from stroke at study baseline. All participants were enrolled in the study between 1990 and 1993 and have subsequently been monitored for occurrence of stroke, transient ischemic attack, and other diseases.

Results: The risk of stroke and ischemic heart disease increased with increasing serum uric acid levels, especially in persons without hypertension *(chapter 2.1)*. The risk of hemorrhagic stroke increased with decreasing glomerular filtration rate (GFR) or worsening kidney function. There was no relation between GFR and risk of ischemic stroke *(chapter 2.2)*. We assessed the relation between hemodynamic parameters of the middle cerebral artery measured with transcranial Doppler ultrasonography and risk of stroke, and found no relation between vasomotor reactivity (the change in blood flow velocity in response to a vasodilatatory stimulus, in our case inhalation of carbon dioxide) and risk of stroke. In contrast, the risk of stroke increased with increasing flow velocity *(chapter 2.3)*. Use of cyclo-oxygenase (COX)-2 selective non-steroidal anti-inflammatory drugs (NSAIDs) and COX-nonselective NSAIDs

increased the risk of stroke *(chapter 2.4)*.

The Gly460Trp polymorphism of the alpha-adducin gene was associated with atherosclerosis, cardiovascular disease, and stroke, especially in persons with hypertension *(chapter 3.1)*. The M235T polymorphism of the angiotensinogen gene (AGT) was associated with higher systolic and diastolic blood pressure and with more white matter lesions on MRI scan, but not with stroke. Associations with the C573T polymorphism of the angiotensin II type 1 receptor (AT1R) were less clear *(chapter 3.2)*. There was no relation between genetic variation in the estrogen receptor alpha (ESR1) gene and risk of stroke *(chapter 3.3)*. Variation in the fibrinogen alpha and gamma genes was possibly associated with risk of ischemic and hemorrhagic stroke. This relation was however weak and needs confirmation *(chapter 3.4)*.

The proportion of strokes that is explained by the well-established modifiable causal factors hypertension, smoking, atrial fibrillation, and diabetes together was 54%. This is the proportion of strokes that at maximum might be prevented when all these etiologic factors would be eliminated from the population. This relatively small proportion might increase to about 68% if several less-well established risk factors prove to be causal and modifiable *(chapter 4.1)*. C-reactive protein (CRP), an exemplary emerging risk factor, was associated with stroke, but nevertheless did not improve the prediction of stroke in individual patients *(chapter 4.2)*.

Many patients suffer from transient attacks with neurological symptoms (transient neurological attacks or TNAs) that do not fulfill diagnostic criteria for TIA and are therefore considered benign. To investigate whether this assumption is correct, we classified all TNAs in 3 groups: focal TNAs (better known as TIAs) with only focal symptoms, nonfocal TNAs with nonfocal symptoms only, and mixed TNAs with a mixture of both focal and nonfocal symptoms during the same episode. We confirmed that focal TNAs were associated with an increased risk of subsequent stroke. Surprisingly, nonfocal TNAs indicated an increased risk of subsequent stroke and vascular dementia. The prognosis was particularly bad for mixed TNAs: these were associated with an increased risk of subsequent stroke, ischemic heart disease, dementia, and vascular death *(chapter 5)*.

Men with depressive symptoms had a higher risk of stroke than men without depressive symptoms, and our results suggested that it were not the men with depressive disorder but those with depressive symptoms in absence of depressive disorder who had the highest risk of stroke. Among women there was no relation between depressive symptoms and stroke *(chapter 6.1)*. Pre-stroke cognitive function did not influence the relation between stroke and poststroke dementia *(chapter 6.2)*.

Conclusions: Only half of all strokes can theoretically be prevented by modification of currently known causes of stroke. Important new potential causal pathways that came to the fore in this thesis were related to the functioning of the kidneys, including uric acid effects,

glomerular filtration rate, and sodium handling. Also pathology of the large intracranial arteries appeared to be important. NSAIDs with effects on COX2 increase the risk of stroke and should therefore be prescribed reluctantly.

Only factors that are very strongly associated with stroke improve the prediction of risk of stroke for individuals in the general population. Most highly prevalent risk factors, including CRP, lack this characteristic. Current recommendations to use patients' CRP levels to estimate their future risk of cardiovascular disease seem erroneous. The best predictor for stroke remains age; risk scores based on additional characteristics do not improve prediction very much. However, patients with focal, nonfocal, and mixed TNAs are at higher risk of stroke than persons who have never experienced a TNA. Also men with depressive symptoms were at higher risk of stroke than men without depressive symptoms. The increased risk of dementia in stroke patients is not explained by pre-stroke cognitive function.

8.2.
Nederlandse samenvatting

Achtergrond en doel: Gedurende de laatste decennia zijn er vele factoren ontdekt die verschillen tussen individuen en die gerelateerd zijn aan de snelheid van arteriële degeneratie (atherosclerose en lipohyalinose), embolusvorming, en bloedingsneiging. Sommige van deze factoren kunnen worden gebruikt om iemands kans op een beroerte in te schatten en andere factoren kunnen zelfs gemodificeerd worden om zo de kans op beroerte te beïnvloeden. We weten echter slechts van een klein deel van de beroertes hoe deze ontstaan zijn en kunnen niet voorkomen dat vandaag de dag jaarlijks 41 duizend Nederlanders getroffen worden door een beroerte, waardoor dit in ons land de belangrijkste oorzaak van invaliditeit en de derde doodsoorzaak is. De studies in dit proefschrift zijn erop gericht om meer inzicht te krijgen in de pathofysiologische mechanismen die tot beroerte leiden en in de mogelijkheden om personen met een grote kans op beroerte te identificeren. De pathofysiologische mechanismen waar in dit proefschrift de nadruk op lag zijn betrokken bij de homeostase van bloeddruk en bloedvolume en bij atherosclerose in verschillende arteriële domeinen. Daarnaast hebben we, omdat het optreden van TIA (transient ischemic attack) één van de beste indicatoren van een hoog risico op beroerte is, onderzocht of aanvallen met neurologische symptomen die niet voldoen aan de typische diagnostische criteria van TIA ook geassocieerd zijn met de kans op toekomstige ziekten. Omdat cerebrovasculaire schade niet alleen tot beroerte en TIA maar ook tot stoornissen in cognitie en stemming kan leiden hebben we tenslotte de relatie tussen beroerte, depressie en dementie beschreven.

Methoden: De studies in dit proefschrift zijn gebaseerd op de Rotterdam Studie, wat een prospectieve cohortstudie in de algemene bevolking is. De studies zijn uitgevoerd in 7.721 deelnemers van 55 jaar en ouder die aan het begin van de studieperiode (tussen 1990 en 1993) nog geen beroerte hadden gehad. Alle deelnemers zijn gevolgd in de tijd om te zien of ze een beroerte, TIA, of andere ziekte kregen.

Resultaten: De kans op beroerte en ischemische hartziekte nam toe naarmate de urinezuurconcentratie in het plasma hoger was, met name bij mensen zonder hypertensie *(hoofdstuk 2.1)*. Het risico op hersenbloedingen nam toe met afnemende glomerulaire filtratiesnelheid (GFR) oftewel naarmate de nierfunctie slechter was. Er was geen relatie tussen GFR en de kans op een herseninfarct *(hoofdstuk 2.2)*. We hebben de relatie tussen hemodynamische parameters van de arteria cerebri media, gemeten met transcraniële Doppler echografie, en het risico op beroerte onderzocht. We vonden geen relatie tussen vasomotore reactiviteit (de verandering in bloedstroomsnelheid ten gevolge van een vasodilatatoire stimulus, in ons geval inhalatie van koolstofdioxide) en kans op beroerte. De kans op beroerte nam echter wel toe als de basale stroomsnelheid in de arteria cerebri media toenam *(hoofdstuk 2.3)*. Het

gebruik van cyclo-oxygenase (COX)-non-selectieve NSAIDs (non-steroidale anti-inflammatoire geneesmiddelen) en met name COX-2-selectieve NSAIDs verhoogde de kans op beroerte *(hoofdstuk 2.4)*.

Het Gly460Trp polymorfisme van het alpha-adducine-gen was geassocieerd met atherosclerose, cardiovasculaire aandoeningen en beroerte, met name onder mensen met hypertensie (hoofdstuk 3.1). Het M235T polymorfisme van het angiotensinogeen-gen (AGT) was geassocieerd met hogere systolische en diastolische bloeddruk en met meer witte-stoflaesies op de MRI-scan, maar niet met beroerte. Associaties met het C573T polymorfisme van de angiotensine II type 1 receptor (AT1R) waren minder duidelijk *(hoofdstuk 3.2)*. Er was geen relatie tussen genetische variatie in het oestrogeenreceptor alpha-gen (ESR1) en de kans op een beroerte *(hoofdstuk 3.3)*. Variatie in de fibrinogeen alpha en gamma genen was mogelijk geassocieerd met het risico op ischemische en hemorrhagische beroertes. Deze relatie was echter zwak en behoeft bevestiging *(hoofdstuk 3.4)*.

Het percentage beroertes dat verklaard wordt door de goed onderzochte modificeerbare causale factoren hypertensie, roken, atriumfibrilleren en diabetes samen was 54%. Dit is het maximale percentage beroertes dat voorkomen zou kunnen worden als deze factoren compleet geëlimineerd worden in de populatie. Dit relatief lage percentage zou kunnen toenemen tot ongeveer 68% als verschillende minder goed onderzochte risicofactoren causaal en modificeerbaar blijken te zijn *(hoofdstuk 4.1)*. C-reactief proteïne (CRP), een voorbeeld van een risicofactor die momenteel erg in de belangstelling staat, was geassocieerd met beroerte, maar desondanks droeg het niet bij aan de inschatting van de kans op beroerte van individuele patiënten *(hoofdstuk 4.2)*.

Veel patiënten lijden onder tijdelijke aanvallen met neurologische symptomen (transient neurological attacks of TNA's) die niet voldoen aan de diagnostische criteria voor TIA, daarom wordt in het algemeen aangenomen dat ze goedaardig zijn. Om uit te zoeken of deze aanname correct is hebben we alle TNA's in drie groepen onderverdeeld: focale TNA's (beter bekend als TIA's) met alleen focale symptomen, nonfocale TNA's met alleen nonfocale symptomen, en 'mixed' (gemengde) TNA's met een mix van focale en nonfocale symptomen tijdens dezelfde aanval. We bevestigden dat de kans op een beroerte verhoogd is na een focale TNA. Verrassend genoeg was de kans op beroerte en vasculaire dementie verhoogd na een nonfocale TNA. De prognose van 'mixed' TNA's was het slechtst: het optreden hiervan was geassocieerd met een verhoogde kans op beroerte, ischemische hartziekte, dementie, en vasculaire dood *(hoofdstuk 5)*.

Mannen met depressieve symptomen hadden meer kans om een beroerte te krijgen dan mannen zonder depressieve symptomen, en onze resultaten suggereerden dat het niet de mannen met een depressieve stoornis maar degenen met depressieve symptomen zonder depressieve stoormis waren die de grootste kans maakten op een beroerte. Bij vrouwen was er geen relatie tussen depressieve symptomen en beroerte *(hoofdstuk 6.1)*. De cognitieve functie voorafgaand aan een beroerte had geen invloed op de kans op dementie ten gevolge van een

beroerte *(hoofdstuk 6.2)*.

Conclusies: Slechts de helft van alle beroertes kan in theorie voorkomen worden door modificatie van de op dit moment bekende oorzaken van beroerte. Belangrijke nieuwe oorzakelijke mechanismen die naar voren kwamen in dit proefschrift waren gerelateerd aan het functioneren van de nieren, zoals effecten van urinezuur, glomerulaire filtratiesnelheid en natriumexcretie. Ook is pathologie van de grote intracraniële arteriën van belang. NSAIDs met COX2-effecten verhogen de kans op beroerte en moeten daarom terughoudend voorgeschreven worden.

Alleen factoren die heel sterk met beroerte geassocieerd zijn verbeteren de voorspelling van de kans op beroerte in individuen in de algemene bevolking. De meeste hoogprevalente risicofactoren, inclusief CRP, hebben deze eigenschap niet. Actuele richtlijnen om de CRP-spiegels van patiënten te gebruiken om hun toekomstig risico op cardiovasculaire ziekten in te schatten lijken daarom onjuist. De beste voorspeller van beroerte blijft leeftijd; risicoscores die gebaseerd zijn op additionele karakteristieken verbeteren de voorspelling niet aanzienlijk. Desalniettemin hebben patiënten met focale, nonfocale, en mixed TNA's een grotere kans op beroerte dan personen die nooit een TNA gehad hebben. Ook hebben mannen met depressieve symptomen een grotere kans op beroerte dan mannen zonder depressieve symptomen. De verhoogde kans op dementie in patiënten met een beroerte wordt niet verklaard door de cognitieve functie voorafgaand aan de beroerte.

8.3.
Publications and manuscripts

Incidence and prognosis of transient neurological attacks. Bos MJ, Van Rijn MJE, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. *JAMA 2007 Dec;298(24):2877-2885*

High serum C-reactive protein level is not an independent predictor for stroke. Bos MJ, Schipper CM, Koudstaal PJ, Witteman JC, Hofman A, Breteler MM. *Circulation 2006 Oct;114(15):1591-8*

Serum uric acid is a risk factor for myocardial infarction and stroke. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. *Stroke 2006 Jun;37(6):1503-7*

Transcranial Doppler hemodynamic indices and risk of stroke. Bos MJ, Koudstaal PJ, Witteman JCM, Hofman A, Breteler MMB. *Stroke 2007 Sep;38(9):2453-8*

Decreased glomerular filtration rate is a risk factor for hemorrhagic but not for ischemic stroke. Bos MJ, Koudstaal PJ, Hofman A, Breteler MM. *Stroke 2007 Dec;38(12):3127-32*

Plasma homocysteine is a risk factor for recurrent vascular events in young patients with an ischaemic stroke or TIA. Bos MJ, van Goor MPJ, Koudstaal PJ, Dippel DWJ. *J Neurol 2005 Mar;252(3):332-7*

Variation in estrogen receptor alpha gene does not influence risk of stroke. Bos MJ, Schuit S, Koudstaal PJ, Hofman A, Uitterlinden A, Breteler MMB. *In press, Stroke*

Depressive symptoms, depressive disorder, and risk of stroke. Bos MJ, Lindén T, Koudstaal PJ, Hofman A, Skoog I, Breteler MMB, Tiemeier H. *In press, J Neurol Neurosurg Psychiatry*

Alpha-adducin polymorphism and the risk of atherosclerosis, cardiovascular and cerebrovascular disease. van Rijn MJ, Bos MJ, Yazdanpanah M, Isaacs A, Arias-Vasquez A, Koudstaal PJ, Hofman A, Witteman JC, van Duijn CM, Breteler MM. *Stroke 2006 Dec;37(12):2930-4*

Polymorphisms of the Renin-Angiotensin System and the risk of atherosclerosis, cardiovascular and cerebrovascular disease. van Rijn MJE, Bos MJ, Isaacs A, Yazdanpanah M, Arias-Vásquez A, Koudstaal PJ, Witteman JC, Hofman A, Breteler MMB, van Duijn CM. *J Neurol Neurosurg Psychiatry. 2007 Oct;78(10):1083-7*

Pre-stroke cognitive performance, incident stroke and risk of dementia. The Rotterdam Study. Reitz C, Bos MJ, Hofman A, Koudstaal PJ, Breteler MMB. *Stroke 2007 Nov [epub ahead of print]*

Insulin-like growth factor I promoter polymorphism, risk of stroke, and survival after stroke: the Rotterdam study. van Rijn MJ, Slooter AJ, Bos MJ, Catarino CF, Koudstaal PJ, Hofman A, Breteler MM, van Duijn CM. *J Neurol Neurosurg Psychiatry 2006 Jan;77(1):24-7*

Retinal vessel diameters and risk of stroke: the Rotterdam Study. Ikram MK, de Jong FJ, Bos MJ, Vingerling JR, Hofman A, Koudstaal PJ, de Jong PT, Breteler MM. *Neurology 2006 May 9;66(9):1339-43*

TGF-beta 1 polymorphisms and risk of myocardial infarction and stroke: the Rotterdam Study. Sie MP, Uitterlinden AG, Bos MJ, Arp PP, Breteler MM, Koudstaal PJ, Pols HA, Hofman A, van Duijn CM, Witteman JC. *Stroke 2006 Nov;37(11):2667-71*

Unrecognized myocardial infarction and the risk of stroke: the Rotterdam Study. Ikram MA, Hollander M, Bos MJ, Kors JA, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. *Neurology 2006 Nov;67(9):1635-9*

Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. *Circulation 2006 Feb;113(5):657-63*

Incidentie van cerebrovasculaire ziekte in Nederland in 2000. Bots ML, Berger-van Sijl M, Jager-Geurts MH, Bos M, Reitsma JB, Breteler MMB, de Bruin A. *Brochure Dutch Heart Foundation*

Aortic arch and carotid calcification in subjects with a history of stroke. The Rotterdam Study. Odink AE, van der Lugt A, Bos MJ, Hofman A, Krestin GP, Koudstaal PJ, Breteler MMB, Witteman JCMW. *Submitted*

CRP-Gene Haplotypes, Serum CRP Levels and Cardiovascular Mortality: The Rotterdam Study. Reitz C, Kardys I, Bos MJ, Koudstaal PJ, de Maat MPM, Witteman JCM, Hofman A, Breteler MMB. *Submitted*

Impact of potentially modifiable etiologic factors on the burden of stroke. Bos MJ, Koudstaal PJ, Hofman A, Breteler MM. *Submitted*

Variation in fibrinogen FGA and FGG genes and risk of stroke. Bos MJ, Cheung E, Leebeek FWG, Koudstaal PJ, Breteler MMB, De Maat MP. *Submitted*

COX-selectivity of non-steroidal anti-inflammatory drugs and risk of stroke. Haag MDM, Bos MJ, Hofman A, Koudstaal PJ, Breteler MMB, Stricker BHC. *Submitted*

8.4.
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8.5.
About the author

Michiel Bos was born in The Hague *('s-Gravenhage)*, the Netherlands, in 1980. In 1998 he graduated from the Alfrink College in Zoetermeer and entered medical school at Erasmus University Rotterdam the same year. After his propaedeuse (cum laude) he entered the Master of Science program Clinical Epidemiology of the Netherlands Institute for Health Sciences, and attended the 2002 summer program of the Harvard University School of Public Health. He obtained his master's degrees in medicine and in clinical epidemiology in 2002 after finishing a research project on determinants of the prognosis of young patients with a minor stroke or transient ischemic attack at the department of Neurology. During his medical internships he started the work described in this thesis at the departments of Epidemiology & Biostatistics and Neurology of Erasmus Medical Center, Rotterdam. In 2005 he obtained his medical doctor degree. He presented his work among others at the International Stroke Conference 2003 (New Orleans, USA), at the annual meeting of the American Academy of Neurlogy 2007 (Boston, USA), and at the European Stroke Conference 2007 (Glasgow, UK). He plans to start working as a neurology resident at Erasmus Medical Center in July 2008.