Determinants of primary open-angle glaucoma

a genetic-epidemiologic approach

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Determinants of primary open-angle glaucoma

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Determinanten van primair open kamerhoekglaucoom

een genetisch-epidemiologische benadering

Proefschrift

ter verkrijging van de graad van doctor aan de
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	Prof.dr. M.D. de Smet
Copromotor:	Dr. A.A.B. Bergen

Voor mijn ouders

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1

INTRODUCTION

1

Introduction

Primary open-angle glaucoma (POAG) is a clinically and genetically heterogeneous eye disease, which involves the death of retinal ganglion cells and their axons due to an apoptotic process. Clinically, this neurodegeneration is characterised by an excavation of the optic nerve, referred to as glaucomatous optic neuropathy (GON), and a glaucomatous visual field defect (GVFD), in combination with open and normal anterior chamber angles. The prevalence of POAG varies around 1% in subjects 55 years and older in the western world, increasing from 0.1% in persons aged 55 to 2% in those over age 80.[1-4]

It is generally acknowledged that POAG is a complex disease.[5-7] In the majority of patients, POAG is thought to be the result of a combination of multiple genetic and non-genetic risk factors.[6] Different combinations of risk factors result in a heterogeneous group of disorders, together classified as POAG. Only in a small subset of families, POAG is caused by mutations in a single gene. The aim of this thesis is to investigate the role of several potential risk factors in the etiology of POAG, using a genetic-epidemiological approach.

Risk factors and pathogenesis-current status

Besides age, well-known risk factors for POAG include myopia, African descent, an elevated IOP.[8,9] The latter factor has been described extensively[10] and most therapy is presently directed at lowering the IOP, as it is the major risk factor that can be influenced. However, is has been shown in population-based studies, that in 30-50% of patients the IOP is within the statistically normal range.[1,3,10] In addition, the proportion of patients whose visual fields deteriorate despite treatment varies from 5% to 80%, with an average of approximately 10% per year.[11,12] These observations point to other factors involved in the development of glaucomatous damage.

One hypothesis is that a decreased blood flow leads to ischemia of the optic nerve head. Much has been written on the factors responsible for a decreased blood flow. Various vascular characteristics such as blood pressure, perfusion pressure, antihypertensive treatment, and vasospasms, have been studied extensively.[13,14]

However, data are contradictory and difficult to interpret. Moreover, most data available are clinic-based and few data are available from population-based studies.[15-19]

The recent finding that the loss of ganglion cells was caused by the process of programmed cell death (apoptosis) introduced a new area of research into glaucoma.[20] Following this innovative insight, alternative management of this disease may develop. In the future, neuroprotective therapy might be directed at preserving ganglion cells from death, independent of the level of IOP.[12]

A major risk factor is a positive family history of glaucoma.[21,22] Individuals with a first-degree relative with POAG have a nine times increased risk over those who do not have an affected relative,[23] and monozygotic twin pairs were shown to be more often simultaneously affected than dizygotic ones.[24] The identification of the first glaucoma disease gene (myocilin (MYOC), GLC1A) gave a new impulse to the research of POAG. This gene was originally identified in a family with juvenile onset POAG, segregating in an autosomal dominant manner.[25] However, it has been shown in clinic-based studies, that MYOC mutations were present in 3-5% of adult onset POAG patients.[25,26] Phenotypes, resulting from different mutations in this gene appeared to vary among patients.[27] Even in patients carrying the same mutation, different intrafamiliar phenotypes were described, which suggest the involvement of other factors than MYOC mutations alone in the development of POAG.[28,29]

Since the identification of MYOC, five other genomic regions have been linked to POAG in independent families (GLC1B to GLC1F).[30] These five additional POAG loci have been found in families where the disease segregated as an autosomal dominant trait and where the disease started in adult patients. The level of IOP was high in most POAG patients for all loci except GLC1E, where 12 of 15 POAG patients in this family had an IOP < 21 mm Hg. Because of the heterogeneous character of POAG, it is expected that even more disease genes will be identified.

Clinical, epidemiological and genetic investigations into POAG

Glaucoma studies are difficult to compare due to the lack of an internationally accepted definition of POAG. Often, POAG is discussed as a single disease, although it is generally acknowledged, that it is actually a group of diseases. It would be a step forward in classifying POAG according to aetiology, when we know

which genes are involved. As a consequence, one would expect that in the coming years part of the POAG group will not be named POAG any more but e.g. myocilin-based open-angle glaucoma (OAG).

Although a classification based on clinical features is unsatisfactory at the moment, clinical phenotyping is in itself an important step on the way to identifying genes. Until a more exact classification according to aetiology is reached, a world-wide-accepted clinical definition is necessary. In the Rotterdam Study, we proposed to define POAG as a combination of a GON with a GVFD.[4] The GON criteria were based on the distributions of optic disc characteristics in the population. This definition may be fine-tuned when incidence data become available.

In the subset of POAG families in which linkage with specific genomic regions (GLC1A to GLC1F) was found, POAG is segregating as an autosomal dominant trait. However, the majority of POAG families do not reveal a clear-cut Mendelian inheritance pattern. The heterogeneous character of POAG complicates research into the genetic causes, because one phenotype may be caused by different genetic factors. Reversibly, one genetic defect may cause different phenotypes with different levels of penetrance. Additionally, genetic and non-genetic factors may influence the expression of a gene.

Another problem complicating the genetic dissection of POAG is the late age of onset. It is difficult to identify individual families with a sufficient number of affected relatives for linkage studies, because parents and siblings are often deceased and children have not reached the age of onset yet. This implies that other methods than traditional linkage analysis will have to be pursued in the search for POAG disease genes.

Outline of thesis

In the first part of this thesis, potential environmental and genetic risk factors for POAG are examined in the Rotterdam Study, a prospective population-based cohort study among subjects 55 years and older. In its second part, the population of a genetic isolate is investigated, using a combined test for linkage and linkage disequilibrium.

Glaucoma cases with pseudoexfoliation were not specifically excluded in the baseline phase of the Rotterdam Study. Investigators disagree about whether pseudoexfoliative glaucoma should be named POAG and prevalence figures in glaucoma populations are reported up to 66% in Scandinavia, but also 12% in the

US and Australia.[3,31] As we did not see any pseudoexfoliation during follow up, and because we specifically excluded pseudoexfoliative glaucoma in the POAG patients from the genetically isolated population, we refer to the disease status as POAG in this thesis.

In chapter 2.1, a proposal for an international definition of POAG is presented as well as the prevalence of POAG in the Rotterdam Study based according to this definition. The association between early menopause and POAG is described in chapter 2.2, suggesting the involvement of hormonal factors in the disease etiology. In chapter 2.3, we investigate the relation between blood pressure, arterial stiffness, and POAG. A new method to estimate the genetic risk in an individual is demonstrated in chapter 3. This study was performed in a family study based on case and control probands derived from the Rotterdam population. In chapter 4, the prevalence of MYOC mutations was studied in a sample of POAG cases from the population-based Rotterdam Study. In addition, we described a family retrieved from this population, where POAG co-segregated with a mutation in this gene.

The exclusion of POAG candidate loci in a sample of patients from a genetically isolated population was reported in chapter 5.1. In chapter 5.2, a genome scan performed in this sample is described, two regions are proposed, and possible candidate genes are selected.

Finally, the main findings and methodological issues of all studies described in this thesis are discussed in chapter 6, with recommendations for future research.

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2

EPIDEMIOLOGICAL STUDIES IN THE ROTTERDAM STUDY

2.1

Definitions and prevalences of primary open-angle glaucoma in the Rotterdam Study

Abstract

PURPOSE- To create a quantitative basis for diagnostic criteria for primary open-angle glaucoma (POAG), and to propose an epidemiologic definition for POAG based on these, and to determine the prevalence of POAG in a general white population.

METHODS- Of the 7,983 subjects 55 years of age or older participating in the population-based Rotterdam Study, 6,756 subjects participated in the ophthalmic part of this study (6,281 independently living subjects and 475 in nursing homes). The criteria for the diagnosis of POAG were based on ophthalmic and semi-automated Imagenet estimations of the optic disc such as vertical cup-to-disc ratio (VCDR), minimal width of neural rim, or asymmetry in VCDR between both eyes, and visual field testing with kinetic Goldmann perimetry. All criteria for the diagnosis of POAG were assessed in a masked way independently of each other.

RESULTS- Mean VCDR on ophthalmoscopy was 0.3 and with Imagenet 0.49, and the 97.5th percentile for both was 0.7. The prevalence of glaucomatous visual field defects was 1.5%. Overall prevalence of definite POAG in the independently living subjects was 0.8% (95% confidence interval [CI] 0.6, 1.0; 50 cases). Prevalence of POAG was double that in women (odds ratio 2.1, 95% CI 1.2, 3.6). Different commonly used criteria for diagnosis of POAG resulted in prevalence figures ranging from 0.1% to 1.2%.

CONCLUSION- The overall prevalence of POAG in the present study was comparable to most population-based studies. However, prevalence figures differed by a factor of 12 when their criteria for POAG were applied to this population. A definition for definite POAG is proposed: a glaucomatous optic neuropathy in eyes with open angles in the absence of history or signs of secondary glaucoma characterized by glaucomatous changes based on the 97.5 percentile for this population, together with glaucomatous visual field loss. In the absence of the latter or of a visual field test, it is proposed to

speak of probable POAG based on the 99.5th or possible POAG based on the 97,5th percentiles of glaucomatous disc changes for a population under study.

Introduction

Primary open-angle glaucoma (POAG) ranks third among causes of incurable visual impairment in the Western world.[1-3] Despite prevalence figures in white subjects ranging from 0.8% to 3.0%[1, 4-14], little is known about its etiology. This may be partly due to the lack of a worldwide epidemiologic definition of, or standard for diagnosis of POAG[1-15] (Table 1). As a result many (epidemiologic) studies are difficult to compare, because of the different criteria and methods used for diagnosis, hampering meta-analyses and the search for risk factors.

It is nowadays generally accepted that POAG is an optic neuropathy characterized by cupping of the optic nerve head, with corresponding nerve fiber loss and visual field defects but that there is no consensus about cut-off points for normal disc measurements. An elevated intraocular pressure (IOP) is considered to be a risk factor for POAG, as well as the presence of a first-degree relative with glaucoma.[16] For the diagnosis POAG congenital forms of glaucoma have to be excluded, as well as secondary causes of glaucoma such as pseudoexfoliation.

The aim of the present study was to quantify in a masked way the prevalence of determinants of POAG in a white population, to propose diagnostic criteria for POAG, and to study the influence of various diagnostic criteria for POAG on the prevalence of POAG. Although pseudoexfoliative glaucoma was not excluded at baseline, we refer to the disease status as POAG, because pseudoexfoliation was not observed at follow up.

Materials and methods

Population

The present study was performed as the ophthalmic part of the Rotterdam Study, a prospective cohort study of all residents, 55 years of age and older.[17] Results of a prevalence study in a subset of the examined population using different criteria for POAG have been published previously.[10] The study was performed according to

Table 1
Different criteria for primary open-angle glaucoma

Baltimore Eye Survey [7]
Definite, probable and uncertain classification. Best available from eight, sometimes not quantified, different disc criteria (CDR ≥ 0.8, or difference between OU ≥ 0.3 or 0.4). VF defect not explainable by other causes. No IOP criterion.

Barbados Eye study[9]
VF, optic disc, and ophthalmic examination criteria. Seven combinations possible. Definite: at least on succession 2 abnormal VF together with 2 of 3 of following criteria: CDR ≥ 0.7, asymmetry ≥ 0.2, rim width ≤ 0.1, notching, disc hemorrhage. If not: suspect. IOP no criterion.

Beaver Dam Eye Study[8]
At least two of the following criteria: VF defect not explainable by other causes, CDR ≥ 0.8, or an asymmetry in CDR ≥ 0.2, IOP ≥ 22 mm Hg, or IOP-lowering treatment.

Blue Mountains Eye Study[11]
GVFD not explainable by other causes, combined with VCDR ≥ 0.7, or asymmetry in VCDR between both eyes ≥ 0.3.

Egna-Neumarkt study[13]
At least 2 of the following criteria with open angle: GVFD, IOP ≥ 22 mm Hg and 1 of the following disc criteria: CDR ≥ 0.7, asymmetry > 0.2, difference in VCDR and HCDR > 0.2, notching, disc hemorrhage, excavation reaching disc margin.

Framingham Study[23]
VF defect not explainable by other causes (only in selected part of the population), combined with VCDR ≥ 0.6, or asymmetry in VCDR between both eyes ≥ 0.2.

Melbourne visual impairment project[14]
No strict criteria due to uncertainty of diagnostic criteria. Panel discussion with 6 ophthalmologists grading in none, possible, probable, or definite POAG. Criteria: past POAG history, IOP > 21 mm Hg, VF defect including enlarged blind spot, CDR ≥ 0.7 or asymmetry ≥ 0.3.

Ponza Glaucoma study[12]
GVFDs and 1 of the following criteria: IOP > 20 mm Hg, CDR ≥ 0.5, or asymmetry ≥ 0.2. Suspect if questionable VF loss.

Rotterdam Study (2000 criteria)
If present in at least 1 eye with open angle and no history or sign of secondary glaucoma. No IOP criteria.
Definite POAG: GVFD combined with at least possible GON: VCDR ≥ 0.7, or asymmetry between both eyes ≥ 0.2 or a minimal rim width < 0.1.
Probable POAG: (1) GVFD without possible GON or (2) absence of GVFD or of any VF test with probable GON: VCDR ≥ 0.9, or asymmetry ≥ 0.3, or minimal rim width < 0.05.
Possible POAG: possible GON and no GVFD.

CDR: cup-disc ratio; GON: glaucomatous optic neuropathy; GVFD: glaucomatous visual field defect; HCDR: horizontal cup-disc ratio; IOP: intraocular pressure; OD: right eye; OD: left eye; OU: both eyes; POAG: primary open angle glaucoma; VCDR: vertical cup-disc ratio; VF: visual field.

the declaration of Helsinki and was approved by the Medical Ethics Committee of the Erasmus University. Written informed consent was obtained from all participants. All residents were asked to participate in an extensive home interview, after which an appointment was made for a medical examination, including a complete ophthalmologic one.

Ophthalmologic examination

The ophthalmologic examination was performed by three ophthalmologic residents and two technicians (Table 2). After perimetry, mydriatic drops were administered in both eyes, irrespective of the anterior chamber angle depth or history of glaucoma,[18] for lens and fundus examination, and photography. At the end of the first phase a miotic was administered in both eyes to counteract the mydriasis.

Optic disc measurements

Stereo transparencies from both eyes of all individuals were digitized and analyzed by two technicians with the semiautomated Topcon Image Analyzer (Imagenet), using the module for the retinal nerve fiber layer height. The system's hardware, its software modules and reproducibility of measurements have been described previously.[19,20] For both ophthalmoscopy and Imagenet the distribution of the measurements of the vertical cup-disk ratio (VCDR) and the asymmetry of the VCDR between both eyes together with their 97.5th and 99.5th percentiles were determined. The neural rim width was only determined with Imagenet and was defined as the proportion of the diameter of the rim section, measured at each of 36 equally spaced points on the optic disc border, in relation to the total optic disc diameter.

Proposed definitions for probable and possible glaucomatous optic neuropathies

Because cupping of the optic nerve head is the hallmark for glaucomatous optic neuropathy (GON), we chose to use this term. However, it does not imply that a person with GON definitely has glaucoma.

Probable GON was defined as the presence of at least one of the following characteristics: a VCDR, or asymmetry in VCDR between both eyes, or minimum width of the neural rim equal to or surpassing the 99.5th percentile of the population concerned. Possible GON was defined as the above and greater than or equal to the 97.5th but less than the 99.5th percentile of the population.

Table 2
Ophthalmologic examination and Open-Angle Glaucoma screening: The Rotterdam Study, 1990-1993

Examination type/method/tool	Manufacturer/Method Reference/Specifications
Phase I	
Autorefraction	Topcon RMA 2000*
Best corrected visual acuity	Lighthouse Visual Acuity Chart (2nd edition)
Keratometry	Topcon OM-4 Ophthalmometer*
Slit-lamp examination	Topcon SL-3E slitlamp*
Chamber angle slit-lamp estimation	Van Herick[29]
IOP measurement	Goldmann applanation tonometer[30], †
Visual field screening of both eyes separately	modified 52-point supra threshold screening test central 24° radius (Humphrey VFA)‡
Mydriatic drops	Tropicamide 0.5% and phenylephrine 5%
Color transparencies macular area	35° field; TRC-50VT camera* in mydriasis
Stereo simultaneous color transparencies optic disc	20° field; TRC-SS2 camera* in mydriasis
Ophthalmoscopy	Direct and indirect (AusJena ophthalmoscope, Zeiss bonoscope) in mydriasis
Miotic drop	One drop of thymoxamine-hydrochloride 0.5%
Phase II:	
Visual field screening	As in phase I in case of unreliable or defective VF
Phase III:	
Visual field determination	In case of unreliable or defective VF in phase II: kinetic Goldmann perimetry†, experienced perimetrist
IOP measurement	Goldmann applanation tonometer [30], †
Gonioscopy (Shaffer)	Goldmann 3-mirror contactlens†

IOP: intraocular pressure
VF: visual field
VFA: visual field analyzer
* Tokyo Optical Co, Tokyo, Japan
† Haag Streit, Bern, Switzerland
‡ Zeiss, Oberkochen, Germany

Visual field screening and determination

The visual field (VF) screening during the first phase (Table 2) reduced examination time and the chance of rim artifacts. Three or more contiguously missed points on the screening test (≥ 4 when blind spot was included) were taken as evidence for a VF defect. In case of a defective or unreliable VF test, VFs were retested with the same screening test in the second phase, about 2 weeks later. Subjects with a VF defect or unreliable test in the second phase of the study, underwent kinetic Goldmann perimetry on both eyes, performed by a skilled perimetrist in the third phase, some weeks later. Also, in cases with a Goldmann VF defect gonioscopy was performed to exclude cases with narrow angles. All subjects with glaucomatous VF defects had normal open anterior chamber angles. VF testing was unreliable or impossible in the institutionalized subjects, mainly due to physical and mental disabilities.

All Goldmann VF charts were independently graded by six different graders (three senior ophthalmologists, two residents, one perimetrist) according to a special grading protocol. Graders were at first masked to all clinical data and optic disc appearances. Classification of the defects was solely based on the shape and localization of the defect. With regard to glaucomatous VF defects (GVFDs) special attention was put on a nasal step, paracentral defects, arcuate scotomas, central rests, remaining peripheral islands, and temporal nerve fiber bundle defects. For fields with inconsistent classifications (30%) a consensus was reached among the graders. The fundus and optic disc transparencies were examined in a masked way for clues for retinal causes of VF defects and, if present, for the expected location of the VF defect. For exclusion of other nonglaucomatous causes of VF defects all other data available in the Rotterdam Study was used, including questionnaire data on and neurologic examination of all subjects, and (history) data from general practitioners including reports from all medical specialists who had treated the subject in the past.

Definition of glaucomatous visual field loss

Glaucomatous VF defect was defined as any Goldmann VF defect for which no other (neur)ophthalmologic cause could be found (see previous paragraph), thus excluding, for example, hemianopias and quadrantanopias.

Definitions for definite, probable, and possible POAGs, ocular hypertension, and elevated IOP

The following POAG definitions hold for a subject in whom in one or both eyes an open angle was present in the absence of a history or signs of angle closure or secondary glaucoma.

Definite POAG is the presence of a GVFD in combination with at least possible GON.

Probable POAG is either the presence of a GVFD in the absence of a GON or the absence of a GVFD with a probable GON.

Possible POAG is the presence of possible GON in the absence of either a GVFD or a VF test.

For logistic reasons subjects underwent a glucose tolerance test (GTT; by the cardiovascular research group) approximately 20 minutes before IOP measurement in the first testing phase. This GTT was carried out by giving an oral glucose load of 75g in 200 ml of water, and was performed on all nondiabetic subjects who had not had a gastrectomy.

The IOP was not used in the definition of POAG, neither was the use of IOP-lowering medication or the performance of an IOP-lowering (laser) operation in the absence of our criteria for POAG. Ocular hypertension was defined as an IOP > 21 mm Hg (or ≤ 21 mm Hg with any form of IOP-lowering treatment) in the absence of a GVFD or a GON. IOP values were adjusted for the IOP-lowering effect of the GTT.

Data analysis

Although the distribution of IOP and VCDR was not completely gaussian we thought it sound to assume a normal distribution of the large numbers in our study. Their 97.5th and 99.5th percentiles, also corrected for disc area quartiles and age strata, were parametrically calculated for each eye separately for the whole cohort, including the POAG cases. These percentiles were rounded up or down to the closest one decimal (for the minimum neural rim width to two decimals), in an attempt to include all POAG cases. In analyses in which Imagenet data were combined with ophthalmoscopic data, the latter was only used when the Imagenet data were missing or unreliable (n = 84).

Table 3
Response figures of the Rotterdam Study, 1990-1993

Age category, y	55-59	60-64	65-69	70-74	75-79	80+	Total
Independently living subjects:							
Total eligibles	1480	1761	1737	1606	1286	1291	9161
Total examined	1172 (79.2)	1421 (80.7)	1327 (76.4)	1157 (72.0)	834 (64.9)	583 (45.2)	6494 (70.9)
Ophthalmologically examined	1162 (78.5)	1399 (79.4)	1278 (73.0)	1108 (69.0)	795 (61.8)	594 (41.8)	6281 (68.6)
Men	483 (75.1)	616 (79.1)	594 (75.7)	452 (69.5)	315 (63.0)	166 (44.0)	2626 (70.5)
Women	679 (81.0)	783 (79.5)	684 (70.8)	656 (68.6)	480 (61.1)	373 (40.8)	3655 (67.2)
Nursing homes:							
Total eligibles	1	4	14	29	125	941	1114
Total examined	1 (100)	3 (75.0)	12 (85.7)	20 (69.0)	72 (57.6)	527 (56.0)	635 (57.0)
Ophthalmologically examined	0 (0.0)	1 (25.0)	8 (57.1)	12 (41.4)	60 (48.0)	394 (41.9)	475 (42.6)
Men	0 (0.0)	0 (0.0)	4 (50.0)	4 (50.0)	18 (48.6)	93 (54.7)	119 (53.1)
Women	0 (0.0)	1 (33.3)	4 (66.7)	8 (38.1)	42 (47.7)	301 (39.0)	356 (40.0)

Numbers in parentheses are percentages of the number of eligible subjects in each category.

Prevalence figures of GVFDs; definite, probable, and possible POAGs; elevated IOP; and ocular hypertension were calculated by 5-year age-categories and by gender. Prevalence figures of definite, probable, and possible POAGs were calculated using disc data obtained by ophthalmoscopy, by Imagenet and both. To estimate the influence of age and gender on these prevalence figures, logistic regression analysis was used. The odds ratio (OR) was used in these analyses as an approximation of the relative risk. Sensitivity, specificity, and predictive values of different cut-off points for VCDR for the presence of a GVFD and, thus, POAG were calculated. All analyses were adjusted for age and gender when appropriate and were performed separately for the independently living subjects and for those living in nursing homes.

Finally, definitions of definite POAG used in other population-based studies (Table 1) were, as far as available and common to those in our study, applied to our data.

Results

Population

Interview data were collected for 78% (n = 7,983) of the eligible persons (n = 10,275; independently living subjects plus nursing home subjects). The overall response rate for the center visit was 69% (n = 7,129). A total of 6,756 subjects participated in the ophthalmic part of the study. Table 3 shows the response figures, focused on the ophthalmologic examinations. The availability of ophthalmologic data in nursing homes was limited.

Distribution of optic disc dimensions

The distribution of the optic disc dimensions in the independently living subjects, determined by Imagenet and ophthalmoscopy, is shown in Table 4. The mean VCDR for ophthalmoscopy was 0.3, for Imagenet 0.49. Mean VCDR was significantly higher with Imagenet than with ophthalmoscopy. Mean VCDR, its asymmetry between both eyes, and mean minimal rim width were not significantly different in independently living subjects and those in nursing homes (data not shown). The influences of disc area and age on those disc measures are given in Tables 5 and 6, respectively. The 97.5th percentile of the VCDR was similar for

Table 4
Distribution of optic disc dimensions in independently living subjects determined by Imagenet and ophthalmoscopy

	Imagenet (SE) n = 5,681	Ophthalmoscopy (SE) n = 6,199
Mean VCDR	0.49 (0.0018)	0.30 (0.0024)
Median asymmetry in VCDR	0.06	0.00
Mean minimal neural rim width	0.17 (0.001)	not assessed
	Percentage of subjects	**Percentage of subjects**
Disc dimension VCDR ≥		
0.4	76.7	43.2
0.5	55.0	19.6
0.6	26.5	9.0
0.7	5.1	4.0
0.8	0.4	1.6
0.9	0.0	0.7
Asymmetry in VCDR ≥		
0.2	7.5	5.8
0.3	1.3	1.6
0.4	0.1	0.6
Minimal neural rim width <		
0.25	80.5	Not assessed
0.20	58.2	
0.15	26.2	
0.10	4.1	
0.05	0.1	

See Table 1 for abbreviations

Table 5
Influence of disc area measured by Imagenet on 97.5th percentile of vertical cup-disc ratio, separately for right and left eyes

Right Eyes		Left Eyes	
Disc area (quartiles)	**VCDR**	**Disc area (quartiles)**	**VCDR**
< 2.11 mm^2	≥ 0.68	< 2.07 mm^2	≥ 0.68
2.11 - 2.39 mm^2	≥ 0.71	2.07 - 2.36 mm^2	≥ 0.71
2.39 - 2.71 mm^2	≥ 0.73	2.36 - 2.68 mm^2	≥ 0.73
≥ 2.71 mm^2	≥ 0.76	≥ 2.68 mm^2	≥ 0.75
Overall	≥ 0.73	Overall	≥ 0.73

right and left eyes and differed 0.05 between the lowest and highest quartiles of disc area. Subjects 75 years of age or older had on ophthalmoscopy on average a 0.1 higher VCDR than those between 55 and 75 years of age. Table 7 shows the 97.5th and 99.5th percentiles for VCDR, asymmetry in VCDR and minimal neural rim width together with the chosen cut-off points for criteria for GON, based on the findings in Tables 4, 5, and 6. The 97.5th percentile of the VCDR for both

Table 6
Influence of age on 97.5th percentile of VCDR and asymmetry in it between both eyes both for ophthalmoscopy and Imagenet

Age (years)	VCDR OD		VCDR OS		Asymmetry	
	Imagenet	Ophthal-moscopy	Imagenet	Ophthal-moscopy	Imagenet	Ophthal-moscopy
55-64	0.72	0.7	0.72	0.7	0.26	0.2
65-74	0.73	0.7	0.72	0.7	0.26	0.2
75-84	0.74	0.8	0.74	0.8	0.30	0.2
85+	0.77	0.8	0.74	0.8	0.31	0.2

See Table 1 for abbreviations. Al data indicate greater than or equal to.

Table 7
Percentiles of optic disc dimensions in independently living subjects and derived cut-off points leading to criteria for probable and possible glaucomatous optic disc neuropathy

	Imagenet n = 5,619		Ophthalmoscopy n = 6,199	
Percentiles	97.5	99.5	97.5	99.5
VCDR ≥	0.73	0.78	0.7	0.9
Chosen cut-off point ≥	0.7	0.8	0.7	0.9
Asymmetry in VCDR ≥	0.26	0.34	0.2	0.3
Chosen cut-off point ≥	0.2	0.3	0.2	0.3
Minimal neural rim width ≥	0.08	0.05	not assessed	
Chosen cut-off point <	0.1	0.05		

Cut-off points derived from Tables 4,5, and 6.

Probable GON was defined as based on the 99.5th percentiles: a disc with a VCDR ≥ 0.9 with ophthalmoscopy or ≥ 0.8 for Imagenet, or an asymmetry in VCDR ≥ 0.3 between both eyes either on ophthalmoscopy or with Imagenet, or a minimal neural rim width < 0.05 on Imagenet.

Possible GON was defined as a VCDR ≥ 0.7, or an asymmetry ≥ 0.2 between both eyes either on ophthalmoscopy and Imagenet, or a minimal rim width < 0.1 with Imagenet.

ophthalmoscopy and Imagenet was ≥ 0.7 (as it was in a different substudy on this population for the Heidelberg Retina Tomograph). The cut-off point for asymmetry in VCDR between both eyes was ≥ 0.2 for both ophthalmoscopy and Imagenet. The chosen cut-off points for definitions of GON derived from Table 7, thus were used for definitions of POAG in the Rotterdam Study (Table 1).

Prevalence of glaucomatous visual field defects

The prevalence of glaucomatous visual field defects is shown in Table 8. The odds for men to have a GVFD were twice higher than for women (OR 2.0, 95% CI 1.3, 3.1). GVFDs were present in 8.6% of all subjects with a VCDR ≥ 0.7. This prevalence increased to 38% in subjects with a VCDR ≥ 0.8, and to 60% in subjects with a VCDR ≥ 0.9.

Prevalence of definite, probable, and possible POAG

Table 9 shows the overall prevalence of definite, probable, and possible POAGs for the various age groups derived from combined Imagenet and ophthalmoscopic data. When Imagenet was not available or unreliable, ophthalmoscopic data were used. Tables 10 and 11 show the data derived when these techniques were separated. Between the cases in Tables 10 and 11 there was overlap but not complete concordance. Of the independently living subjects, 50 had definite POAG (0.8%, 95% CI 0.6, 1.0%) with an OR of 2.1 (95% CI 1.2, 3.6) for men versus women. The risk estimates between POAG, age, and gender remained the same when the POAG cases defined with Imagenet or with ophthalmoscopy were analyzed separately or by pooling (see Tables 9 through 11).

Table 8
Prevalence of glaucomatous visual field defects

Age (years)	Men (%)	Women (%)	Total (%)
55-59	2/474 (0.4)	0/668 (0.0)	2/1142 (0.2)
60-64	5/602 (0.8)	4/763 (0.5)	9/1365 (0.7)
65-69	11/579 (1.9)	7/658 (1.1)	18/1237 (1.5)
70-74	13/424 (3.1)	11/628 (1.8)	24/1052 (2.3)
75-79	8/282 (2.8)	7/444 (1.6)	15/726 (2.1)
80+	10/149 (6.7)	9/312 (2.9)	19/461 (4.1)
Total	49/2510 (2.0)	38/3473 (1.1)	87/5983 (1.5)

Numbers indicate subjects.
Glaucomatous visual field defect was defined as any Goldmann VF defect

Table 9
Prevalence of POAG: The Rotterdam Study, 1990-1993--Imagenet data combined with ophthalmoscopic data

Independently living subjects

Age, y	Men (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Women (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Total	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG
55-59	483	1 (0.2)	1 (0.2)	7 (1.4)	72 (14.9)	679	0 (0)	0 (0)	10 (1.5)	94 (13.8)	1162	1 (0.1)	1 (0.1)	17 (1.4)	166 (14.2)
60-64	616	4 (0.6)	1 (0.2)	10 (1.6)	83 (13.5)	783	1 (0.1)	3 (0.4)	10 (1.3)	114 (14.6)	1399	5 (0.4)	4 (0.3)	20 (1.4)	197 (14.1)
65-69	594	5 (0.8)	6 (1.0)	6 (1.0)	98 (16.5)	684	6 (0.9)	1 (0.1)	17 (2.5)	105 (15.4)	1278	11 (0.8)	7 (0.5)	23 (1.8)	203 (15.9)
70-74	452	6 (1.3)	7 (1.5)	5 (1.1)	57 (12.6)	656	7 (1.1)	4 (0.6)	8 (1.2)	97 (14.8)	1108	13 (1.2)	11 (1.1)	13 (1.2)	154 (13.9)
75-79	315	6 (1.9)	2 (0.6)	11 (1.1)	51 (16.2)	480	3 (0.6)	4 (0.8)	16 (3.3)	70 (14.6)	795	9 (1.1)	6 (0.8)	27 (3.4)	121 (15.2)
80+	166	6 (3.6)	4 (2.4)	5 (3.0)	39 (23.5)	373	5 (1.3)	4 (1.1)	10 (2.7)	60 (16.1)	539	11 (2.0)	8 (1.5)	15 (2.8)	99 (18.4)
Total	2626	28 (1.1)	21 (0.8)	44 (1.7)	400 (15.2)	3655	22 (0.6)	16 (0.4)	71 (1.9)	540 (14.8)	6281	50 (0.8)	37 (0.6)	115 (1.8)	940 (15.0)
SEM		0.0020	0.0018	0.0025	0.0069		0.0013	0.0011	0.0022	0.0058		0.0011	0.0010	0.0017	0.0044
*Dependently living subjects**															
55-59	0					0					0				
60-64	0					1					1				
65-69	4					4					8				
70-74	4				1 (25.0)	8				1 (12.5)	12				2 (16.7)
75-79	18				3 (16.7)	42			2 (4.8)	6 (14.3)	60			2 (3.3)	9 (15.0)
80+	93			4 (4.3)	21 (22.6)	301			18 (6.0)	42 (14.0)	394			22 (5.6)	63 (16.0)
Total	119			4 (3.4)	25 (21.0)	356			20 (5.6)	49 (13.8)	475			24 (5.1)	74 (15.6)
SEM		-	-	0.0165	0.0375	-	-	-	0.0122	0.0183		-	-	0.0100	0.0166

Relative risk estimates for definite POAG in independently living subjects: gender (men compared with women), adjusted for age (OR 1.99; 95% CI 1.13, 3.51) and age (per year increase), adjusted for gender (OR 1.09; 95% CI 1.05, 1.12).

* In nursing homes no VF testing was performed. Thus, only probable and possible POAGs could be diagnosed.

Probable POAG GON: probable POAG based on probable GON; probable POAG GVFD: probable POAG based on GVFD.

Table 10
Prevalence of POAG: The Rotterdam Study, 1990-1993—Ophthalmic disc: data only

Age, y	Men (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Women (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Total	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG
Independently living subjects															
55-59	483	0 (0)	2 (0.4)	4 (0.8)	31 (6.4)	679	0 (0)	0 (0)	10 (1.5)	40 (5.9)	1162	0 (0)	2 (0.1)	14 (1.2)	71 (6.1)
60-64	616	3 (0.5)	2 (0.3)	10 (1.6)	41 (6.7)	783	1 (0.1)	3 (0.4)	7 (0.9)	58 (7.4)	1399	4 (0.3)	5 (0.4)	17 (1.2)	99 (7.1)
65-69	594	5 (0.8)	6 (1.0)	6 (1.0)	45 (7.6)	684	6 (0.9)	1 (0.1)	10 (1.5)	55 (8.0)	1278	11 (0.9)	7 (0.5)	16 (1.3)	100 (7.8)
70-74	452	9 (2.0)	4 (0.9)	10 (2.2)	19 (4.2)	656	8 (1.2)	3 (0.5)	10 (1.5)	53 (8.1)	1108	17 (1.5)	7 (0.6)	20 (1.8)	72 (6.5)
75-79	315	3 (1.0)	5 (1.6)	10 (3.2)	26 (8.3)	480	2 (0.4)	5 (1.0)	10 (2.1)	39 (8.1)	795	5 (0.6)	10 (1.3)	20 (2.5)	65 (8.2)
80+	166	6 (3.6)	4 (2.4)	2 (1.2)	22 (13.3)	373	4 (1.1)	5 (1.3)	7 (1.9)	33 (8.8)	539	10 (1.9)	9 (1.7)	9 (1.7)	55 (10.2)
Total	2626	26 (1.0)	23 (0.9)	42 (1.6)	184 (7.0)	3655	21 (0.6)	17 (0.5)	54 (1.5)	278 (7.6)	6281	47 (0.7)	40 (0.6)	96 (1.6)	462 (7.4)
SEM		0.0019	0.0018	0.0024	0.0050		0.0013	0.0011	0.0020	0.0044		0.0011	0.0010	0.0015	0.0033
*Dependently living subjects**															
55-59	0					0					0				
60-64	0					1					1				
65-69	4					4					8				
70-74	4					8					12				
75-79	18				2 (11.1)	41			2 (4.9)	3 (7.3)	59			2 (3.4)	5 (8.5)
80+	93			6 (6.5)	7 (7.5)	297			13 (4.4)	22 (7.4)	390			19 (4.9)	29 (7.4)
Total	119			6 (5.0)	9 (7.6)	351			15 (4.3)	25 (7.1)	470			21 (4.5)	34 (7.2)
SEM		-	-	0.0201	0.0243	-	-	-	0.0108	0.0138		-	-	0.0095	0.0120

Relative risk estimates for definite POAG in independently living subjects: gender (men compared with women), adjusted for age (OR 1.94; 95% CI 1.08, 3.47) and age (per year increase), adjusted for gender (OR 1.09; 95% CI 1.05, 1.12).
* In nursing homes no VF testing was performed. Thus, only probable and possible POAGs could be diagnosed.
See Tables 1 and 9 for definitions and abbreviations.

Table 11
Prevalence of POAG: The Rotterdam Study, 1990-1993—Imagenet data only

Independently living subjects

Age, y	Men (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Women (n)	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG	Total	Definite POAG	Probable POAG GVFD	Probable POAG GON	Possible POAG
55-59	483	1 (0.2)	1 (0.2)	6 (1.4)	67 (15.4)	620	0 (0)	0 (0)	7 (1.1)	85 (13.7)	1054	1 (0.1)	1 (0.1)	13 (1.2)	152 (14.4)
60-64	616	4 (0.7)	1 (0.2)	8 (1.4)	80 (14.2)	722	0 (0)	4 (0.6)	6 (0.8)	108 (15.0)	1285	4 (0.3)	5 (0.4)	14 (1.1)	188 (14.6)
65-69	594	2 (0.4)	9 (1.7)	5 (0.9)	90 (16.7)	626	4 (0.6)	3 (0.5)	14 (2.2)	100 (16.0)	1165	6 (0.5)	12 (1.0)	19 (1.6)	190 (16.3)
70-74	452	3 (0.8)	10 (2.5)	0 (0)	55 (13.8)	592	4 (0.7)	7 (1.2)	7 (1.2)	93 (15.7)	991	7 (0.7)	17 (1.7)	7 (0.7)	148 (14.9)
75-79	315	4 (1.6)	4 (1.6)	6 (2.4)	44 (17.5)	399	3 (0.8)	4 (1.0)	15 (3.8)	64 (16.0)	651	7 (1.1)	8 (1.2)	21 (3.2)	108 (16.6)
80+	166	2 (1.6)	8 (6.6)	5 (4.1)	36 (29.5)	262	4 (1.5)	5 (1.9)	7 (2.7)	49 (18.7)	384	6 (1.6)	13 (3.4)	12 (3.1)	85 (22.1)
Total	2626	16 (0.7)	33 (1.4)	30 (1.3)	372 (16.1)	3221	15 (0.5)	23 (0.7)	56 (1.7)	499 (15.5)	5530	31 (0.6)	56 (1.0)	86 (1.6)	871 (15.8)
SEM		0.0017	0.0025	0.0024	0.0077		0.0012	0.0015	0.0023	0.0064		0.0010	0.0013	0.0017	0.0049
*Dependently living subjects**															
55-59	0					0					0				
60-64	0					1					1				
65-69	4					2					6				
70-74	4				1 (25.0)	7				1 (14.3)	11				2 (18.1)
75-79	18				3 (23.1)	24				5 (20.8)	37				8 (21.6)
80+	93			1 (1.8)	17 (29.3)	169			8 (4.7)	33 (19.5)	226			9 (3.9)	50 (22.1)
Total	119			1 (1.3)	21 (26.9)	203			8 (3.9)	39 (19.2)	281			9 (3.2)	60 (21.4)
SEM		-	-	0.0128	0.0506	-	-	-	0.0137	0.0277		-	-	0.0105	0.0245

Relative risk estimates for definite POAG in independently living subjects: gender (men compared with women), adjusted for age (OR 1.63; 95% CI 0.80, 3.32) and age (per year increase), adjusted for gender (OR 1.09; 95% CI 1.04, 1.13).

* In nursing homes no VF testing was performed. Thus, only probable and possible POAGs could be diagnosed.

See Tables 1 and 9 for definitions and abbreviations.

In nursing homes no VFs were tested. In these subjects only probable or possible POAG could be diagnosed based on optic disc appearance. The prevalence of possible POAG was comparable to prevalence figures of possible POAG in the independently living subjects in the same age categories.

IOP distribution in this population

Although IOP was not used for the diagnosis of POAG in this study, we will present our data on IOP here for comparison with other studies. Our IOP data were influenced by the GTT. The IOP-lowering effect of the GTT was studied by comparing the IOPs of subjects who had undergone a GTT with those of subjects who had not (those who refused and diabetic subjects). Subjects with a GTT had a significantly lower mean IOP (-1.13 mm Hg, 95% CI -1.41, -0.84) than subjects without GTT (similar in diabetic subgroup and refuser subgroup). Unadjusted for the effect of the GTT the mean IOP (subjects with IOP-lowering treatment were excluded) was 14.5 mm Hg (95% CI 14.46, 14.61). After correction for the IOP-lowering effect of the glucose solution, the mean IOP was 15.6 mm Hg (95% CI 15.48, 15.64). The cumulative distribution of IOP (adjusted for the GTT) is shown in Figure 1. There were no significant IOP differences between independently living subjects and subjects in nursing homes ($p = 0.185$, adjusted for age and gender), or between men and women, and there was no clinically significant change in IOP with increasing age.

The prevalence figures of elevated IOP (> 21 mm Hg) are shown in Table 12 for the independently living subjects. The OR for men to have an IOP > 21 mm Hg compared with women was 1.35 (95% CI 1.10, 1.66). Ocular hypertension was present in 5.6% of participants and also was more prevalent in men than in women (OR 1.26; 95% CI 1.02, 1.56). Again, the prevalence of ocular hypertension in subjects in nursing homes was not significantly different from the prevalence in independently living subjects ($p = 0.48$, adjusted for age and gender).

Of the 50 diagnosed POAG cases (using the combined Imagenet and ophthalmoscopy data) 23 subjects (OR 46.0%; 95% CI 45.9, 46.1) were previously known to have POAG and received IOP-lowering treatment. Of the remaining 27 POAG cases, only three had an IOP > 21 mm Hg.

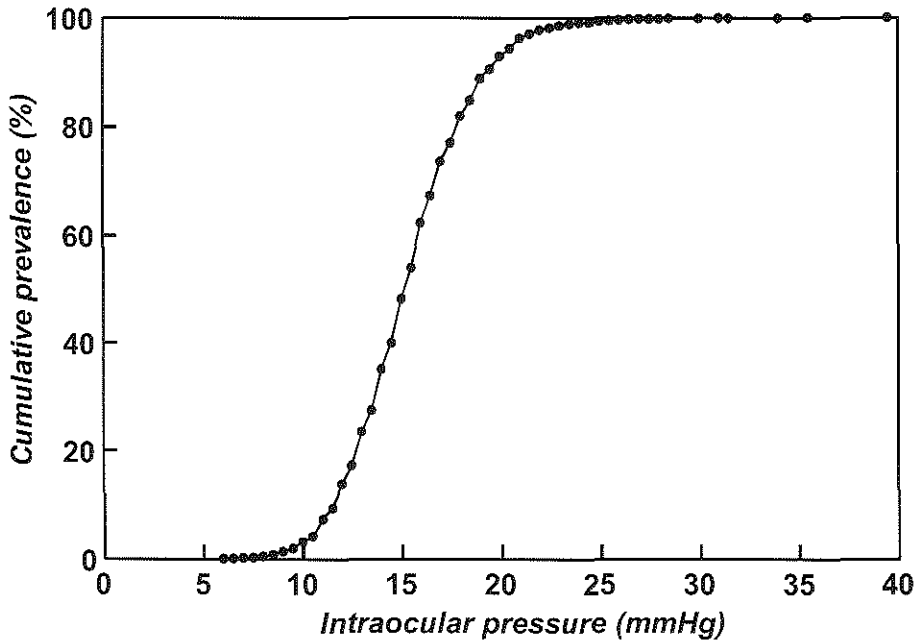

Figure 1
Cumulative distribution of the IOP adjusted for influence of a previously taken GTT in 5977 independently living subjects without IOP-lowering therapy. The 2.5th percentile corrected for the GTT was both for right and left eyes < 10 mm Hg, the 97.5th percentile > 22 mm Hg. Uncorrected for the GTT these were < 9 mm Hg and 21mm Hg, respectively.

On the other hand, of the 242 independently living subjects with IOP-lowering treatment, only 13 (OR 8.7%, 95% CI 5.1, 12.2) had definite POAG when using only ophthalmoscopic data, and 23 (9.5%, 95% CI 5.8, 13.2) subjects had definite POAG using the combined Imagenet and ophthalmoscopy data. The sensitivity of elevated IOP for detection of POAG was calculated only in the newly diagnosed POAG cases (because the IOPs at the time of diagnosis of the known POAG cases were not available). The sensitivity was 11.1% (3 of 27 cases had an elevated IOP) and the specificity 98.0% (5,827 of 5,943 subjects had no definite POAG). The predictive value of an IOP > 21 mm Hg for the detection of POAG was only 2.5%; the predictive value of an IOP ≤ 21 mm Hg for its absence was 99.6%.

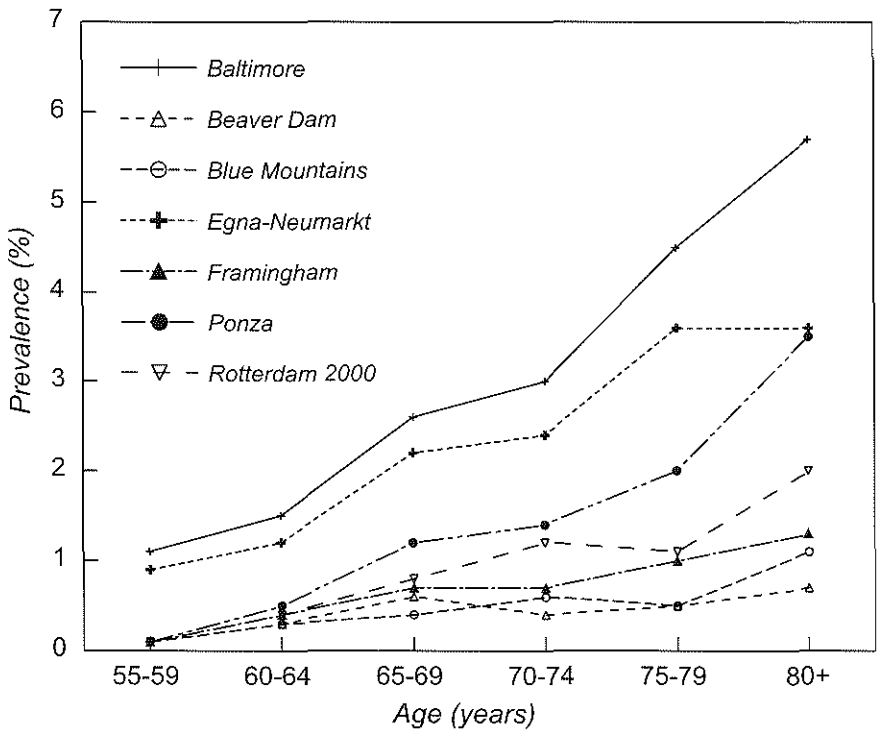

Figure 2
Variation in prevalence figures of POAG in the Rotterdam Study when different criteria for the definition of POAG, as used by other population-based studies, were applied to the Rotterdam data.

Figure 2 shows the variation in prevalence of POAG by age in our study, when POAG definitions from other large population-based studies were applied to our data. This resulted in prevalence figures varying between 0.1% and 1.4% in the youngest age-categories to prevalence figures between 0.9% and 5.9% in the oldest ones.

Discussion

In this article we have given the rationale for the following proposal for an international definition of POAG in epidemiologic research: POAG is a disorder characterized by a GVFD, in combination with probable or possible GON based on cut-off points approximating the 99.5th and 97.5th percentiles, respectively, in that population in at least one eye of a subject with open chamber angle, and no history

or sign of angle closure or secondary glaucoma. Thus an algorithm may be created leading to the diagnosis of definite, probable, or possible POAG (see addendum). If other research groups use a similar approach, one is free to pool definite and probable POAG or not.

When looking at Table 1 and reference 15, it seems that we have not made much progress in defining glaucoma since Donders coined the term glaucoma simplex in 1861.[21] It, thus, seems like a risky enterprise to start defining criteria for POAG nearly 150 years later. On the other hand, it is clear also from Figure 2 that there is a need for valid comparisons between studies. Current variations in definition allow wide variations in prevalence data, as well as justification for treatment. In this study, we define cut-off values for POAG determinants based on statistical grounds. This means that on arbitrary statistical grounds a division is made between normal and abnormal discs. We realize that this might be artificial and that some subjects may falsely be defined as healthy or abnormal. However, because of the large variation in POAG definitions in epidemiologic and/or clinical research, we think that it is for the time being a good starting point to use such a definition for better comparison and pooling of study results. In due time further refinement of the definition may become possible when more incidence data become available. Using 97.5th or 99.5th percentiles to define abnormality does not mean that we used these criteria to define someone as being diseased. Only in combination with other signs we propose the term definite POAG. We felt that our database allowed for the definition of POAG on statistical grounds and that such an approach may become a starting point for future diagnostic fine-tuning. This may not be too far away because some large population-based studies are working on incidence data on POAG.

Although pseudoexfoliative glaucoma was not excluded at baseline, we refer in this version of the manuscript to the disease status as POAG, because pseudoexfoliation was not observed at follow up.[11,13] In the published version, we refer to the disease status with OAG.

In this study we present prevalence figures for POAG combining GON data obtained by Imagenet and ophthalmoscopy. We think the Imagenet data are the more reliable data, especially for follow-up and risk factor analyses. However, because the Imagenet module for the optic disc is not available any more, and neither is the simultaneous stereoscopic Topcon TRC-SS2 camera, also essential for this module, we also present prevalence figures based on only ophthalmoscopic

optic disc data for comparison with other studies. We found in a substudy that Imagenet and the Heidelberg Retina Tomograph had much higher correlations for the estimation of the VCDR than ophthalmoscopy, showing that ophthalmoscopy is less reliable than these semiautomated apparatus, even carried out by trained examiners. Still we felt that in daily practice ophthalmoscopy will be the method of choice during the coming years. Therefore, in choosing cut-off points for the VCDR and other disc measures we looked primarily at feasibility, and tried to choose cut-off points that were also ophthalmoscopically assessible. Thus, to create as simple as possible a definition for POAG, based on GVFD and optic neuropathy, we propose as a cut-off point for a statistically abnormal, and thus arbitrarily pathologic, possible GON a VCDR $\geq$ 0.7, asymmetry in VCDR between both eyes $\geq$ 0.2, or neuroretinal rim width $<$ 0.1 for data obtained by ophthalmoscopy. The latter was not assessed in this study by ophthalmoscopy but would probably be necessary in other studies to detect discs with local notching of the rim. From Tables 5 and 6 one may see that for the largest discs the cut-off for pathologic VCDR might have been chosen as 0.8, and the same holds for those 75 years of age and older. All these subdivisions make the definition more and more complicated and that is why we propose to keep as the cut-off point for a possible GON a VCDR $\geq$ 0.7.

Hitherto, some studies did not specify whether one used information on IOP or disc measures to grade VF defects as glaucomatous.[2,8,13] Two were masked in this respect[9,11] and one was not.[14] Similarly, before deciding whether a subject had POAG, all studies mentioned in Table 1 looked at the combined data of a case while we tried to do so by combining strictly defined determinants without subjective overall evalution at the end. We believed that this would lead to less assessment bias. On the other hand, this resulted in small differences in prevalence of POAG when the Imagenet or ophthalmoscopy data were used.

Our overall prevalence of definite POAG of 0.8% (with combined use of Imagenet and ophthalmoscopic data; 0.7% when only using ophthalmoscopic data) and its rise with age is comparable to prevalence figures of the Framingham Study[24] (1.2%), of the Baltimore Eye Survey[7] (1.1%), and among the white subjects of the Barbados Eye Study[9] (0.8%). The Beaver Dam Eye Study and the Blue Mountains Eye Study, on the other hand, found a higher overall prevalence, 2.1%[8] and 3.0%,[11] respectively. The prevalence of definite plus probable POAG in the Rotterdam Study was 3.2% and this may explain the gap. Several more reasons for these

differences exist. All studies mentioned in Table 1 but the Egna-Neumarkt and the Rotterdam Study used for final assignment to glaucoma diagnosis a review of all data by one or more principal investigators, glaucoma specialists, or ophthalmologists. In this study we combined the VF and optic disc data, and this led straightforward to one of three diagnostic categories (apart from normals) without additional influence on the final results. The discrepancy with the Beaver Dam Study could be explained by their wider criteria for POAG. Other sources for differences between studies include sampling and perimetry techniques, screening methods for glaucoma, subjective interpretation of examination data, diagnostic criteria, age distributions, and real geographic contrasts in prevalence due to differences in lifestyle or genetic drift.

The VF screening and grading procedure in our study resulted in a prevalence of 1.5% of GVFDs compatible with POAG. This is comparable with the findings of the Framingham Study (1.4%, screening in a subset only, enlargement of blind spot excluded),[25] but lower than that found in Australia (3.1%).[11] The Blue Mountains Eye Study used, after screening, Humphrey full threshold perimetry (C30-2), which is more sensitive than kinetic Goldmann perimetry,[26] especially in glaucoma where it might detect up to 21% more defects.[27] Full threshold automated perimetry is nowadays considered to be the gold standard for VF examination, but at baseline in 1990 we felt that especially in older subjects it may create more false-positive errors compared with Goldmann perimetry. This might be because of poor fixation that accounted for 9% of inadequate Humphrey fields versus 2% at the Goldmann perimeter.[27] Between threshold Humphrey perimetry and kinetic Goldmann perimetry there is 88% concordance when both tests appeared reliable.[27] Because the Humphrey algorithms also have changed in the meantime and because we now perform both Humphrey 30-2 and Goldmann perimetry in a follow-up study, a more valid comparison between both methods will be possible within 1 year from now. It also has been shown that supra threshold perimetry identifies about two thirds of all cases identified by full-threshold perimetry.[28] Using this latter test our prevalence of definite POAG might have risen to approximately 1.4%. Even then there still would have been a twofold difference in prevalence by comparison with the Blue Mountains Eye Study. Given the variation in techniques and differences between various studies we believe that conclusions on geographic differences are for the time being not justifiable.

Our study differed from other large population-based studies with regard to the use of Imagenet to assess the optic nerve. Imagenet used strict criteria for defining the cup margins, based only on topographic data, thus reducing variation due to different observers. This makes it also particularly interesting for follow-up studies.[21] We found a higher mean VCDR on Imagenet measurements (0.49) compared with studies using other methods for examining the optic disc (mean VCDR 0.28[5], 0.3[10] using ophthalmoscopy by several examiners, 0.36[8] and 0.43[11] by grading of photographs). As a result, the prevalence of an enlarged VCDR was also higher in our study than in other studies (VCDR ≥ 0.4: 76.7% compared with 27.1%[5], 37.0%[8]). However, our prevalence of a VCDR ≥ 0.7 (5.1%) was only slightly different from the findings of the Blue Mountain Eye Study (5.0%), which examined stereo transparencies with a viewer.[11] Also, asymmetry in VCDR between both eyes was more prevalent in our study, compared with findings of other studies (4.6% asymmetry ≥ 0.2[23], 0.7% asymmetry ≥ 0.3[11]).

The relation between POAG and gender is still controversial. In Framingham[5] and Barbados[9] a higher prevalence of POAG was found in men, which matchdour finding. However, in the Blue Mountains Eye Study a (borderline significant) higher OR of 1.55 for POAG was found for women,[11] and in Baltimore[7] and Beaver Dam[8] no difference was found. It might be that in younger subjects the association between POAG and gender is not yet present. It would seem possible that if the study cohort had a greater proportion of younger the gender risk would disappear.

Our study did not show any correlation between age, gender or IOP. This is in contrast This is in contrast to previously published results,[1,4,29] but is in agreement with others.[1,5,8] Our findings do agree with prevalence data on IOP and VCDR in nursing home inhabitants.[30] However, the response in the nursing homes was low, especially in the older subjects, increasing the risk of selection bias. This could explain our lower POAG prevalences contrasting findings with that study.[30] One could adjust the prevalence rates for probable and possible POAGs in the nursing homes by raising them by 25% similar to the lower response rates in these homes than in the independently living subjects.

In conclusion, the overall prevalence of definite POAG in the Rotterdam Study was 0.8%, which is comparable to findings of other population-based studies on whites. The OR for men to have POAG was higher than for women. There was a significant increase in prevalence of POAG with increasing age. The overall prevalence of POAG varied 12-fold with different criteria and screening algorithms. We

hope that standardizing diagnostic procedures and our proposed definitions will improve future (epidemiologic) glaucoma research.

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Addendum:

Decision tree for classifying primary open-angle glaucoma

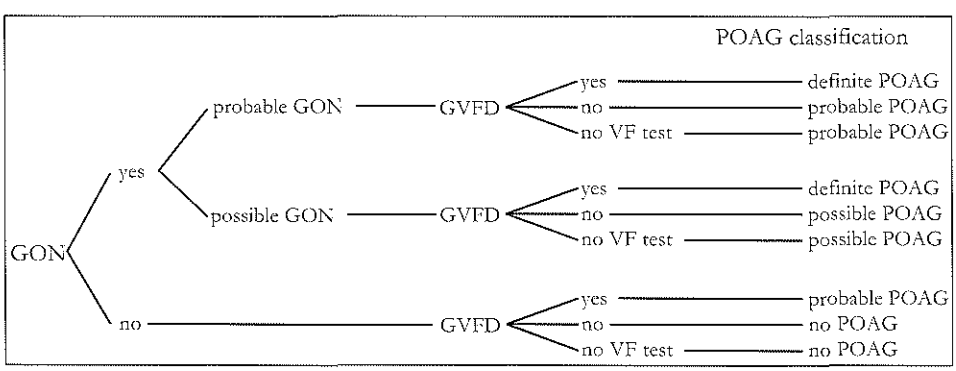
in subjects who have at least in one and the same eye an open anterior chamber angle and no history or signs of angle closure or secondary glaucoma

The diagnosis POAG depends on the presence or absence of a glaucomatous optic neuropathy (GON) and/or a glaucomatous visual field defect (GVFD). Because a GON usually appears earlier and is easier to assess than a GVFD, the decision tree starts with GON and afterwards includes presence or absence of a GVFD.

The diagnosis of GON is based on the 97.5th and 99.5th percentiles of the various disc measures in the population concerned.

Category of GON	VCDR	Asymmetry in VCDR between both eyes	Minimal neural rim width
Possible GON (97.5th percentile)	0.7	0.2	0.1
Probable GON (99.5th percentile)	0.8	0.3	0.05

Definite POAG is the presence of a GVFD in combination with a probable or possible GON.
Probable POAG is the presence of a GVFD in absence of a GON or the presence of a probable GON in the absence of a GVFD or of any VF test.
Possible POAG is the presence of a possible GON in the absence of a GVFD.



GON: glaucomatous optic neuropathy; GVFD: glaucomatous visual field defect.
See text for explanation

2.2

Primary open-angle glaucoma and age at menopause

Abstract

The authors examined the association between age at menopause and primary open-angle glaucoma among women aged ≥ 55 years in the population-based Rotterdam Study (1990–1993). Information on age and type of menopause was obtained by interview. Subjects (n = 3,078) were stratified into three categories according to age at menopause: < 45 years, 45–49 years, and ≥ 50 years, with the last group serving as the reference group. Diagnosis of primary open-angle glaucoma was based on the presence of a glaucomatous visual field defect and glaucomatous optic neuropathy. Primary open-angle glaucoma was diagnosed in 78 women with a natural menopause and 15 women with an artificial menopause. In the category of natural menopause, women who went through menopause before reaching the age of 45 years had a higher risk of primary open-angle glaucoma than the reference group (odds ratio = 2.6; 95% confidence interval: 1.5, 4.8), after adjustment for age and use of hormone replacement therapy. Among women who went through menopause between the ages of 45 and 49 years, the odds ratio was 1.1 (95% confidence interval: 0.7, 2.0). These findings suggest that early menopause is associated with a higher risk of primary open-angle glaucoma.

Introduction

Primary open-angle glaucoma (POAG) is characterized by a progressive loss of ganglion cells and their axons, resulting in glaucomatous optic neuropathy in combination with corresponding visual field defects. In the Western world, primary open-angle glaucoma is the third largest cause of irreversible visual impairment.[1] Despite the important implications of visual field loss, little is known about the pathogenesis and etiology of POAG. Elevated intra-ocular pressure (IOP) is an important risk factor,[2-4] but in 30–50 percent of POAG patients IOP is within the normal range; therefore, other causative factors must be present.[5]

Following our observation that the prevalence of POAG was twice as high in men as in women,[4] we hypothesized that one of the possible explanations for this difference is a protective effect of female sex hormones. This hypothesis is strengthened by the finding that IOP is higher among postmenopausal women than among men of the same age[6,7] and premenopausal women.[8] Furthermore, hormone replacement therapy has been suggested to lower IOP.[9,10]

To our knowledge, no population-based study has been performed to investigate this possible protective effect of female sex hormones, of which age at menopause is one indicator, on POAG. Thus, the purpose of the current study was to test our hypothesis and to determine whether age at menopause is associated with the prevalence of POAG.

Materials and methods

Population

The Rotterdam Study is a population-based prospective follow-up study of residents of a suburb of Rotterdam, the Netherlands, aged ≥ 55 years in which chronic cardiovascular, neurologic, locomotor, and ophthalmologic diseases are being investigated.[11] The present cross-sectional study was performed with baseline data, which were collected from 1990 to mid-1993. The study was approved by the Medical Ethics Committee of Erasmus University (Rotterdam), and written informed consent was obtained from all participants.

Of all eligible residents (n = 10,275) who were invited to take part in the study, 7,983 subjects (78 percent) participated in an extensive home interview (Table 1). Because the ophthalmic part of the Rotterdam Study started later than the other three sections, 6,872 subjects were eligible for the ophthalmologic study. Of the 6,756 subjects who participated in the eye examination, 4,032 were female. Nursing home residents (n = 366) were excluded, because visual field testing could not be carried out. Independently living women for whom we had no data on optic disc parameters (n = 24) or on visual field testing of at least one eye (n = 111) were also excluded. In addition, women who were premenopausal or who could not remember the date or cause of their last menstrual period (n = 189) were excluded. Complete menopausal and ophthalmologic data were available on 3,342 women.

Table 1
Procedure used to select the study population in the baseline phase of the Rotterdam Study, 1990-1993

Study group	No.	Exclusions	Characteristics of subgroup
Eligible persons	10,275	2,292 nonparticipants	
Participants in Rotterdam Study	7,983	1,111 with no physical examination or before start of ophthalmologic study	
Persons eligible for ophthalmologic study	6,872	116 with no eye examination	
Persons examined ophthalmologically	6,756	2,724 males	
Females	4,032	135 living independently	24 with no data on optic disc 111 with no perimetry or incomplete screening
		366 living in nursing home	No perimetry
Persons with complete ophthalmologic data	3,531	189 premenopausal or age or cause of menopause unknown	
Postmenopausal women	3,342	173 with menopause after cessation of contraceptive use 91 with menopause induced by medication	
Final study group	3,078		2,469 with natural menopause 609 with artificial menopause 576 with menopause induced by surgery 33 with menopause induced by irradiation

In the present study, women who reported experiencing menopause after stopping the use of oral contraceptives (n = 173) were excluded, because for these women true age at menopause was not known. Women who stopped menstruating after taking medication (n = 91) were also excluded, because data on the use and effects of medications with a specific influence on menopause were not available at the time of the analyses. The present analysis included 2,469 women who experienced a natural menopause and 609 women who experienced menopause after surgery or irradiation therapy.

Interview

Data on menopause, menarche, and use of hormone replacement therapy were obtained by self-report during the home interview, which was conducted by a trained research assistant. For women with natural menopause, age at menopause was defined as self-reported age at the time of last menstruation. For all women reporting menopause after gynecologic surgery or radiation therapy and for those reporting any other operations before age 50 that might have led to menopause, information on the exact date and type of operation was verified using general practitioners' records, which included correspondence from medical specialists. Data on the use of hormone replacement therapy were likewise validated. Age at menarche was defined as age at the date of the first menstrual period, and duration of exposure to endogenous sex hormones was defined as the difference in years between menarche and menopause.

Measurements

The complete eye examination included testing of visual acuity, ocular refraction, and visual fields, measurement of IOP, and slit-lamp examination, as well as indirect ophthalmoscopy.[4] For IOP, three measurements were taken and the median was used.[12] For assessment of the optic disc, stereo transparencies from both eyes were digitized and analyzed using the module for retinal nerve fiber layer height of the Topcon image analyzer Imagenet.[4,13] Disc area, vertical cup-disc ratio, and the smallest neural rim width were automatically calculated from the topographic data.[14] Visual field testing was performed as described by Wolfs et al.[4]

Visual field grading and classification

Six graders (three senior ophthalmologists, two residents, and one perimetrist) independently graded all Goldmann visual field charts according to shape and localization of the defect.[4] The graders were masked with regard to other clinical data such as medical history or optic disc characteristics. The cause of the visual field defect was determined using data from the home interview, ophthalmologic examination, and neurologic assessment and additional information obtained from the medical records of general practitioners and specialists. Glaucomatous visual field loss was defined as a visual field defect compatible with nerve fiber bundle defects (nasal step, paracentral defect, arcuate scotoma, temporal nerve fiber bundle defect, remaining peripheral island and a central remnant) after exclusion of other retinal or neuroophthalmologic causes.

Diagnosis

Although pseudoexfoliative glaucoma was not specifically excluded in the baseline phase of the study, we refer to the disease status as POAG, because at follow up no cases with pseudoexfoliative glaucoma were found. POAG was classified into three categories: definite, probable, and possible.[4] For the current analysis, we included only patients with definite and probable POAG.

Definite POAG was defined as the presence of a glaucomatous visual field defect in combination with a possible or probable glaucomatous optic neuropathy, based on the 97.5th and 99.5th percentiles of the distributions of the measurements in this population, respectively. The 97.5th percentile for vertical cup-disc ratio was 0.7, that for asymmetry between the vertical cup-disc ratios of both eyes was 0.2, and that for minimal neural rim width was 0.10. For the 99.5th percentile, these values were 0.8, 0.3, and 0.05, respectively. Probable POAG was defined either as the presence of glaucomatous visual field loss in the absence of a possible glaucomatous optic neuropathy or as a probable glaucomatous optic neuropathy, without glaucomatous visual field loss.

IOP was not included in our criteria for the diagnosis of POAG, but elevated IOP was included in the analysis because of its importance as a risk factor for POAG.[15] We considered the IOP elevated when applanation tonometry was over 21 mm Hg in one or both eyes or when treatment had been given to lower the IOP.

Data analysis

We studied the association between age at menopause and POAG in the following ways. First, we compared baseline characteristics between the women included in the analysis and those excluded from the analysis because of missing data. For this, both logistic and linear multivariate regression analyses were used. Next, women were stratified into three categories according to age at menopause: < 45 years, 45–49 years, and ≥ 50 years. The last group was regarded as the reference group. Odds ratios and 95 percent confidence intervals were calculated for definite and POAG and for elevated IOP, by age at menopause, using multivariate logistic regression analysis.

Relative risks were then calculated for women who had experienced either natural or artificial menopause. To examine the effect of ovarian estrogen production, we made a distinction in the artificial menopause group between women whose menopause started after irradiation therapy or bilateral oophorectomy and those whose menopause resulted from hysterectomy with or without unilateral oophorectomy. In all analyses, adjustments were made for age, hypertension, diabetes mellitus, use of hormone replacement therapy at any time, and duration of hormone replacement therapy (in months).[15]

Results

Baseline characteristics of the women who were included in the analyses and those who were excluded from the analyses are shown in Table 2. Excluded women were older. In these women, systolic blood pressure was higher after age adjustment, and percentage of women who had ever used hormone replacement therapy was lower. Mean age at menopause, mean vertical cup-disc ratio in the right eye, and duration of use of hormone replacement therapy did not differ between the groups.

Characteristics of women who experienced a natural menopause are given by age category in Table 3. Age at the time of examination ranged from 55 years to 94.5 years (mean = 68.8 years), and age at menopause varied from 30 years to 60 years (mean = 49.7 years). Women whose menopause occurred before age 45 were older; a higher percentage of them had used hormone replacement therapy at some time, and a higher percentage had used it for at least 1 year. In these women, the prevalence of definite and probable POAG and of elevated IOP was higher.

Table 2
Age-adjusted baseline characteristics of women included in analyses and women excluded from analyses (because of complete and incomplete ophthalmologic and menopausal data, respectively), The Rotterdam Study, 1990-1993

Characteristic	Included women (n = 3,342)		Excluded women (n = 699)	
	Mean or %	SD†	Mean or %	SD†
Mean age (years)	68.1	8.1	80.7*	10.1
Mean age at menopause (years)	48.8	4.9	48.7	5.8
Mean time since menopause (years)	19.3	9.5	32.5	11.0
Mean duration of exposure to endogenous hormones (years)	35.2	5.2	34.7	6.2
% with ever use of HRT†	7.9		2.1*	
% with use of HRT for ≥ 1 year				
Mean systolic blood pressure (mm Hg)	139.2	22.4	143.5*	25.1
Mean diastolic blood pressure (mm Hg)	73.3	11.1	72.0	13.8
% with hypertension	40.6		36.7	
Mean intraocular pressure in right eye (mm Hg)	15.7	3.1	15.5	4.3
% with elevated intraocular pressure	10.4		11.4	
Mean vertical cup: disc ratio in right eye	0.50	0.1	0.50	0.1

* $p < 0.05$.
† SD, standard deviation; HRT, hormone replacement therapy.

Table 3
Characteristics of women with natural menopause, by age group, The Rotterdam Study, 1990-1993

Characteristic*	Age group (years)		
	≥ 50 (n = 1,555)	45–49 (n = 650)	< 45 (n = 264)
Mean age (years)	68.4 (8.4)†	69.2 (8.4)	70.0 (7.6)
% with ever use of HRT‡	6.8	6.5	7.4
% with use of HRT for ≥ 1 year	2.4	3.0	5.4
% with definite open-angle glaucoma	0.4	0.6	1.1
% with probable POAG‡, based on a glaucomatous visual field defect	0.4	0.3	2.3
% with probable POAG‡, based on a glaucomatous optic neuropathy	1.9	2.0	3.4
% with elevated intraocular pressure	10.4	8.9	13.3

* see text for definitions and criteria.
† Numbers in parentheses, standard deviation.
‡ HRT, hormone replacement therapy; ‡ POAG, primary open-angle glaucoma.

Definite (n = 13) and probable POAG based on either a glaucomatous visual field defect (n = 14) or a glaucomatous optic neuropathy (n = 51) was diagnosed in 78 subjects (Table 4). Women who experienced menopause before the age of 45 years had a significantly higher risk of POAG (odds ratio (OR) = 2.6; 95 percent confidence interval (CI): 1.5, 4.8) than the reference group. Women who experienced menopause between the ages of 45 and 49 years did not have a significantly increased odds ratio (OR = 1.1; 95 percent CI: 0.7, 2.0). When we considered the small number of cases with definite open-angle glaucoma (n = 13), the odds ratio for women with menopause before age 45 was 3.5 (95 percent CI: 0.8, 14.8) and that for women with menopause between ages 45 and 49 was 1.8 (95 percent CI: 0.5, 6.7).

We next examined the relation between age at menopause and probable POAG, based on a glaucomatous visual field defect or a glaucomatous optic neuropathy (Table 5). The odds ratio for women with menopause before age 45 was 2.5 (95 percent CI: 1.3, 4.8) and that for women with menopause at age 45–49 years was 1.0 (95 percent CI: 0.6, 1.9), in comparison with the reference group.

Duration of lifetime exposure to endogenous sex hormones ranged from 15 years to 49 years (mean = 36.1 years). The odds ratio for POAG decreased significantly by 5 percent per year of exposure to endogenous sex hormones (OR = 0.95; 95 percent CI: 0.90, 0.99) and by 6 percent per year for each year of age at menopause (OR = 0.94; 95 percent CI: 0.90, 0.99). Additional adjustment for use of hormone replacement therapy did not markedly change our estimates.

Table 4
Odds ratios for definite and probable* primary open-angle glaucoma, by age at natural menopause, The Rotterdam Study, 1990-1993

Age at menopause (years)	Primary open-angle glaucoma		Odds ratio†	95% confidence interval
	Absent	Present		
≥ 50	1,276	41	1.0‡	
45–49	541	19	1.1	0.7, 2.0
< 45	210	18	2.6	1.5, 4.8

* Probable primary open-angle glaucoma was based on either a glaucomatous visual field defect or a glaucomatous optic neuropathy.
† Adjusted for age and use of hormone replacement therapy.
‡ Reference category.

Table 5
Odds ratios for probable* open-angle glaucoma, by age at natural menopause, The Rotterdam Study, 1990-1993

Age at menopause (years)	Primary open-angle glaucoma		Odds ratio†	95% confidence interval
	Absent	Present		
≥ 50	1,276	35	1.0‡	
45–49	541	15	1.0	0.6-1.9
< 45	210	15	2.5	1.3-4.8

* Probable primary open-angle glaucoma was based on either a glaucomatous visual field defect or a glaucomatous optic neuropathy.
† Adjusted for age and use of hormone replacement therapy.
‡ Reference category.

There were 609 women who had experienced an artificial menopause. Among women who had experienced menopause after irradiation therapy or bilateral oophorectomy with or without hysterectomy (n = 209), definite or probable POAG was diagnosed in seven women: four women with menopause at or after age 50 and three women with menopause between ages 45 and 49 years. POAG was not present in the women who had experienced menopause before age 45. In the category of women who had undergone a hysterectomy with or without unilateral oophorectomy (n = 400), these numbers were one, two, and five, respectively. Numbers were too small among women with an artificial menopause to calculate odds ratios. When we analyzed all women, regardless of cause of menopause (n = 3,078), the odds ratio for POAG was 1.8 (95 percent CI: 1.0, 3.0) among women with menopause before age 45 and 1.1 (95 percent CI: 0.7, 1.9) among women with menopause at age 45-49, in comparison with the reference group. In all analyses, additional adjustments for use of hormone replacement therapy, hypertension, diabetes, and elevated IOP did not markedly change our results.

Table 6
Odds ratios for elevated intraocular pressure, by category of age at natural menopause, for women with natural menopause and women who experienced artificial menopause after undergoing irradiation therapy or a bilateral oophorectomy, The Rotterdam Study, 1990-1993

Age at menopause (years)	Elevated intraocular pressure		Odds Ratio*	95% confidence interval
	Absent	Present		
≥ 50	1,450	172	1.0†	
45-49	659	62	0.8	0.6-1.1
< 45	293	42	1.2	0.8-1.8

* Adjusted for age, hormone replacement therapy and hypertension.
† Reference category.

Hormone replacement therapy

Among women with natural menopause or menopause after irradiation therapy or bilateral oophorectomy (n = 2,678), 188 women had taken hormone replacement therapy for some period of time. Mean duration of use was 2.5 years (maximum = 24 years). Of women who used hormone replacement therapy, three had definite or probable open-angle glaucoma. The risk of POAG was lower in women who had used hormone replacement therapy than in women who never had, but this estimate was not significant (OR = 0.54 (95 percent CI: 0.17, 1.74), adjusted for age and age at menopause).

Intraocular pressure

The odds ratio for elevated IOP among women who experienced either natural or artificial menopause before age 45 years was 1.2 (95 percent CI: 0.8, 1.8), and the odds ratio among those with menopause between ages 45 and 49 years was 0.8 (95 percent CI: 0.6, 1.1), in comparison with the reference group (Table 6). Additional adjustments for hypertension and hormone replacement therapy did not change the odds ratios.

Discussion

In this population-based study, women who experienced an early natural menopause had an increased risk of POAG. In interpreting this finding, some methodological issues must be addressed.

Women included in the present analysis may have differed from those excluded. We excluded subjects with incomplete ophthalmologic or menopausal data; these women were mostly older and living in a nursing home, where visual field screening was not possible. After we adjusted for age, there was no difference in most baseline characteristics between the two groups, but the percentage of use of hormone replacement therapy was lower and the mean systolic blood pressure was higher in the group of excluded subjects. Since age is an important risk factor for POAG, exclusion of an older set of women probably causes underestimation of the prevalence of the disease. Mean age at menopause and mean vertical cup-disc ratio were comparable in the two groups; therefore, we do not think selection bias had a large effect on our results.

Data on age and cause of menopause were self-reported. The distribution of ages at menopause in our study was comparable to the distributions reported by two leading studies on age at menopause in the Netherlands.[16,17] Still, misclassification could have occurred because of the time period between age at menopause and age at examination. Since we have no reason to assume that women with POAG would have reported an earlier age at menopause, misclassification would have been non-differential, and this would have given an underestimation of the true effect.

The main finding of our study was that women who had a natural menopause before the age of 45 had a significantly higher risk of POAG than those who had a natural menopause at age 50 or above. This is in agreement with our hypothesis that female endogenous sex hormones protect against POAG. However, the risk was not significantly elevated among women who experienced menopause between the ages of 45 and 49 years. This can be explained by a smaller contrast in hormonal status between the two groups. Probably only very early menopause is relevant for an increased risk of POAG. We cannot exclude the possibility that the small number of cases rendered our findings insignificant. When we considered only definite POAG, both in women who had menopause before age 45 and in those who had menopause between ages 45 and 49, the odds ratios were increased. The small number of cases probably explains the lack of significance in this analysis.

Another variable of possible importance is the interval between menarche and menopause. We studied the relation between menarche and POAG (1-year-later menarche: OR = 1.05; 95 percent CI: 0.92, 1.19). The reason for such a small effect and wide confidence intervals could be misclassification due to inaccurate assessment of age at menarche. Possibly, in contrast to cessation of hormonal activity

during middle age, the start of hormonal activity in youth has no effect or a weaker effect on risk of open-angle glaucoma. We also studied the interval between menarche and menopause, but this gave us results similar to those for age at menopause. For these reasons, we chose age at menopause as our indicator of exposure to endogenous female hormones. For analyses including other parameters of endogenous or exogenous exposure to hormones, such as artificial menopause and use of hormone replacement therapy, the data should be interpreted with care, since the numbers of cases in these categories were small.

There was no significant difference in risk of elevated IOP between women in different categories of age at menopause. This finding was expected from our hypothesis. No appreciable difference in risk was predicted, because any hypothesized IOP-lowering effect of estrogens and progestogens would be a direct effect, independent of age at menopause. In the literature, evidence is found for a direct effect of endogenous hormonal changes on aqueous humor circulation: Most authors agree that in pregnancy, with altered hormonal status, intraocular pressure decreases significantly.[18-20] This reversible effect is most likely explained by an increased outflow facility through the trabecular meshwork.[19]

The relation between POAG and gender is still controversial. In the Baltimore Eye Survey,[21] the Beaver Dam Eye Study,[22] and the Blue Mountains Eye Study,[23] no significant difference was found between prevalences of primary open-angle glaucoma in men and women. However, in the Framingham Eye Study,[24] the Barbados Eye Study,[3] and the Rotterdam Study,[4] up to a twofold higher prevalence was found in men. Many differences between these studies have been discussed.[4] The change over time in use of hormone replacement therapy, and thus a protective effect of exogenous sex hormones, could explain the gender difference.

Several biologic mechanisms could explain the association between early menopause and POAG. It may be assumed that the decrease in estrogen and progesterone levels after menopause may play a key role, and therefore biologic mechanisms influenced by these hormones may be involved. It is known that estradiol increases endothelial nitric oxide levels by enhancing the activity of the enzyme nitric oxide synthase III.[25-28] Several investigators have reported that nitric oxide induces a decrease in IOP--for example, by relaxation of the trabecular meshwork.[29-32] Moreover, as a vasodilator, nitric oxide may have an effect on the blood supply of the optic nerve and the basal vascular tone in uveal, retinal, and choroidal circulation.[32-37]

There is evidence that progesterone has the properties of a glucocorticoid antagonist.[38] Glucocorticoids are known to elevate IOP.[39,40] Progesterone may inhibit the ocular hypertensive effect of endogenous glucocorticoids by competing for the receptor-binding site. These receptors have been located in human trabecular meshwork cells[41] and rabbit iris-ciliary body cells,[42] binding both glucocorticoids and progesterone.

In the population-based Rotterdam Study, age at natural menopause was associated with the presence of POAG. For both etiologic and therapeutic reasons, further research into the effects of endogenous and exogenous exposure to female sex hormones would be of interest.

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2.3

Blood pressure, arterial stiffness, and primary open-angle glaucoma

Abstract

The association between blood pressure and primary open-angle glaucoma is still debated, as results are inconsistent between studies. We investigated blood pressure and arterial stiffness in relation to primary open-angle glaucoma among 5,732 subjects of the population-based Rotterdam Study. Primary open-angle glaucoma cases were classified into those with and without an intraocular pressure > 21 mm Hg (high-tension and normal-tension primary open-angle glaucoma, respectively). Arterial stiffness was determined by pulse wave velocity and common carotid distensibility. Associations were evaluated with logistic regression analysis, adjusted for age, gender, body mass index, education and smoking. Positive associations with high-tension primary open-angle glaucoma were found for systolic blood pressure (> 160 mm Hg: odds ratio: 2.9, 95% confidence interval: 1.1-7.6 compared to the reference group) and pulse pressure (> 80 mm Hg: odds ratio: 4.5, 95% confidence interval: 1.6-12.8), in subjects without treatment for systemic hypertension, and for low diastolic blood pressure (< 65 mm Hg: odds ratio: 3.0, 95% confidence interval: 1.1-8.2) and low diastolic perfusion pressure (< 50 mm Hg: odds ratio: 4.8, confidence interval: 1.3-17.7) in subjects treated for systemic hypertension. Increased arterial stiffness was associated with an increased risk of high-tension primary open-angle glaucoma, (relative risk (95% confidence interval) for the highest tertile of pulse wave velocity: 2.2 (0.9-5.5), and for the lowest tertile of distensibility: 2.7 (1.0-7.4), compared to their reference categories). No association was observed for normal-tension primary open-angle glaucoma. The findings of this study show that high systolic blood pressure, high pulse pressure, low diastolic blood pressure, and arterial stiffness are related to high-tension primary open-angle glaucoma.

Introduction

The vascular etiology of primary open-angle glaucoma (POAG) has been studied extensively. Still, the relation between risk factors such as systemic hypertension, systolic and diastolic blood pressure (BP), and POAG remains controversial. Studies have found associations between high[1-3] and low[1,4] levels of BP and POAG, while others did not find any relation.[5,6] Reasons for these contradictory results may be the complexity of the mechanisms possibly involved, the difference in design of studies, and the difference in definitions of POAG used in studies.

In the elderly, systolic hypertension is the most observed type of hypertension, which is found to be associated with the phenomenon of arterial stiffness. Recently, it has been suggested that arterial stiffness is not an innocent process in ageing, but may be related to ischemic heart disease and stroke.[7,8]

The objective of the present study was to examine systolic and diastolic BP, pulse pressure, as well as arterial stiffness, in relation to POAG.

Methods

Study population

The Rotterdam Study is a prospective cohort study among 7,983 subjects aged 55 years and older.[9] The baseline examination phase took place from 1990 to 1993 and the third phase between 1997 and 1999. The study was approved by the Medical Ethics Committee of the Erasmus University and written informed consent was obtained from all participants.

Measurements

A standardized eye examination was performed during the baseline phase of the study.[10] Optic disc transparencies were analyzed with Imagenet (Topcon, Tokyo) and ophthalmoscopy.[11] Visual fields were screened with the Humphrey Field Analyzer (Dublin, California) and defects were confirmed by Goldmann perimetry.[10]

Although pseudoexfoliative glaucoma was not excluded at baseline, we refer to the disease status as POAG, because pseudoexfoliation was not observed at follow up. POAG was classified into definite, probable, and possible.[10] According to the presence of an elevated IOP, defined as an IOP > 21 mm Hg or the use of IOP-

lowering treatment, POAG was stratified in high-tension POAG (htPOAG) and normal-tension POAG (ntPOAG).

BP was measured with a random-zero sphygmomanometer.[12] Pulse pressure was defined as the difference between systolic and diastolic BP; perfusion pressure as the difference between BP and IOP. Systemic hypertension was defined as a systolic BP ≥ 160 mm Hg, diastolic BP ≥ 100 mm Hg, or use of antihypertensive medication.

Information on medical history, alcohol, smoking, and education was obtained during the interview. A positive history of myocardial infarction or stroke was confirmed by reviewing medical records or by ECG. Diabetes was defined as a non-fasting serum glucose ≥ 11.1 mmol/l or the use of antidiabetic medication. Non-fasting total and high-density lipoprotein (HDL) cholesterol were determined using an automatic enzymatic procedure.

Arterial stiffness was assessed during the third examination phase by measuring pulse wave velocity with tonometry and common carotid artery distensibility by measuring the vessel wall motion with a Duplex scanner.[13]

Statistical analysis

Risk factors were investigated with logistic regression analysis, with definite and probable POAG cases as the case group and subjects without POAG as the reference group, repeated for htPOAG and ntPOAG separately. Odds ratios were computed as measures of relative risks (RR's).

Systolic, diastolic BP and pulse pressure were examined per 10 mm Hg and in four categories, using predefined cut-off points. For diastolic BP, we expected a J-shaped relation.[1,2,14] After inspection of the results, we chose the second category (65-74 mm Hg) as reference group. As we expected an inverse relationship for diastolic perfusion pressure, we chose the highest category (≥ 65 mm Hg) as reference group. Indicators of arterial stiffness were stratified in tertiles. As confounders were included: age; gender; smoking (categorized as never, former or current smoking); education (dichotomised in ≤ versus > primary education); and body mass index (weight/height^2).

Study population

Since the ophthalmic part of the Rotterdam Study started later than the other groups, 6,872 subjects participated and 6,756 (98.3%) had ophthalmic data for the diagnosis of POAG. Of these, 6,624 subjects were available for analyses, since 132 subjects had no data on BP. Data on pulse wave velocity were available for 3,364 subjects and data on common carotid distensibility for 2,996 subjects.

Results

Baseline characteristics are given for definite (n = 50) and probable POAG cases (n = 176) and for subjects without POAG (n = 5,516) in Table 1. Subjects with possible POAG (n = 1014) were excluded from all analyses. Definite POAG cases were older, and had more men, a higher IOP, and a higher pulse wave velocity than subjects without POAG.

Table 1
Characteristics of the Study Population by Primary Open-angle Glaucoma Status

Characteristic	No POAG	Probable POAG	Definite POAG
No.	5,516	176	50
Age, y	69.0 ± 9.0	73.0 ± 9.6	73.5 ± 7.3
Women, %	60	61	44
Intraocular pressure, mm Hg	16.0 ± 3.4	16.9 ± 5.9†	18.0 ± 4.5†
Systolic blood pressure, mm Hg	139.2 ± 22.4	144.0 ± 24.4	144.6 ± 21.6
Diastolic blood pressure, mm Hg	73.6 ± 11.6	74.7 ± 12.3	71.7 ± 12.1
Hypertension*, %	34	42	44
Antihypertensive treatment, %	32	40	40
Body mass index, kg/m^2	26.3 ± 3.7	26.3 ± 3.2	25.8 ± 3.2
Current smoker, %	24	20	22
Former smoker, %	41	38	40
Higher than primary education, %	62	58	62
Pulse wave velocity*, m/s	13.5 ± 3.0	13.9 ± 2.7	16.1 ± 5.0
Distensibility coefficient*, 10^{-3}/kPa	10.6 ± 4.4	9.4 ± 4.0	8.7 ± 5.7

Values are given as proportion or as mean ± SD. POAG indicates primary open-angle glaucoma
* see methods section for definitions
† different from subjects with no POAG, $p < 0.05$, adjusted for age and gender

Table 2
Association between blood pressure, pulse pressure per 10 mm Hg and primary open-angle glaucoma in subjects without blood pressure-lowering treatment

Blood pressure	Total	Intraocular pressure	
Per 10 mm Hg		≤ 21 mm Hg	> 21 mm Hg
Controls/POAG cases	3,600/139	3,600/101	3,600/38
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Systolic BP	1.06 (0.98-1.15)	1.05 (0.96-1.15)	1.16 (1.01-1.34)
Diastolic BP	1.04 (0.90-1.21)	1.08 (0.91-1.29)	1.01 (0.78-1.33)
Pulse pressure	1.06 (0.98-1.14)	1.04 (0.92-1.17)	1.25 (1.05-1.50)

Both definite and probable POAG cases were included in case group.
Relative risks are adjusted for age, gender, smoking, body mass index, and education.
POAG indicates primary open-angle glaucoma; BP, blood pressure; RR, relative risk; CI, confidence interval

Associations with POAG for systolic and diastolic BP and pulse pressure are given in Table 2 and Table 3. In these two tables, results are only shown for subjects without treatment for systemic hypertension, because of the difficulty in discriminating the effect of systemic BP on POAG from the effect of BP lowering treatment on POAG. Increases of 10 mm Hg in systolic BP and pulse pressure were associated with an increased risk of htPOAG (RR 1.16, 95% CI 1.01-1.34 and RR 1.25, 95% CI 1.05-1.50, respectively) (Table 2). For diastolic BP, no association was found with htPOAG and a statistically non-significant positive association was found with ntPOAG (RR 1.08, 95% CI 0.91-1.29). When only definite POAG cases were included in the case group, the RR's of htPOAG for systolic BP and for pulse pressure increased additionally (RR 1.23, 95% CI 1.01-1.51, and RR 1.30, 95% CI 1.00-1.68, respectively).

Results of BP parameters examined in four categories are shown in Table 3; we found increasing risks of htPOAG for higher levels of systolic BP and higher levels of pulse pressure (Table 3). Subjects in the highest category of BP (> 160 mm Hg) had an RR of 2.9 (95% CI 1.1-7.6) of htPOAG, compared to the reference group. Subjects in the highest category of pulse pressure (> 80 mm Hg) had an RR of 4.5 (95% CI 1.6-12.8) of htPOAG, compared to those with pulse pressure < 55 mm Hg. No association was found with ntPOAG for systolic BP, diastolic BP or pulse pressure. Since analyses between systolic perfusion pressure and POAG yielded results similar to those between systolic blood pressure and POAG, results of the former analyses were not shown.

Table 3
Association between blood pressure, perfusion pressure in four categories and primary open-angle glaucoma in subjects without blood pressure-lowering treatment

Blood pressure	Intraocular pressure	
	≤ 21 mm Hg	> 21 mm Hg
Controls/POAG cases	3600/101	3600/38
Systolic BP, mm Hg	RR (95% CI)	RR (95% CI)
< 130	Reference	Reference
130-144	1.3 (0.8-2.2)	1.5 (0.5-4.1)
145-160	0.8 (0.4-1.5)	2.5 (1.0-6.5)
> 160	1.2 (0.7-2.2)	2.9 (1.1-7.6)
Diastolic BP, mm Hg		
< 65	1.0 (0.6-1.8)	1.1 (0.5-2.5)
65-74	Reference	Reference
75-85	1.0 (0.6-1.7)	0.7 (0.3-1.6)
> 85	1.3 (0.7-2.4)	1.1 (0.4-2.8)
Pulse pressure, mm Hg		
<55	Reference	Reference
55-64	1.4 (0.8-2.5)	1.0 (0.3-3.6)
65-80	1.3 (0.7-2.3)	2.0 (0.7-6.0)
> 80	1.3 (0.7-2.5)	4.5 (1.6-12.8)

Both definite and probable POAG cases were included in case group.
Relative risks are adjusted for age, gender, smoking, body mass index and education
POAG indicates primary open-angle glaucoma; BP, blood pressure; RR, relative risk; CI, confidence interval

Since we expected a perfusion problem in particular for low diastolic BP, we separately examined the association between diastolic BP and POAG. As any effect of low diastolic BP could particularly be observed in subjects treated for systemic hypertension, we examined the relation between diastolic BP, diastolic perfusion pressure, and POAG in the treated group (Table 4). The risk of htPOAG increased when the diastolic BP decreased, with an RR of 3.0 (95% CI 1.1-8.2) for a diastolic BP < 65 mm Hg, compared to those with diastolic BP between 65 and 75 mm Hg. No significant association was found between diastolic BP and ntPOAG (data not shown). Subjects with a diastolic perfusion pressure < 50 mm Hg had an RR of 4.8 (95% CI 1.3-17.6), compared to those with diastolic perfusion pressure ≥ 65 mm Hg.

Table 4
Association between diastolic blood pressure, diastolic perfusion pressure and primary open-angle glaucoma in subjects taking blood pressure-lowering treatment

Characteristic	RR (95% CI)
Diastolic blood pressure, mm Hg	
< 65	3.0 (1.1-8.2)
65-75	Reference
75-85	1.4 (0.5-4.3)
≥ 85	1.0 (0.2-3.9)
Diastolic perfusion pressure, mm Hg	
< 50	4.8 (1.3-17.6)
50-57.5	2.1 (0.5-9.1)
57.5-65	1.8 (0.4-7.6)
≥ 65	Reference

Both definite and probable POAG cases were included in case group.
RR indicates relative risk; CI, confidence interval

Table 5
Association between arterial stiffness and primary open-angle glaucoma

Indicators of arterial stiffness	Total	Intraocular pressure	
		≤ 21 mm Hg	> 21 mm Hg
Pulse wave velocity, m/s			
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Controls/POAG cases	2,735/91	2,735/58	2,735/33
< 13.2	Reference	Reference	Reference
13.2–15.3	1.7 (1.0-2.8)	1.8 (1.0-3.4)	1.7 (0.6-4.6)
≥ 15.3	1.2 (0.7-2.0)	0.7 (0.3-1.7)	2.2 (0.9-5.5)
Distensibility, 10^{-3}/kPa			
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Controls/POAG cases	2,417/79	2,417/52	2,417/27
< 7.4	1.9 (1.0-3.4)	1.4 (0.7-3.1)	2.7 (1.0-7.4)
7.4–9.9	1.3 (0.7-2.4)	1.7 (0.9-3.3)	0.6 (0.1-2.3)
≥ 9.9	Reference	Reference	Reference

Both definite and probable POAG cases were included in case group.
RR indicates relative risk; POAG, primary open-angle glaucoma; CI, confidence interval

The presence of systemic hypertension was associated with a weakly increased risk of definite or probable POAG (RR 1.3, 95% CI 1.0-1.7). In those without antihypertensive treatment, the risk of htPOAG increased, but both estimates were not significant (RR 1.5, 95% CI 0.7-3.0). No association was observed between systemic hypertension and ntPOAG.

An increased pulse wave velocity and a low distensibility coefficient, both indicative for a high arterial stiffness, were associated with an increased risk of htPOAG in a dose-response relationship (Table 5). The lowest category of distensibility (< 7.4 10^{-3}/kPa) reached statistical significance (RR 2.7, 95% CI 1.0-7.4, compared to distensibility ≥ 9.9 10^{-3}/kPa).

Discussion

In this population-based study, we found that an increased systolic BP and an increased pulse pressure were associated with a higher risk of htPOAG in subjects who were not treated for systemic hypertension, while no association was found for diastolic BP. A low diastolic BP and a low diastolic perfusion pressure were associated with an increased risk of htPOAG in subjects with treatment for systemic hypertension. Furthermore, indicators of arterial stiffness were associated with htPOAG. We found no significant association between ntPOAG and systolic BP, diastolic BP, pulse pressure or arterial stiffness.

The present study has a cross-sectional design, which limits the causal interpretation of our findings. We have no evidence that the examined risk factors were present before POAG occurred. However, it is very unlikely that our results are caused by an effect of POAG on systemic BP or arterial stiffness. Therefore, the determinants can be interpreted as risk factors of POAG. Furthermore, we consider it unlikely that the association between low diastolic BP, diastolic perfusion pressure, and htPOAG in subjects treated for systemic hypertension is caused by the selective systemic antihypertensive treatment of POAG patients.

For the definition of POAG, we used the criteria described before.[10] All participants underwent a standardised ophthalmological examination where features of POAG were assessed separately in a masked fashion to ensure an unbiased diagnosis. The level of IOP was not included in these criteria, which enabled us to investigate risk factors separately in cases with ntPOAG and those with ntPOAG.

The distinction between IOP > 21 and ≤ 21 mm Hg is artificial for a continuous measure such as IOP. However, the cut-off point of 21 mm Hg

represents the 97.5 centile in our population[10] and is a commonly used cut-off point for elevated IOP. It is generally acknowledged, that POAG is considered a multifactorial disease, where it is likely that different combinations of factors are responsible for the different phenotypes of the disease.[15] In POAG accompanied by a normal IOP, other factors may be of importance than in POAG accompanied by a high IOP. Therefore, risk factors that are involved in a subset of the heterogeneous group of POAG may better be identified when analysing these subsets, instead of analysing the total group. Ophthalmological data were collected during the baseline phase of the study, while arterial stiffness was assessed in the third phase. As POAG is not related to survival (PH Borger et al, unpublished data, 2001),[16] it is unlikely that the different moments of measurements have biased our results. Arterial stiffness may be associated with an increased risk of cardiovascular disease.[7,8] As a consequence, subjects with a high level of arterial stiffness may have died before the third phase of the Rotterdam Study, which may have led to an underestimation of the association between arterial stiffness and POAG.

Several population-based studies have investigated the relation between BP and POAG.[1,2,4,17] Our findings of an association between high systolic BP, low diastolic perfusion pressure and htPOAG were also observed in the Egna-Neumarkt Study,[2] but in that study, the association between systolic BP and htPOAG was weak. In the Baltimore Eye Survey[1] and the Barbados Eye Study,[17] htPOAG was not separately examined, which complicates exact comparison with our findings. A modest positive association between systolic BP and the total group of POAG cases was found in the Baltimore Eye Survey.[1] The Barbados Eye Study[17] found no association with systolic BP, but reported low systolic BP/IOP ratios to be associated with POAG. Both studies found a strongly increased risk of POAG with low diastolic perfusion pressures, which is partly in agreement with our finding, because we found this association specifically in htPOAG and in subjects with treatment for systemic hypertension. No reports exist on the association between pulse pressure and POAG.

Although vascular factors often have been proposed as important risk factors of ntPOAG, we found no relation between ntPOAG and systolic BP, diastolic BP, pulse pressure or arterial stiffness. Our findings contradict several clinic-based case-control studies, which report that low systolic or diastolic BP are associated with ntPOAG,[18-21]. One explanation for the absence of an association in our study, while clinic-based studies did find an association, could be the absence of extremer levels

of BP in clinic-based studies. However, clinic-based studies are also more prone to selection-bias, which may be another explanation for the differences between our population-based study and clinic-based studies. Our results are in agreement with the only population-based study that examined ntPOAG apart from htPOAG,[2] and that found, like our study, no significant association between systolic or diastolic perfusion pressure and ntPOAG. The absence of an association with ntPOAG may be expected from the positive association between systolic BP and IOP: in contrast, in subjects with a normal IOP no increased systolic BP is present. Although our findings of ntPOAG need to be confirmed in other population-based studies, we may conclude that the mechanisms involved in the etiology of htPOAG may, at least partly, be different from those of ntPOAG.

Two mechanisms could be responsible for our finding of a higher risk of htPOAG with increased systolic BP, pulse pressure, and arterial stiffness. Firstly, in elderly subjects, an isolated systolic hypertension with normal or even low diastolic BP levels is often observed, resulting in a high pulse pressure and accompanying arterial stiffness. A perfusion pressure above or below a critical range may disturb the autoregulation.[14] We hypothesize that a high pulse pressure and arterial stiffness may lead to disruption of autoregulation. A disturbed autoregulation is observed in POAG patients.[22,23] When in subjects with a defective autoregulation the IOP increases, vessels may not be able to respond to changes in BP to maintain perfusion. This may lead to ischemia and optic nerve damage. Ischemia has been proposed by many investigators as a cause of POAG, as in these patients, the blood flow in the optic nerve head, retina, and choroids is reduced.[24-26]

A second explanation for the association between high systolic BP, pulse pressure, arterial stiffness, and htPOAG, may be a direct effect on the optic nerve head. The axons of the ganglion cells enter the optic nerve head embedded in a structure called the cribriform plate. Assuming that a high systemic pulse pressure produces a high pulse pressure in the eye, this may lead to more shearing forces in the cribriform plate and degeneration of the axons and the retinal ganglion cells.

The finding of an association between low diastolic BP and low diastolic perfusion pressure and htPOAG in subjects treated for systemic hypertension may be explained by a low perfusion of the optic nerve. A level of low perfusion may in particular be reached in case of an elevated IOP. It is known that if the diastolic BP drops below a critical level, cardiovascular mortality and morbidity increases, which has been called "a J-shape phenomenon".[27] This would occur especially under

antihypertensive treatment, demonstrating a harmful effect of artificially decreasing BP. Visual field progression in POAG patients has been found to be associated with nocturnal dips in systemic BP,[19,28] in particular when receiving systemic antihypertensive therapy[29]or in combination with a high nocturnal IOP.[30] Our findings confirm that (over) treatment of systemic hypertension may be a risk factor for htPOAG.

In the present study, we found a weaker association between systemic hypertension and POAG than in our previous study.[31] This is probably explained by the use of new criteria for both parameters. There is a discrepancy between the weak association between systemic hypertension and POAG, and the associations between systolic and diastolic BP and POAG. The different directions of the effects of systolic and diastolic BP on POAG may cause this discrepancy. A high level of systolic and a low level of diastolic BP were associated with an increased risk of htPOAG, while hypertension was defined as high levels of either systolic or diastolic BP.

It has been suggested, that the increased risk of systolic BP and pulse pressure for POAG is explained through an association with IOP,[17] as a significant association between systolic BP and IOP is well established.[1,2,31,32] Although the association between systolic BP, pulse pressure and POAG may partly be explained through IOP, we consider the increase in IOP too small (0.23 mm Hg increase in IOP per 10 mm Hg increase in systolic BP)[31] to contribute largely to the risk of POAG.

We showed a strong positive relation between htPOAG and systolic BP and pulse pressure in subjects not treated for systemic BP, an inverse relation with diastolic BP in subjects treated for systemic BP, and a relation between between htPOAG and arterial stiffness. Our results confirm the findings of other studies, and support the role of these risk factors and their consequences in the etiology of POAG.

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3

MYOCILIN MUTATION ANALYSIS

3

Myocilin mutations in a population-based sample of cases with primary open-angle glaucoma

Abstract

AIMS/BACKGROUND- To investigate the prevalence of myocilin (MYOC) mutations in a population-based sample of primary open-angle glaucoma (POAG) cases and to describe a family with both juvenile and adult onset POAG caused by a mutation in MYOC.

METHODS- The MYOC gene was screened in cases derived from the Rotterdam Study in the Netherlands. Definite POAG was defined as a glaucomatous optic neuropathy together with a glaucomatous visual field defect. Upon the identification of the Asn480Lys mutation in one case, seven additional family members were studied. To test for a founder effect with earlier reported families with this mutation, the haplotypes of the MYOC flanking markers D1S2851, D1S242, D1S218, and D1S1165 were compared.

RESULTS- Seven sequence alterations in MYOC were found in 14 of 47 POAG cases; six of these were also found in controls. In one case, an Asn480Lys mutation was found. In the family members of the latter patient, the phenotype ranged from a glaucomatous optic neuropathy without visual field defect in a 70-year old patient to severely affected optic discs and a remaining peripheral island in a 34-year old patient; those without the mutation had no signs of POAG. Haplotype analysis suggested a different origin of the mutation.

CONCLUSIONS- The prevalence of MYOC mutations (2.2%) among POAG patients was similar to that found in hospital-based studies. Although mutations in MYOC are rare, relatives carrying this mutation run a high risk of developing the disease. Instead of submitting all members of a family with the Asn480Lys mutation to frequent follow up, medical care can be restricted to those carrying the mutation.

Introduction

Primary open-angle glaucoma (POAG) is clinically characterised by a glaucomatous optic neuropathy (GON) and a glaucomatous visual field defect (GVFD) in eyes with open chamber angles and no history or signs of secondary glaucoma. Its prevalence increases from 0.1% in subjects aged 55, to 2.0% in subjects aged 80 or over.[1] In addition to age, risk factors include a high intraocular pressure (IOP), ethnic origin and myopia.[2-4] A major risk factor is a positive family history.[5-7] It is now generally accepted that POAG is a complex disease, which is a result of multiple and interactive genetic and environmental effects. Although six chromosomal loci[8-13] and five possibly involved genomic regions[14] for POAG have been described, the only causative gene known at present is myocilin[15] (MYOC) which codes for the MYOC protein.[16,17]

The normal function of MYOC is largely unknown. MYOC is expressed in multiple eye tissues, including the trabecular meshwork,[16] ciliary body and sclera,[18] axons of ganglion cells and several parts of the optic nerve head,[19,20] as well as in nonocular tissues.[18] It is not clear how mutations in MYOC cause glaucoma. Since the MYOC-related phenotype is characterised by an elevated IOP, the outflow of aqueous humour may be obstructed.[21] The mutated protein may either disturb the normal cytoskeletal function,[22] or contribute to extracellular matrix affecting the outflow.[17] Increased MYOC expression in trabecular meshwork was found in POAG and pseudoexfoliative glaucoma.[23] Since expression of MYOC in the optic nerve head was shown, Karali et al. suggested that the protein might be involved in glaucomatous damage in this tissue.[19]

While myocilin has been implicated in families with autosomal dominant juvenile glaucoma, mutations have also been described in sporadic patients with adult onset POAG. Among patients with a family history of POAG, the prevalence of MYOC mutations was 4.4%, and among sporadic POAG patients 2.6-4.6%.[15,24,25] In total, more than 30 different disease-causing mutations have been identified in large samples of patients from different ethnic backgrounds.[25] The age of onset, the penetrance and the severity of the disease vary considerably among subjects with mutations in MYOC.

So far, all mutation analyses have been carried out in samples of POAG patients from hospital-based studies, which are not representative of POAG in the general population. To know the prevalence of MYOC mutations in POAG in the general

population, it is necessary to screen a sample of POAG cases that is drawn from a population-based study, not selected by level of IOP, age at diagnosis or family history.

We investigated the prevalence of MYOC mutations in POAG cases from the population-based Rotterdam Study in the Netherlands. We also described a family derived from our current study with both juvenile and adult onset POAG caused by a mutation in MYOC and investigated whether this mutation was based on a European founder effect.

Material and methods

Cases

Cases were derived from the Rotterdam Study, a follow-up study of determinants and prognosis of chronic diseases in the elderly.[26] All residents, aged 55 years and older, living in one suburb of Rotterdam, the Netherlands, were invited to participate. The present study was performed in baseline data, which were collected from 1990 to mid 1993. The study has been approved by the Medical Ethics Committee of Erasmus University (Rotterdam) and written informed consent was obtained from all participants.

Although pseudoexfoliative glaucoma was not specifically excluded in the baseline phase of the study, we refer to the disease status as POAG, because at follow up no cases with pseudoexfoliative glaucoma were found.

The complete eye examination included testing of best-corrected visual acuity, measurement of IOP, slitlamp examination, direct and indirect ophthalmoscopy, fundus photography, and simultaneous stereo photography of the optic discs on colour transparencies.[1] Optic disc parameters were assessed with the Topcon image analyser Imagenet. Visual fields were screened with a modified 52-point suprathreshold test (Humphrey VFA) and confirmed with kinetic Goldmann perimetry (Haag Streit, Bern Switzerland).[1]

POAG was classified into three categories: definite, probable and possible. Definitions and cut-off points have been described before.[1] In short, definite POAG was defined as a possible or probable GON together with the presence of a GVFD. Probable POAG was defined as the presence of a probable GON without a GVFD or the presence of a GVFD without a possible GON.

To perform the present mutation analysis in the sample of cases derived from the Rotterdam Study, only cases with definite POAG were included. The age of onset of the disease was not determined. An elevated IOP was considered present when either the IOP was over 21 mm Hg during our examination or IOP-lowering treatment had been used, including medical, laser or surgical therapy. Unaffected relatives and spouses from families who participated in prior linkage studies were included as controls.[27] Sequence changes, which were supposed to be disease causing, were tested among 100 controls; those that were previously described as polymorphisms were tested in at least 50 controls.

After detection of the Asn480Lys mutation in one POAG case, who was deceased in the meantime of the study, relatives were contacted through another member of the family. Since the history of glaucoma was known in the family, most relatives had been attending an ophthalmologist. For most participating relatives and for relatives who were deceased at the time of the investigation, data were retrieved from medical charts.

DNA analysis

Genomic DNA was isolated from the peripheral blood lymphocytes. The three exons of the MYOC gene, the flanking splice sites, and the promoter regions that were proven to be functionally important[28] were screened by single strand conformational polymorphism (SSCP) analysis on a 6% acrylamide gel with 0 and 5% glycerol. For the first and the third exon, three pairs of primers and for the second exon one pair of primers were used. Except for one pair of primers of the first exon (F: ccaagcctctgcaatgaggt) and one of the second exon (F: ctggccggcagcctatttaa), primers were described before.[18] Microsatellite markers were PCR amplified using standard procedures.[29] Nucleotide sequences were determined by direct sequencing of both strands of the PCR product.

For seven family members of the POAG case carrying the Asn480Lys mutation, DNA was obtained. These persons were screened for the presence or absence of the mutation by direct sequencing. To investigate the possibility of a founder effect with the patients described by Brézin[30] the alleles of four markers in the region of the disease gene were compared between these patients and the patients of our family. The markers D1S2851, D1S242, D1S218, and D1S1165 were tested.

Results

Cases

Definite POAG was present in 50 subjects of those who were ophthalmologicaly examined (n = 6,756). DNA was available of 47 of them. Mean age of these 47 cases was 73.3 years, ranging from 58.7 to 88.5 years and 26 (55%) were male. A positive family history of first-degree relatives was reported by 14 (30%) patients. Of the 47 cases, 23 were previously known to have POAG and received IOP-lowering treatment. Of the remaining 24 newly identified cases, six had an IOP > 21 mm Hg.

Mutation screening

In the 47 cases from the Rotterdam Study, seven sequence alterations in MYOC were found (Table 1). One case had a previously reported heterozygous Asn480Lys mutation in the third exon, which could not be found among control persons (n = 100). The Arg76Lys change in the first exon was found in 10 cases and in 30

Table 1
Sequence changes in myocilin found in 47 primary open-angle glaucoma patients from the Rotterdam study (1990-1993) and in unrelated controls

Sequence change	Location	Fraction of patients of Rotterdam Study with variant[a]	Fraction of unrelated controls with variant[a]	Fraction of controls with variant from other reports
Bp -127 (T→C)	promoter	2/40	6/94	not applicable
Bp -83 (G→A)	promoter	8/40	30/94	112/686[24,25]
Arg76Lys	exon 1	10/46	30/103	18/288[24,25] 23/132[21]
Tyr122Tyr	exon 1	1/46	2/86	2/793[24]
Tyr347Tyr	exon 3	2/45	1/51	34/793[24]
Lys398Arg	exon 3	1/45	3/111	1/60[38] 7/793[24,25]
Asn480Lys	exon 3	1/45	0/100	not applicable

[a] The number of patients differs among parts of exons, because only results that have been confirmed in our laboratory twice are reported. The samples without unambiguous results of cases or controls have been omitted from the table.

controls. In at least eight cases with this change, an additional bp -83 (G→A) change in the promoter region was detected, which was also found in the same 30 controls.

Another sequence change in the promoter region (bp -127 T→C) as well as a Lys398Arg change in the third exon was found both in cases and controls. Two other variations resulted in synonymous codon changes (Tyr122Tyr and Tyr347Tyr).

Myocilin Asn480Lys: Clinical investigation and genetic analysis

Upon the identification of the Asn480Lys mutation in one female case, we initiated the investigation of additional family members. A pedigree was constructed (Figure 1) including one additional case with definite POAG and one case with probable POAG (Table 2).

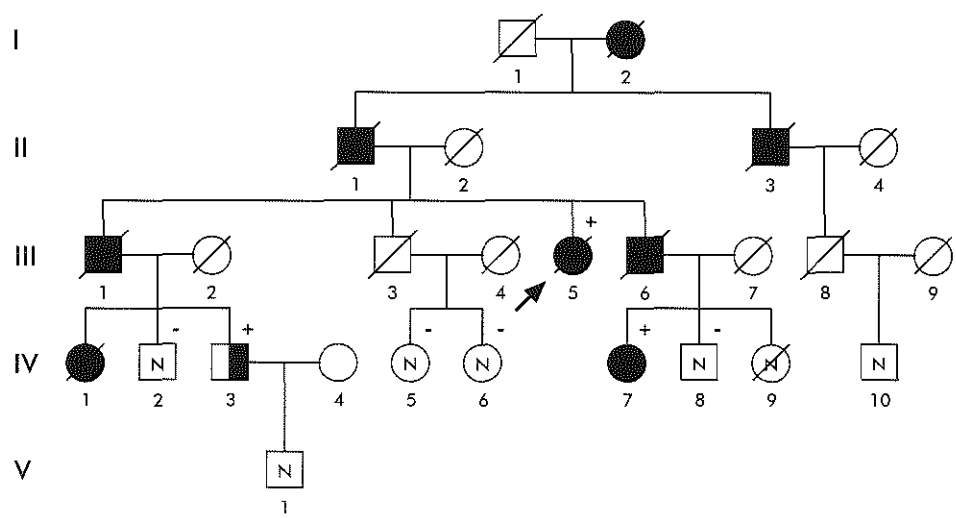

Figure 1

Square = male; circle = female; solid symbol = definite POAG; hatched symbol = probable POAG; open symbols = disease status unknown; symbol with N = unaffected individual; + sign = Asn480Lys mutation present; - sign = Asn480Lys mutation absent; diagonal line through symbol = individual is deceased.

Subjects from generation I and II and subjects who were deceased at the time of the investigation (III-1, III-6, IV-1) have not been examined. The definition of POAG in these subjects is based on history from relatives or on data from medical charts.

The female proband (III:5) had a history of POAG being present around age 40. Despite medical therapy and surgery, visual field loss deteriorated. When she came to our attention through participating in the Rotterdam study, she was 88 years old and was using medical therapy to lower the IOP. She had lost sight in her left eye due to a haemorrhage during glaucoma surgery. The other eye showed a GVFD of a large part of the upper hemisphere.

Patient IV:7 was diagnosed with definite POAG. When she visited an ophthalmologist for the first time at age 34, she had severely excavated discs in both eyes, with a glaucomatous quadrant anopia in the right eye, an IOP of 31 mm Hg in the right eye, and 21 in the left eye. Also in this patient, visual fields and optic discs deteriorated, despite medical therapy and surgery. At the time of our study, visual field testing showed a remaining temporal island of the right eye and an arcuate defect of the left eye. Probable GON was present in both eyes, existing of a total excavation in the right eye and an excavation locally extending to the disc margin in the left eye.

Table 2
Clinical features of affected relatives of pedigree with Asn480Lys mutation in myocilin

Subject	Diagnosis	Age at diagnosis	GON	GVFD	Maximum introcular pressure	Therapy
III:1	POAG	± 30	+	+	52	medical, laser, surgery
III:5	definite POAG	40	+	+	30	medical, surgery
III:6	POAG	"middle age"	+	+	unknown	Unknown
IV:1	POAG	"middle age"	+	+	30	medical, laser
IV:3	probable POAG	40	+	-	52	laser, medical, surgery
IV:7	definite POAG	34	+	+	31	medical, laser, surgery

GON = Glaucomatous optic neuropathy, defined as a vertical cup-to-disc ratio ≥ 0.7 or an asymmetry between vertical cup-to-disc ratios of both eyes ≥ 0.2
GVFD = Glaucomatous visual field defect
POAG = Primary open-angle glaucoma; definite POAG is defined as a GON plus a GVFD; probable POAG is defined as a GON without a GVFD or a GVFD without a GON
+ = Present; - absent

Patient (IV:3) showed a less severe phenotype. Since age 30 he had been treated for a high IOP, with medical, laser and surgical therapy. At the time of our investigation, he had a probable GON with normal visual fields at age 70.

Medical records of subjects III-1, III-6 and IV-1 showed strong evidence for definite POAG. In the ophthalmic records, a probable or possible GON was described and a GVFD was present. Since these persons were deceased at the time of our investigation, DNA for analyses was not available and it was not possible to screen for the presence of the mutation. An additional number of three persons, deceased at the time of investigation, had a history of POAG according to relatives (I:2, II:1, and II:3), but no medical records were available to verify the diagnosis.

Molecular analysis of the DNA of the definite and probable POAG patients of this pedigree revealed cosegregation of the Asn480Lys mutation and gene-flanking markers with the disease phenotype (Figure 1). The haplotypes of four gene-flanking markers in the MYOC region differed between our patients and the six POAG patients with the Asn480Lys mutation from Northern France.

Discussion

In a sample of 47 POAG cases of a population-based study one definite disease-causing mutation in the MYOC gene (Asn480Lys) has been identified. Six additional sequence changes were considered to be polymorphisms, because they were found in both cases and controls. All sequence changes were observed before by other research groups, except for the putative promoter polymorphism bp -127 (T→C). The latter is, however, apparently not located in a regulatory element of the promoter.[17,28]

The Arg76Lys change in 10 of our cases was reported before to be a polymorphism. In one study, the Arg76Lys segregated with a non-disease associated haplotype in a POAG family[31] and in other studies it was found in controls as well.[24,25] In eight of the cases carrying Arg76Lys also the bp -83 (G→A) change was found. This co-occurrence of the bp -83 (G→A) and Arg76Lys polymorphisms in the same subjects has been reported in a Chinese population,[21] where these changes always occurred together. It was not reported in Caucasians before. A potential functional effect of the latter phenomenon on the glaucoma phenotype remains to be elucidated.

As most POAG-causing MYOC mutations, the Asn480Lys mutation is located in the third exon of the MYOC gene. This is a domain homologous to olfactomedin, a protein secreted by nasal epithelium.[18] The Asn480Lys mutation is situated near a casein kinase II site and causes a change in charge in the local residue and a gain of α-helix in the structural conformation of the MYOC protein.[32] A POAG pedigree segregating this mutation was previously described by Adam et al.[18,30]

Upon examination of seven relatives of our case carrying the Asn480Lys mutation, we identified two additional affected subjects. The phenotypes of the patients varied, ranging from GON but no visual field damage in a 70-year old patient to a severely affected optic disc and a remaining temporal rest in a 34-year old patient. The four subjects without the mutation had no GON, GVFD or elevated IOP. These findings are consistent with observations reported by Brézin et al,[30] who found variable expressivity among carriers with the same mutation, ranging from an unaffected carrier at age 60 to severe POAG at age 23. Obviously, other genetic or environmental factors must be responsible for these differences in phenotypes.

Mutations in different families may have an independent origin, or may be inherited from the same founder, many generations ago. Haplotypes corresponding to the MYOC locus were different between our family and a family from Northern France carrying the Asn480Lys mutation.[30] From these data it can be concluded that the mutation in our family is most likely a recurrent one.

A difficulty in glaucoma studies has been the lack of a gold clinical standard to define POAG. A world-wide-accepted definition would facilitate comparison of different studies. The classification of POAG we used was proposed in the World-wide Glaucoma meeting, Hopkins, Baltimore 2000. A strength of our study is the assessment of all characteristics in a masked way and the use of objective criteria to define POAG, independent of any previous glaucoma diagnosis, treatment or IOP.[1] A world-wide conclusive classification based on objective criteria is necessary to unravel the factors involved in the heterogeneous group of diseases named POAG.

When investigating the contribution of a gene to a disease phenotype in the general population, a population-based approach has an advantage over hospital-based studies. Since, in hospital-based studies, most patients are probably identified by the occurrence of an elevated IOP, samples of these patients tend to be biased towards the type of POAG with a high IOP. In our study, the proportion of cases with an IOP within normal limits was 38%, which is similar to other population-

based studies.[1,33,34] Using a population-based approach selection bias towards a specific type of POAG is minimised.

Our approach also involves a difficulty, because the number of cases is limited by the prevalence of the disease and our strict clinical definition. Since the prevalence of definite POAG cases in our study was 0.8%, which is comparable to other population-based studies,[4,33,35-37] the total number of cases in our sample was relatively low.

From this study, it can be concluded that the prevalence of MYOC mutations within the population-based sample of the Rotterdam study (2.2%) is approximately equal to that found in hospital-based studies.[15,21,24,25] Although mutations in the MYOC gene are only responsible for a minority of POAG patients, it is important to realise that in a family with POAG caused by a MYOC mutation, relatives carrying this mutation run a high risk of developing the disease and need frequent follow up. At the same time, relatives not carrying the mutation have a risk not higher than that in the general population and need less follow up.

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4

FAMILY STUDY

4

Family score as indicator of genetic risk of primary open-angle glaucoma

Abstract

OBJECTIVES- To assess the genetic risk of primary open-angle glaucoma (POAG) in individuals by calculating a family score (FS), which summarizes the information about all relatives including their disease status, age, gender and degree of kinship. Furthermore, to examine the contribution of genetic factors to the occurrence of POAG with and without an increased intraocular pressure (IOP).

METHODS- Case and control probands, derived from the Rotterdam Study, underwent the same eye examination as their relatives. FS's were calculated by summing the differences between observed and expected values for each relative, corrected for the degree of kinship. The expected values were based on age- and gender-specific prevalences of POAG in the Rotterdam population. The risk of POAG for FS was calculated by comparing FS's in case and control probands using logistic regression analysis, with adjustments for IOP.

RESULTS- Of 37 case probands 42 siblings, 2 half-siblings, and 86 children were available for analyses, and of 83 control probands there were 95 siblings, 2 half-siblings, and 155 children. FS's ranged from –0.44 to 7.08 in case families and from –0.98 to 2.46 in control families. An increase of one unit FS was significantly associated with a higher risk of POAG (odds ratio 1.59, 95% confidence interval 1.14, 2.23, adjusted for age and gender). Adjustments for IOP did not significantly change the odds ratio.

CONCLUSIONS- These data show that the FS strongly predicts POAG, independent of the IOP. Therefore, the FS is useful to identify individuals with a high genetic risk.

Introduction

Primary open-angle glaucoma (POAG) could be described as a degenerative process of the ganglion cells in the retina. This neurodegeneration is clinically characterised by a glaucomatous optic neuropathy (GON) with a glaucomatous visual field defect (GVFD) and open chamber angles. The prevalence of POAG varies around 1% in individuals 55 years and older in the western world,[1-4] and a positive family history as well as an elevated intraocular pressure (IOP) have consistently been shown to be major risk factors.[5-7] It is generally recognised that genetic factors play an important role. Linkage with several genomic regions has been found in different selected families, where the disease segregates as an autosomal dominant trait.[8-13] Recently, the myocilin gene has been identified, accounting for 3-5% of adult onset POAG.[14] Apart from this small subset of families, the majority of POAG families do not follow a clear-cut Mendelian inheritance pattern. Instead, POAG is considered to be a heterogeneous, multifactorial disease, in which probably multiple genetic and non-genetic factors are involved.[15-18] To quantify the effects of these factors and interaction between them on the aetiology of POAG, family studies are carried out.

A familial aggregation study has recently been performed in siblings, half-siblings and children of POAG cases and controls, drawn from the population-based Rotterdam study.[19] The lifetime risk of POAG in relatives of cases was 9.2 compared to relatives of controls.

Here we use a new approach, which integrates all information from a family to calculate a family score (FS). Houwing-Duistermaat et al.[20] derived that an FS defined as the sum of the weighted difference between the observed and expected value of each relative for a certain disease performs well under various genetic mechanisms. The expected value for each relative is based on the age- and gender-adjusted prevalence in the population where the families are drawn from. The weights are proportional to the degree of kinship between the relative and the proband. This method summarizes the information about all relatives including their disease status, age, gender and degree of kinship.

The first aim of this study was to apply this novel method to our family data of POAG in the Rotterdam Study and to check whether an association exists between the family-derived FS and POAG in the probands. The second aim of the study was to address the issue of clinical heterogeneity in POAG. We examined whether

there was evidence for a difference in contribution of genetic factors to the occurrence of POAG with and without an increased IOP.

Materials and methods

Study population

Design and methods of the familial aggregation study have been described before.[19] Case and control individuals, here to be called case and control probands to discern them from their relatives, were recruited from the baseline phase of the Rotterdam Study. This is a population-based cohort study that aims to assess the occurrence of and the risk factors for chronic diseases in subjects aged 55 years and older.[4,21] The study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Erasmus University. Informed consent was obtained from all participants.

In the present analysis, the group of case probands differed from the group in the original aggregation study, because we recently changed our definitions of POAG.[4] Of the 48 case probands from the original familial aggregation study,[19] 37 probands fulfilled the new POAG criteria. Together with 13 new case probands, a total of 50 case probands had definite POAG. Three of them were deceased at the time of the family study, resulting in 47 eligible case probands for the present study. Of the 135 (87%) control probands who consented to participate in the original familial aggregation study, 23 were excluded, because after resetting the definition of POAG, they met the criteria for possible or probable POAG. Thus, 112 control probands were eligible for the present study. Although pseudoexfoliative glaucoma was not specifically excluded in the baseline phase of the study, we refer to the disease status as POAG, because at follow up no cases with pseudoexfoliative glaucoma were found.

Measurements and diagnosis of POAG

Relatives of case and control probands underwent a standardised eye examination.[4,7] In short, the ophthalmological examination included measurement of IOP, direct and indirect ophthalmoscopy, stereo fundus photography, and simultaneous stereo disc photography. Optic disc characteristics were measured with a digital image analyzer (Imagenet system, Topcon Optical Co, Tokyo, Japan) and

ophthalmoscopy.[22] Visual fields were tested with the Humphrey perimeter (Humphrey Visual Field Analyzer II, model 750, Dublin, California).

POAG cases were classified in three categories according to our new definition:[4] definite, probable, or possible POAG. Definite POAG cases had a probable or possible GON in combination with a GVFD. Probable POAG was defined as either a probable GON without a GVFD, or a GVFD without GON. Possible POAG cases had a possible GON without a GVFD. Only definite POAG cases were included as case probands. Control probands were included when they had neither GON nor GVFD, nor age-related maculopathy. The IOP was defined as elevated if it was > 21 mm Hg during our examination or if treatment to lower the IOP had been used. To investigate whether hypertension or diabetes mellitus could account for the familial risk, either directly, or through their relation with IOP, these disorders were included in the analyses. Hypertension was defined as either a systolic blood pressure ≥ 160 mm Hg, or a diastolic blood pressure ≥ 95 mm Hg, or the use of antihypertensive treatment. Diabetes mellitus was considered present if the non-fasting serum glucose was ≥ 11.1 mmol/l or if antidiabetic medication was used.

Statistical analysis

Baseline characteristics of case and control probands were compared using Students' t-test. For each family an FS was computed, based on the disease status, number, age, and gender of all relatives available for analyses. As a few half-siblings were included, a correction was made for the degree of kinship. The FS was calculated according to the formula described by Houwing-Duistermaat[20]

$$FS = 10\,\Sigma(\Psi_j(O_j - E_j))$$

where the sum (Σ) is taken over the values of all relatives j of a proband, Ψ_j is the kinship coefficient between each relative j and the proband (0.25 for siblings and children of the proband, and 0.125 for half-siblings), O_j is the observed disease status (1 affected, 0 otherwise) and E_j is the expected value for each relative j. Expected values were based on age- and gender-specific prevalences of POAG in the Rotterdam Study, based on Imagenet data combined with ophthalmoscopic data (Table 1);[4] for relatives younger than 55 years a prevalence of 0% was assumed. For the purpose of presentation, we multiplied the sum of all individual

scores by ten. As an example, for a family that consists of relatives *a, b* and *c*, the formula can be written as:

$$FS = 10\ (\Psi_a (O_a - E_a) + \Psi_b (O_b - E_b) + \Psi_c (O_c - E_c))$$

Logistic regression analysis was performed to estimate the risk of POAG in probands given the FS. First, analyses were performed after calculating FS's assigning both relatives with *d*efinite POAG and relatives with *p*robable POAG as affected (FS_{dp}): definitely and probably affected relatives were included as observed cases; expected values were derived from prevalences of definite and probable POAG (Table 1).

Next, the analyses were repeated after calculation of the FS's assigning only *d*efinitely affected relatives as observed cases and excluding relatives with probable POAG (FS_d). Expected values were derived from prevalences of definite POAG. Relatives with possible POAG and without POAG were assigned as unaffected.

Table 1
Prevalences of primary open-angle glaucoma in independently living subjects of a population-based cohort (n = 6281), aged 55 years and over. Imagenet data combined with ophthalmoscopic data, the Rotterdam Study (1990-1993)[4]

Age (years)	Primary open-angle glaucoma		
	Definite*	Probable	Definite and probable†
Men			
55-59	0.002	0.017	0.019
60-64	0.006	0.018	0.024
65-69	0.008	0.020	0.028
70-74	0.013	0.027	0.040
75-79	0.019	0.041	0.060
80+	0.036	0.054	0.090
Women			
55-59	0	0.015	0.015
60-64	0.001	0.017	0.018
65-69	0.009	0.026	0.035
70-74	0.011	0.018	0.029
75-79	0.006	0.042	0.048
80+	0.013	0.038	0.051

* Prevalences used in calculation of the family score, with only definitely affected relatives
† Prevalences used in calculation of the family score, with both definitely and probably affected relatives

Analyses were adjusted for age, gender, diabetes mellitus and hypertension status of the proband. To examine whether an elevated IOP had effect on the relation between FS and POAG, additional adjustments were made for IOP and IOP-lowering treatment. We tested whether the relation between FS and POAG was different for POAG with and without an IOP > 21 mm Hg, by adding an interaction term between FS and IOP (1 elevated, 0 normal).

Results

Response rates and baseline characteristics

Of the 47 eligible case probands, 43 (91%) consented to participate, and of six no relatives were available for analyses. Among the relatives of case probands, 48 (80%) (half) siblings and 87 (95%) children responded, of whom 42 siblings, 2 half-siblings, and 86 children had complete data to diagnose or exclude POAG. Of 112 control probands, 101 (90%) participated, and 110 (81%) (half) siblings and 158 (81%) children responded. Complete data on POAG status were available for 95 siblings, 2 half-siblings, and 155 children. For 18 control probands, no relatives were available for analyses. This resulted in 37 case and 83 control probands with families available for analyses (Table 2A).

Their maximum IOP before or during treatment often was not known. In three of the remaining 18 cases an IOP > 21 mm Hg was measured during our examination. Of control probands five (6%) had IOP-lowering treatment and in seven of the remaining controls, we measured an IOP > 21 mm Hg.

Table 2A
Characteristics of case probands with definite primary open-angle glaucoma and control probands

Characteristic	Case probands (n = 37)	Control probands (n = 83)
Mean age ± SD*	73.2 ± 7.3	75.5 ± 8.3
% Women	37.8	55.4
Mean intraocular pressure, mm Hg	17.3 ± 3.6	16.3 ± 3.1
% intraocular pressure-lowering treatment	51.4	6.1
% Elevated intraocular pressure†	59.5	14.5

*SD: standard deviation

† Elevated IOP was defined as an IOP > 21 mm Hg or the use of IOP-lowering treatment

Table 2B
Characteristics of relatives of case probands and of control probands

Characteristic		Case probands (n = 37)	Control probands (n = 83)
Siblings			
Number		44†	97†
Mean age ± SD*		73.8 ± 7.2	74.3 ± 9.3
% Women		57	66
Mean intraocular pressure, mm Hg		17.7 ± 11.2	13.8 ± 3.0
% Intraocular pressure-lowering treatment		11.4	2.2
Primary open-angle glaucoma‡			
Definite		7 (15.9)	1 (1.0)
Probable		7 (15.9)	10 (10.3)
Possible		12 (27.3)	5 (5.2)
Children			
Number		86	155
Mean age ± SD*		46.1 ± 8.7	48.5 ± 8.9
% Women		47	41
Mean intraocular pressure, mm Hg		15.2 ± 3.2	14.0 ± 2.7
% intraocular pressure-lowering treatment		0.02	0.01
Primary open-angle Glaucoma‡	Definite	1 (1.2)	0
	Probable	2 (2.3)	2 (1.2)
	Possible	18 (20.9)	16 (10.3)

*SD: standard deviation
† Two half-siblings are present among siblings of case probands and two among siblings of control probands
‡ Values in parentheses are percentages

The number of examined relatives varied from one to nine among case probands and from one to 11 among control probands and did not differ significantly (p = 0.29) (Table 2B). The number, mean age and proportion of women were similar in (half) siblings of cases and controls. These characteristics were also similar in their children, except for age: children of cases were on average 2.4 years younger than children of controls (46.1 versus 48.5 years).

Relatives of cases had a higher mean IOP than relatives of controls, especially siblings (17.7 versus 13.8 mm Hg). (Half) siblings of cases also had IOP-lowering treatment more often than those of controls (11% versus 2%). The distribution of case and control families with different numbers of affected relatives is shown in Table 3.

Table 3A
Number (percentage) of families of case and control probands including 0, 1, 2 or 3 relatives with definite or probable open-angle glaucoma

Number of affected relatives	Case families (n = 37)	Control families (n = 83)
0	24 (65)	70 (84)
1	10 (27)	13 (16)
2	2 (5)	-
3	1 (3)	-

Values in parentheses are percentages

Table 3B
Number (percentage) of families of case and control probands including 0, 1, 2 or 3 relatives with definite open-angle glaucoma

Number of affected relatives	Case families (n = 37)	Control families (n = 83)
0	30 (81)	82 (99)
1	6 (16)	1 (1)
2	1 (3)	-
3		-

Values in parentheses are percentages

Family scores

First, FS_{dp}'s were calculated (Figure 1). In families of POAG case probands, the FS_{dp} ranged from –0.44 to 7.08, with a mean FS_{dp} of 0.98. In families of control probands, FS_{dp}'s were lower, ranging from –0.98 to 2.46, with a mean FS_{dp} of 0.24. Logistic regression analysis showed that an increase of 1 unit in FS_{dp} was associated with a significantly increased risk of POAG in the probands (odds ratio (OR) 1.59, 95% confidence interval (CI): 1.14, 2.23), adjusted for age and gender (Table 4). With the inclusion of IOP as a continuous variable, and IOP-lowering treatment, diabetes mellitus and hypertension of the proband as covariates, the risk of POAG was even more increased per unit of FS_{dp} and still significant (OR 1.76, 95% CI 1.15, 2.70). After additional inclusion of the interaction term between FS_{dp} and an elevated IOP as covariate, this term was not associated with POAG (OR 1.10; 95% CI 0.41, 2.94), while FS_{dp} alone was still significantly associated with POAG (OR 1.67, 95% CI 1.05, 2.65).

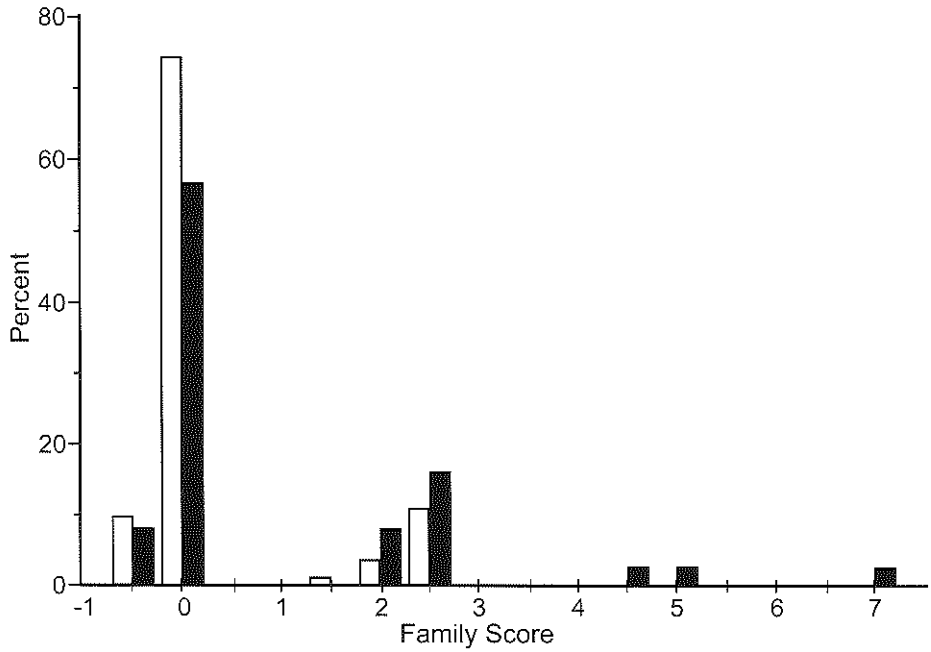

Figure 1
Distribution of family score (FS) in case (n = 37) and control (n = 83) probands. Affected relatives had definite or probable primary open-angle glaucoma. Numbers of FS are midvalues of categories. Range of categories: –0.25 + value, value + 0.25. Black bars represent case probands, white bars represent control probands.

The FS_d in families of case probands ranged from –0.14 to 4.89, with a mean FS_d of 0.48, and in families of control probands ranged from –0.29 to 2.41, with a mean FS_d of –0.01. An increase of 1 unit of FS_d was associated with a higher risk of POAG in the proband than the former FS_{dp} (OR 3.32, 95% CI 1.25, 8.82). Adjusted for all risk factors mentioned above, and after the additional adjustments of IOP and IOP-lowering treatment, the OR did not change and was still statistically significant. Finally the interaction term between FS_{def} and an elevated IOP was not significantly associated with POAG in the proband (p = 0.67), while FS_{def} alone was still significantly associated with POAG.

To illustrate the FS method, we describe three families with different FS_{dp}'s. The family with the highest FS_{dp} (7.08) included eight siblings, of whom three were affected. Two sisters, aged 71 and 72, had definite POAG and one sister, aged 73, had probable POAG. The age- and gender- adjusted prevalence for each of them

Table 4
Odds ratios of primary open-angle glaucoma in the proband by family score

Relatives have definite or probable primary open-angle glaucoma

	Odds ratio* (95% CI)	Odds ratio† (95% CI)
Family score ‡	1.59 (1.14-2.23)	1.76 (1.15-2.70)
Intraocular pressure, mm Hg		1.13 (0.96-1.33)
Diabetes mellitus		4.36 (0.76-24.88)
Hypertension		1.30 (0.48-3.53)

Relatives have definite open-angle glaucoma

	Odds ratio* (95% CI)	Odds ratio† (95% CI)
Family score §	3.32 (1.25-8.82)	3.33 (1.15-9.62)
Intraocular pressure, mm Hg		1.15 (0.98-1.36)
Diabetes mellitus		3.18 (0.58-17.40)
Hypertension		1.77 (0.64-4.85)

* Adjusted for age and gender of the proband
† Adjusted for age, gender, presence of diabetes mellitus, hypertension, intraocular pressure and intraocular pressure-lowering treatment in the proband
‡ Based on probable and definite open-angle glaucoma in relatives
§ Based on only definite open-angle glaucoma in relatives

was 0.029 (Table 1). Subtracting the expected (0.029) from the observed value (= 1) and weighting with Ψ_j (= 0.25) resulted in an individual score of (1 – 0.029) x 0.25 = 0.24275 each. Three unaffected sisters aged 63, 65 and 74 had expected values of 0.018, 0.035, and 0.029 respectively, resulting in individual scores of –0.0045, –0.00875, and –0.00725 after weighting. Two unaffected sons, aged 44 and 41, had an expected value of 0, resulting in an individual score of 0. The sum of these individual scores of all relatives was an FS_{dp} of (0.24275 + 0.24275 + 0.24275 – 0.0045 – 0.00875 – 0.00725 + 0 + 0 =) 0.708. Multiplying by 10 resulted in an FS_{dp} of 7.08.

In a second family, no POAG was observed among all eight siblings, aged between 67 and 80, and two children, aged 48 and 57. Subtracting the expected values from these observed values of 0 resulted in negative individual scores. After weighting with Ψ_j, these individual scores were added up to an FS_{dp} of –0.098 and multiplied by ten to –0.98.

A third family included seven unaffected children. Since they were relatively young (aged 35 to 52), expected values were 0, resulting in individual scores of 0, and, subsequently, an FS_{dp} of 0. This example demonstrates that a family with $FS_{dp} = 0$ does not contain much information about the genetic risk of that family.

Discussion

In this study, we applied a new statistical method to estimate the risk of POAG for a individual, given the disease distribution in relatives. The presence of POAG in siblings and children was quantified by an FS, which took the disease status, number, age, gender, and kinship coefficient of the relatives into account. The FS, which can be interpreted as the genetic risk for that particular individual, was heterogeneous among case probands. We found that an increase in FS was significantly associated with an increased risk of POAG. This association was independent of the presence of an elevated IOP.

FS's are distributed around zero. A positive FS indicates that in a family, more cases are observed than expected and therefore points to genetic risk in that family, and vice versa. The magnitude of the FS depends on the size and age distribution in the family. Small and/or young families do not contain much information about the genetic risk of the proband. This is reflected in the model by the fact that small families produce an FS zero or close to zero. As long as candidate genes are not identified, the risk estimation of POAG for the individuals with these FS's should not be based on the occurrence of POAG in the family, but on other risk factors. Since the sizes and age distributions of the case families were similar to those of the control families confounding by family size was unlikely.

A strength of the study is the fact that all case and control probands and their relatives underwent a standardised ophthalmological examination. Features of POAG were assessed separately in a masked fashion to ensure an unbiased diagnosis. Examining relatives instead of taking a family history from the probands reduces misclassification of disease status due to the insidious course of the disease, lack of knowledge of the disease status in proband or relative, and different and changing definitions of POAG.[7] By recruiting probands from the population-based Rotterdam study, ascertainment bias due to family size and selection bias towards a specific type of POAG was reduced.

Our findings indicate that the contribution of genetic factors to the occurrence of POAG is independent of IOP. Both low and high FS's were observed in case probands with and without an elevated IOP (data not shown). Adjustment for IOP did not change the effect of FS on the risk of POAG; the OR of POAG remained significantly increased. Finally, the interaction term between FS and IOP was not significant, and did not change the effect of FS. Thus, FS had an effect on POAG with and without an elevated IOP, and the effect of FS on POAG was not statistically different within these two subgroups. Our findings match the observation in literature where the occurrence within families of POAG cases with high IOP,[10,11,13,14] low IOP,[12,23] and both[9,24] are reported. However, the design of many studies complicates distinguishing between the genetic influence leading directly to POAG and that leading to POAG through IOP. Genetic factors are involved in IOP, [25-27] while IOP is considered an important risk factor for POAG. In our study, we were able to investigate the effect of IOP apart from POAG because the level of IOP was not included in the definition of POAG and because an elevated IOP was defined independently from the POAG diagnosis.

It is generally accepted that POAG is a multifactorial disease,[15-18] caused by actions of many genes and effects from the environment. However, it is difficult to discriminate whether familial occurrence of a multifactorial disease is the result of shared genetic or environmental factors.[28] It seems unlikely that shared environmental factors early in life contribute to the occurrence of POAG at middle age. Indeed, no environmental factor has consistently been associated with the risk of POAG, neither in clinic-based case-control studies,[18] nor in population-based studies.[29-31] Also, a recent twin study showed concordance of POAG in twins but not in spouses, suggesting the involvement of genetic rather than environmental factors.[27] To identify possible environmental risk factors, further research in cohort studies is necessary. To separate the effects of genetic and environmental factors, and the interactions between these, genetic epidemiological studies in POAG families should be carried out, preferably encompassing more generations.[32] In such studies, the FS can be used to represent the familial risk of an individual. Furthermore, the FS is valuable to select individuals for clinical follow up and for further genetic analysis. Research into genotype-phenotype relations in families will remain necessary, also after identification of a disease-causing genetic defect, to study possible modifier genes or environmental factors influencing its expression.

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5

GENETICALLY ISOLATED POPULATION

5.1

Exclusion of candidate gene loci for adult onset primary open-angle glaucoma using a combined linkage-linkage disequilibrium analysis

Abstract

Primary open-angle glaucoma (POAG) is a genetically heterogeneous eye disorder. In order to localize new genes implicated in POAG, we clinically investigated a sample of patients in a genetically isolated population. Furthermore, we studied whether sharing of any of the known glaucoma candidate chromosomal regions was present among the patients identified.

For the current exclusion analysis, only definite POAG patients were included, defined as a glaucomatous optic neuropathy together with a glaucomatous visual field defect. In total, twenty-seven markers were used to screen the myocilin (MYOC) gene, five candidate POAG loci (GLC1B to F) and five other, potentially involved, genomic regions. A combined likelihood ratio test for linkage and linkage disequilibrium was carried out, with θ representing the recombination fraction, and λ the proportion of disease chromosomes with the associated allele.

A sample of 11 definitely and 15 probably affected individuals were identified. Only the definite POAG patients were included for the current exclusion analysis. Negative lodscores were obtained for all markers; maximizing λ resulted in null values.

It can be concluded that neither the MYOC gene nor other known candidate regions are implicated in POAG in our sample of patients. Based on the population structure and the previous genetic studies in this isolate, we expect this sample to be suitable for a subsequent whole genome scan to identify yet unknown genes for POAG.

Introduction

Primary open-angle glaucoma (POAG) is clinically characterized by an atrophic excavation of the optic nerve, referred to as glaucomatous optic neuropathy (GON), a typical glaucomatous visual field loss (GVFL), and an open anterior chamber angle. Its prevalence is 1% in subjects 55 years and over in the Western world.[1] It is generally recognized that genetic factors are an important component in the etiology of POAG.

Many POAG families do not reveal a clear-cut Mendelian inheritance pattern. Linkage analysis in families implicated the involvement of several chromosomal regions: 1q21-q31,[2] 2cen-q13,[3] 3q21-q24,[4] 8q23,[5] 10p15-p14,[6] 7q35,[7] and possibly 2p12, 14p11, 17p13, 17q24 and 19q13.[8] The only known causative gene is the Myocilin (MYOC) gene (GLC1A) on chromosome 1q.[9]

The problems that arise when investigating the etiology of POAG include the late age of onset, the lack of internationally accepted diagnostic criteria, genetic heterogeneity and possible presence of phenocopies. To address these problems, we identified a sample of POAG patients from a genetically isolated population, and investigated whether sharing of any of the known glaucoma candidate chromosomal regions was present among the definite POAG patients.

Materials and Methods

Patients

The current study was carried out within the same community in which the gene for retinitis pigmentosa type 12,[10] and the gene for pseudoxanthoma elasticum[11] were identified. The existence of this community has been reported for the first time in 1462. The number of inhabitants increased steadily from five in that year to 1,000 in 1830. From that moment on, a rapid growth took place to 20,029 inhabitants in the year 2000. During these centuries, the community was isolated for cultural/religious reasons.

The study was approved by the medical ethical committee of the Academic Medical Center in Amsterdam. All participants gave written informed consent. Individuals were ascertained through registers of ophthalmologists and general practitioners. Ophthalmologic examination included IOP measurement, gonioscopy, ophthalmoscopy, and optic disc photography (35° field, RC-2 fundus

camera, Kowa Corporation, Japan). Visual field tests were retrieved from medical records; if patients with normal fields had not been tested within the last year, a Humphrey 24-2 test was performed (Zeiss Instruments, Oberkocken, Germany).

Both definite and probable POAG patients were identified.[1] Only definite patients were included in the current analyses. Our criteria for GON were slightly adapted for the use of optic disc morphometry. Subjects with visual field loss plus a normal optic disc were excluded. Spouses (n = 6) of the patients and individuals (n = 12) from the same population were included as controls.

DNA analysis

DNA analysis was carried out as described before.[12] MYOC was screened for mutations in the promoter region[13] and all three exons including the flanking splice sites. Primers were used as described before,[14] except for the first (F: ccaagcctctgcaatgaggt) and second exon (F: ctggccggcagcctatttaa).

Statistical Analysis

A combined test for linkage and linkage disequilibrium was carried out.[15] This test, a modification of the procedure described by Terwilliger,[16] examines the likelihood that a marker allele is over-represented on disease chromosomes compared with control chromosomes. Genotypes of control individuals and spouses or, in their absence, children were used for estimation of the control population-frequencies of marker alleles as nuisance parameters in the likelihood calculations.[17]

The logarithm of the likelihood ratio (lod score) was calculated using a modified version of ILINK:[18] $LOG_{10}(L_{\lambda,\theta}/L_0)$, where θ represents the recombination fraction, and λ the proportion of disease chromosomes with the associated allele.[15] An autosomal dominant mode of transmission of the disease was modelled with 50% penetrance and a gene frequency of 0.01. Definite POAG patients were designated affected; the disease status of all other individuals was set to unknown. Likelihood's were maximized for λ, and also calculated for fixed values of λ (0.8 and 0.9), all for θ fixed at 0 (association and linkage present), and compared with the likelihood obtained for $\lambda = 0$ and $\theta = 0.5$ (no association or linkage).

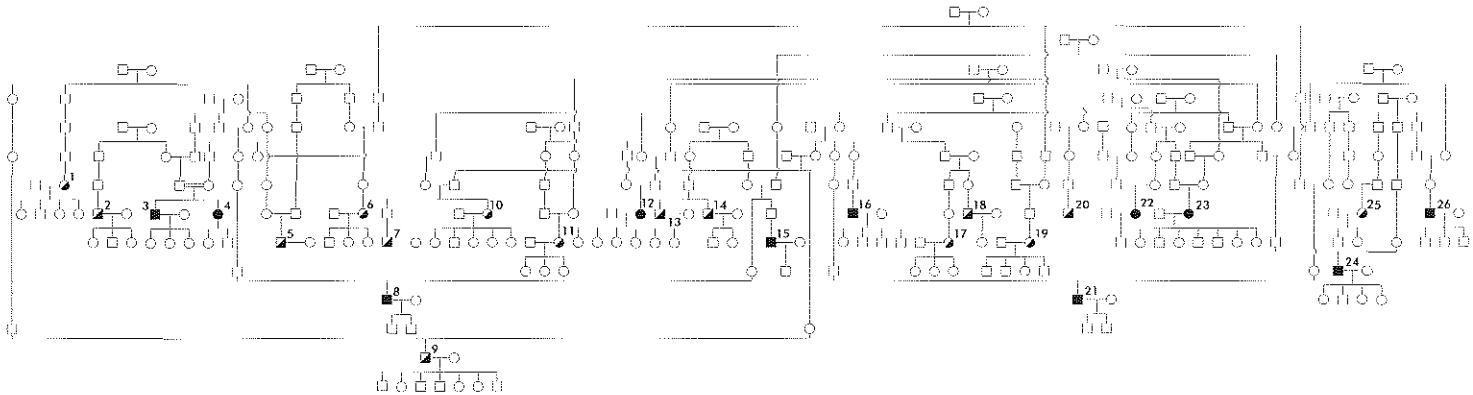

Figure 1

Pedigree with definite and probable primary open-angle glaucoma patients

Definite (solid symbols, n = 11) and probable primary open-angle glaucoma patients (hatched, n = 15) were related. Open symbols: disease status unknown. All individuals in the generations prior to the ones with primary open-angle glaucoma patients were deceased before the investigation started. Their DNA was not available. Only definite patients were included in the current exclusion analysis.

Results

We identified 11 definite and 15 probable POAG patients. Mean age at diagnosis in the definite patients was 68.5 years, ranging from 49 to 82 years. The maximum IOP ranged from 21 to 40 mm Hg. All definite patients received IOP-lowering medication and two had also undergone laser or surgical treatment.

After construction of the pedigree (Fig. 1), we computed what proportion of patients would be expected to share a marker allele in the immediate vicinity of a shared disease gene. Assuming an average distance of 12 generations between the patients and their presumed ancestor and a distance between marker locus and disease gene of at most 2.3 cM (the largest distance between locus boundary and closest flanking marker), λ should exceed 0.8 with a probability of 75%, and it should be greater than 0.5 with a probability of 99.8%. Marker analysis with at least three markers revealed negative lodscores for λ fixed at 0.8 or 0.9 for all candidate POAG regions (Table 1). The maximized value of λ was 0 for all markers except D8S85, which yielded a λ of 0.39 with a lod score of 0.19. Five definite POAG patients were screened for mutations in MYOC. No non-synonymous nucleotide changes were found compared with the wild type sequence.

Discussion

Research into the genetics of POAG has been hampered by the acceptance of an unambiguous clinical definition of POAG. Recently, we proposed a strict definition[1] at the Worldwide Glaucoma Meeting (Hopkins, Baltimore May 2000) which was also used in the current study. Unlike most genetic glaucoma studies, but in agreement with many epidemiological studies,[19-21] our system considers an elevated IOP an important risk factor, but not a criterion for POAG. Our system also differs from the "pedigree probability" score developed by the Glaucoma Inheritance Study of Tasmania,[22] by using objective clinical criteria instead of genetic or clinical information on relatives.

Given the pedigree structure and the young history of our population, it is plausible that many patients will share a POAG risk-allele from a single common ancestor, surrounded by a shared DNA segment.[10] Consequently, a test combining association and linkage can be carried out, which has been successfully used before in genetically isolated populations to exclude loci or map genes of complex disorders.[15,17]

Table 1
Lodscores of markers tested in each candidate region for primary open-angle glaucoma

LOCUS	*MARKERS*	*Lodscore* ($\lambda = 0.8$)	*Lodscore* ($\lambda = 0.9$)
GLC1A	D1S2844	-1.64	-2.87
	D1S445	-0.86	-1.39
	D1S212	-1.07	-2.53
	D1S191	-1.12	-1.75
GLC1B	D2S139	-1.66	-2.33
	D2S2161	-0.70	-1.17
	D2S2175	-1.88	-2.94
	D2S1897	-2.38	-4.36
GLC1C	D3S3637	-3.77	-5.18
	D3S1569	-2.84	-3.20
	D3S1555	-0.39	-1.13
	D3S1279	-1.74	-2.95
GLC1D	D8S167	-4.16	-5.42
	D8S85	-1.20	-2.20
	D8S198	-1.88	-3.15
GLC1E	D10S1745	-2.40	-3.89
	D10S1779	-2.85	-4.44
	D10S1216	-2.61	-3.41
	D10S191	-2.69	-3.53
GLC1F	D7S688	-3.84	-4.98
	D7S2439	-2.80	-3.10
	D7S2546	-3.76	-4.87
Markers[a]	D2S441	-1.06	-1.81
	D14S742	-1.11	-1.79
	D17S926	-3.16	-5.34
	D17S801	-4.03	-5.70
	D19S408	-1.20	-4.13

[a] Markers used in the recently published primary open-angle glaucoma genome scan.[8]

For every candidate region (GLC1A to GLC1F), at least one marker within the locus was tested and at least one marker flanking the locus. Negative lodscores were achieved for each candidate locus, under both the assumption for λ (the proportion of disease chromosomes with the associated allele) = 0.8 and λ = 0.9. The data suggest that these loci are not involved in the development of primary open-angle glaucoma in our sample.

The structure of our population enabled us to collect a large number of individual patients, who were distantly related. We chose control persons from the same population to determine the normal allele distribution within the population. Obviously, the latter may be quite different from outbred populations.
Due to our strict clinical criteria the number of definite patients was small, which reduces power and explains the absence of significance in exclusion of some markers. However, simulation calculations indicate that our analyses should virtually always (99.8%) yield positive evidence with $\lambda > 0.5$ around a true glaucoma risk gene. The fact that none of the analyzed regions gave a $\lambda > 0.4$ strongly argues against involvement of any of these loci. In calculations with fixed λ, values of λ were chosen to be 0.8 and 0.9 to allow for 10 to 20% of phenocopies. Based on the structure of this isolate and the previous genetic studies in this population,[10,11] we expect this sample to be suitable for a subsequent whole genome scan to identify yet unknown genes for POAG.

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5.2

Genome wide search for primary open-angle glaucoma in a genetically isolated population indicates potential susceptibility loci on chromosomes 2p14 and 12q24

Abstract

Primary open-angle glaucoma (POAG) is a complex eye disease. To identify POAG susceptibility genes, we performed a complete genome scan of a sample of POAG patients from a genetically isolated population in the Netherlands.

The clinical definition of definite POAG was based on a glaucomatous optic neuropathy and a glaucomatous visual field defect. Eleven definitely and 15 probably affected POAG patients were identified, as well as 23 individuals with ocular hypertension. The POAG patients were tested with 347 markers distributed over the total genome. A likelihood ratio statistic was carried out with a combined test for linkage and linkage disequilibrium both in the definite patients only and in the total sample of patients. We found lodscores > 1.5 with marker D2S123 on chromosome 2p14 and with marker D12S97 on chromosome 12q24. Additional markers in the regions around D2S123 and D12S97 were tested and haplotype sharing of adjacent markers in affected individuals was observed. These findings potentially suggest the presence of at least two novel POAG loci on chromosome 2p14 and on chromosome 12q24.

Introduction

Primary open-angle glaucoma (POAG) is an etiologically diverse eye disease. POAG is clinically characterized by a glaucomatous optic neuropathy (GON) and a glaucomatous visual field defect (GVFD), together with open and normal anterior chamber angles. In the western world, the prevalence of POAG increases with age from 0.1% in persons aged 55 to 2% in those over age 80.[1-4]. In addition to age, major risk factors include an elevated intraocular pressure (IOP) and a positive family history.[5-8]

It is generally accepted that genetic factors are involved in POAG. During the last decades, several POAG families have been described and different inheritance patterns for the disease have been postulated. The first and, so far, only gene found for POAG is myocilin (MYOC, MIM 601652; GLC1A) on chromosome 1q. This gene was originally identified in a family with autosomal dominant juvenile onset POAG,[9] but mutations also appeared to be present in a small subset (3-5%) of patients with adult onset POAG.[10]

Five additional chromosomal regions have been identified using linkage analysis in unrelated POAG families: 2cen-q13 (GLC1B),[11] 3q21-q24 (GLC1C),[12] 8q23 (GLC1D),[13] 10p15-p14 (GLC1E),[14] and 7q35 (GLC1F).[15] Of these loci, GLC1A, B,[16] and C[17] were confirmed with data from independent POAG family studies. So far, the loci GLC1D, E, and F have not been confirmed. A genome-wide scan in a larger sample of unrelated POAG families and sib pairs showed five additional genomic regions on chromosomes 2p, 14p, 17p, 17q, and 19q to be potentially involved in POAG.[18]

The regions thus far identified in linkage studies were found in a subset of POAG families where the disease segregates as an autosomal dominant trait. However, in the majority of POAG families, the disease does not follow a clear-cut Mendelian inheritance pattern. Instead, POAG is considered to be a complex disease,[19-22] with many genetic and non-genetic factors involved.[23] When searching for genes in a complex disease such as POAG, problems are encountered such as its genetic heterogeneous character, possible presence of phenocopies, unknown mode of transmission and late age of onset of the disease.

Because of these difficulties, we chose a different study design to aid the search of additional genes for POAG.[24] Evaluation of linkage disequilibrium (LD) in genetically isolated populations has proved to be useful to map genes for monogenic diseases.[25-28] In the current study, we attempt to identify susceptibility loci for POAG in a genetically isolated population, because it is expected that these populations are genetically more homogeneous, and that they have larger regions of LD than mixed populations.

Moreover, the genetic isolate enabled us to ascertain individual patients, independent of the disease status of their relatives. Consequently, we could collect a relatively large number of patients who were distantly related.[29] We examined 11 definite and 15 probable POAG patients from a recently genetically isolated population. In a former study (Hulsman et al, manuscript in press), we examined the

known candidate POAG gene loci GLC1A to F, and the five regions identified in a genome scan performed by Wiggs.[18] All loci were excluded in the definite patients with a combined test for linkage and association.[30,31]

In the current paper, we present the results of a whole genome scan in a group of POAG patients from a genetically isolated population. Furthermore, we evaluate two promising regions for haplotype sharing among patients, and propose potential candidate susceptibility genes for POAG within these regions.

Materials and methods

Study population

The current study was carried out in the same community in which the gene for retinitis pigmentosa type 12,[25] and the gene for pseudoxanthoma elasticum[28] were identified. The existence of this community has been reported since 1462. The number of inhabitants increased steadily from 25 in that year to 1,000 in 1830. From that moment on, a rapid growth took place to 20,029 inhabitants in the year 2000. During these centuries, the community was isolated for cultural and religious reasons and immigration has been estimated to be only a few percent. The study was approved by the medical ethical committee of the Academic Medical Center in Amsterdam and written informed consent was obtained from all participants.

Patient recruitment and clinical measurements

Individuals were ascertained through registers of ophthalmologists and general practitioners. Data on family and medical history were obtained from a questionnaire. The eye examination included applanation tonometry, gonioscopy, ophthalmoscopy, and fundus photography of the optic disc and 35° macular field (RC-2 fundus camera, Kowa Corporation LTD, Tokyo, Japan). Papillometry was used to measure the vertical cup-to-disc ratio and the width of the neuroretinal rim.[32] When available, visual field tests were retrieved from medical records of the ophthalmologists, who used a Humphrey or Octopus perimeter. If visual field tests had not been performed within the last year, patients were tested with the 24-2 program of the Humphrey Field Analyser (Humphrey Instruments, San Leandro, California).

For the definition of POAG, we used the criteria from the Rotterdam Study as described by Wolfs,[4] adapted for the use of papillometry instead of Imagenet. In short, definite POAG was defined as a GVFD together with a possible (vertical cup-to-disc ratio ≥ 0.7, asymmetry in vertical cup-to-disc ratio ≥ 0.2, or neuroretinal rim < 0.1) or probable (0.8, 0.3, and 0.05, respectively) GON. Probable POAG was defined as a probable GON with a normal visual field. Unlike Wolfs' definition, we did not define a GVFD with a normal optic disc as probable POAG, because neurological causes of visual field defects could not be excluded as extensively as in the Rotterdam Study. Instead, we excluded those individuals from all analyses. Age at diagnosis was defined as the age of detection of a GVFD in the definite patients, and as the age of detection of a GON in probable patients. Ocular hypertension was defined as a maximum IOP measured in a patient's history > 21 mm Hg or the use of IOP-lowering treatment, without any signs of a GON or a GVFD.

Definite and probable POAG patients were included in the analyses of the present study. We have chosen two phenotypes for genetic analyses; first, a broad category including definite and probable POAG patients, and second, a narrow category, only including definite POAG patients. Individuals from the same population, who showed no signs of POAG, were included as population-matched controls. Likewise, spouses of the patients were included as controls. Spouses were asked for history of eye disease, in particular for the presence of POAG or elevated IOP, but they were not ophthalmologically examined. Blood samples were taken from all patients, their spouses and, if available, at least three children to construct phase-known haplotypes.

DNA analysis

DNA was extracted from white blood cells following standard protocols. Genotyping was performed with an ABI type 377 sequencer. A total of 347 markers were tested covering the autosomal genome, with an average spacing of 10 cM and an average heterozygosity of 78%. The set of markers was obtained from http://www.msz.mdc-berlin.de/markers.html.

Statistical analysis

A combined test for linkage and LD was carried out.[30,31] In a genetically isolated population, a high proportion of patients are likely to share a chromosomal segment containing the disease gene, which they inherited from a common ancestor.[27,29,33,34] Therefore, if in a sample of patients, marker allele frequencies differ significantly from control individuals, the disease locus is most likely located in the chromosomal region flanking that marker. This region is in LD with the marker allele. We used a modification of the procedure described by Terwilliger[35] to examine the likelihood that a marker allele is over-represented on disease chromosomes compared with control chromosomes.[30,31] Genotypes of control individuals and spouses, or, in their absence, children were used for estimation of the control population-frequencies of marker alleles as nuisance parameters in the likelihood calculations.

The logarithm of the likelihood ratio (lod score) was calculated using a modified version of ILINK[36]: $LOG_{10}(L_{\lambda,\theta}/L_{\theta})$, where θ represents the recombination fraction, and λ the proportion of disease chromosomes with the associated allele. Since a priori it is not known which allele will be overrepresented, each allele is considered the potential founder allele. A total likelihood is obtained by summing the likelihoods for each potential founder allele, weighted for their population frequency. The gene frequency was set at 0.01. An autosomal dominant inheritance was assumed, with 50% penetrance.

For each marker, likelihoods were first calculated under the hypothesis of no linkage ($\theta = 0.5$) and no LD ($\lambda = 0$). Then, analyses were repeated with additional estimation of λ, starting from 0.3, 0.6, and 0.9, all for $\theta = 0$. The analyses were performed under both the narrow and the broad phenotype.

In our study, multiple markers with multiple alleles were tested, as beforehand, it was not known which allele of which marker would be in LD with the disease. This increases the chance of false-positive findings,[24] requiring a higher level of significance for evidence of linkage and association. However, there is discussion about the exact threshold in whole genome scans to accept as conclusive evidence. Since our findings are used to give an initial indication for localization of the disease gene, we considered lod scores > 1 interesting for further exploration.

Fine mapping and haplotype analysis of two regions

Two regions around the markers with the highest lod scores under both the narrow and the broader phenotype were further examined by testing extra markers in those regions. Around D2S123 on chromosome 2p14, the markers D2S119, D2S2298, D2S2378, D2S2156, D2S2251, and D2S378 were additionally genotyped. Furthermore, we developed a novel marker, which we denote as D2Sm1, 70 kb downstream from D2S123.

Around marker D12S97 on chromosome 12q24, markers D12S1609, D12S367, and D12S343 were genotyped. PCR reactions were carried out as described before.[37] The additional markers were analysed with the same combined linkage and association method as described above.[30,31] The haplotypes of offspring were used to determine the phase of adjacent marker alleles in patients and spouses, who were included as controls, and, in the absence of a spouse, to reconstruct phase-known haplotypes.

Selection of candidate genes

To evaluate the presence of candidate disease genes in the chromosomal region in linkage disequilibrium with the disease-associated allele, we used the databases of Map view (http://www.ncbi.nlm.nih.gov:80/cgi-bin/Entrez)) and UDB (http://bioinformatics.weizmann.ac.il/udb). Of the positional candidate genes, the function, expression, and possible involvement in POAG were evaluated using the OMIM (http://www3.ncbi.nlm.nih.gov/htbin-post/Omim) and Genecard databases (http://thr.cit.nih.gov:8081/cards/index. html).

Results

Patients

In total, 63 patients were ascertained. Of these, seven were excluded because one of the parents was not born in the genetic isolate (n = 3), data to establish the presence of or exclude POAG were incomplete (n = 1), or because other causes of visual field loss were present (n = 3), such as a cerebrovascular accident, central retinal vein occlusion, or medication. Seven additional patients were excluded because they had other types of glaucoma, including angle closure glaucoma (n = 5), nanophthalmos (n = 1) and glaucoma as part of a syndrome with early menopause and congenital deafness (n = 1), resulting in 11 patients with definite

POAG, 15 with probable POAG, and 23 with ocular hypertension. None of these had any signs of ocular pigment dispersion or pseudoexfoliation, and in all, the anterior chamber angle was open and normal. The individuals with ocular hypertension were not included for present analyses, but they were ascertained for future studies. Thus remained 11 definite and 15 probable POAG patients for the present genome scan (Table 1).

As population-matched controls we included: 14 spouses of patients and five unrelated individuals from the same population who showed no signs of POAG. For nine spouses who were deceased, haplotypes could be reconstructed with the help of data from their children.

The age at diagnosis of the definite POAG patients ranged from 49 to 82 years (mean 68.5, median 72 years), and in the probable patients from 50 to 84 years (mean 63.3, median 64 years). The exact age of onset of the disease was not known, because the disease has an insidious character, not causing symptoms until in a most progressed stage. The mean age at the time of ascertainment was both in definite patients and controls 71.3 years, and in probable patients, 71.7 years. The proportion of males was 7/11 (64%) in the definite patients and 8/15 (53%) in the probable ones. An elevated IOP was present in 10/11 definite patients and 10/15 probables, with a mean IOP 27.1 mm Hg and 24.5 mm Hg, respectively.

All 26 probable and definite POAG patients were related (Figure 1, chapter 5.1). A common founder could be identified who lived in the 16th century, which is 13 to 14 generations back.

Table 1
Clinical features of patients with definite and probable primary open-angle glaucoma

Individual[a]	Sex[b]	Age at diagnosis (yrs)[c]	Intraocular pressure (mm Hg)[d]		Glaucomatous optic neuropathy[e]		Glaucomatous visual field defect[f]		Therapy
			OD	OS	OD	OS	OD	OS	
Patients with definite primary open-angle glaucoma									
3	M	73	28	35	+	+	+	+	medical, laser
4	F	73	21	18	+	+	-	+	medical
8	M	49	28	32	-	+	-	+	medical
12	F	82	19	22	+	+	+	-	medical
15	M	56	23	24	-	+	+	+	medical
16	M	76	23	23	+	+	+	+	medical
21	M	60	40	40	+	+	+	+	surgery, laser
22	F	68	26	26	+	+	-	+	medical
23	F	78	25	26	+	+	+	+	medical
24	M	67	22	22	+	+	+	+	medical
26	M	72	27	27	+	-	+	-	medical
Patients with probable primary open-angle glaucoma									
1	F	74	22	29	+	+	-	-	medical, laser, surgery
2	M	65	30	30	-	+	-	-	medical
5	M	53	33	33	-	+	-	-	medical, laser
6	F	51	19	22	+	-	-	-	medical
7	M	50	14	13	+	-	-	-	-
9	M	84	13	14	-	+	-	-	-
10	F	65	21	21	+	-	-	-	medical
11	F	63	16	16	+	-	-	-	-
13	M	72	20	29	-	+	-	-	medical
14	M	62	28	28	-	+	-	-	medical
17	F	56	26	23	-	+	-	-	medical
18	M	74	33	22	+	+	-	-	medical, laser
19	F	64	21	22	-	+	+[g]	+[g]	medical, laser
20	M	69	33	30	+	-	-	-	medical, laser
25	F	52	16	17	+	-	-	-	medical

⇐ [a] Numbers of individuals refer to numbers in the pedigree in Figure 1, chapter 5.1
[b] F = female; M = male
[c] Age at diagnosis was defined as the age of detection of a GVFD in the definite patients and GON in the probable patients
[d] The intraocular pressure represents the maximum level of intraocular pressure ever measured in a patient's history; OD = right eye; OS = left eye
[e] + = present; - = absent
[f] + = present; - = absent
[g] Early visual field defects, could not be confirmed because patient died

Genome scan results

Lod scores = 0 were produced in 82% of markers, both under the narrow and broad phenotype. The proportion of markers with lod scores between 0 and 0.5 was similar under both phenotypes (15% versus 14%). Markers with lod scores > 1 under either the narrow or broad phenotype are shown in Table 2.

In total, a strong indication for combined linkage and association between POAG and two markers was found (lod scores ≥ 1.5). The highest result under either phenotype was observed with marker D2S123, located on 2p21 at 77 cM.

Table 2
Markers with lodscores ≥ 1 for primary open-angle glaucoma

Marker	Probable and definite POAG[a]		Definite POAG		Location[b]
	Lodscore	Lambda	Lodscore	Lambda	(cM)
D2S123	2.25	0.83	1.32	1.00	77.1
D12S97	1.63	0.44	0.70	0.51	160.9
D4S3042	1.25	0.69	0.59	0.78	81.9
D21S1908	0.45	0.63	1.15	1.00	31.4
D1S252	0.97	0.50	0.98	0.69	155.1

Shown are maximum lod scores using a combined analysis for linkage and association calculated by a modified version of ILINK. Lambda represents the proportion of disease chromosomes with the associated allele.
[a] POAG = primary open-angle glaucoma. Definite POAG was defined as a glaucomatous visual field defect together with a possible or probable glaucomatous optic neuropathy (see text for cut-off points). Probable POAG was defined as a probable glaucomatous optic neuropathy with a normal visual field.
[b] Location indicates the position of the marker in centimorgans from the top of the relevant chromosome on the Whitehead genetic map (www-genome.wi.mit.edu).

Haplotypes in the D2S123 region among definite and probable primary open-angle glaucoma patients

Individual[c]	Marker (position in cM[a])									
	D2s2294 (68.2)	D2s119 (69.0)	D2s2298 (69.5)	D2S2378 (74.0)	D2s2156[b] (77.0)	D2s123[b] (77.1)	D2sm1 (77.2)	D2s2251 (79.8)	D2S378 (82.0)	D2S393 (92.3)
13	17	2	5	5	3	12	2	6	2	13
16	**17**	**2**	**5**	**5**	**3**	**12**	**2**	**6**	**2**	**.**
7	15	2	5	5	3	12	2	6	2	13
12	**17**	**.**	**.**	**5**	**3**	**12**	**2**	**.**	**4**	**12**
23	**11**	**3**	**8**	**5**	**3**	**12**	**2**	**3**	**7**	**14**
26	**11**	**7**	**.**	**5**	**3**	**12**	**2**	**8**	**4**	**13**
20	16	3	16	4	3	12	2	7	4	12
14	16	4	4	1	3	12	2	2	5	11
6	16	7	13	1	3	12	3	2	2	11
15	**16**	**6**	**16**	**7**	**3**	**12**	**3**	**8**	**4**	**11**
24	**16**	**6**	**.**	**4**	**3**	**12**	**3**	**9**	**2**	**12**
25	16	2	3	3	3	12	3	9	2	13
22	**16**	**7**	**10**	**2**	**3**	**12**	**3**	**9**	**2**	**13**
21	**16**	**6**	**16**	**8**	**3**	**12**	**3**	**9**	**2**	**13**

Minimal region of linkage disequilibrium

Maximal region of linkage disequilibrium

Figure 1

Haplotype sharing in the D2S123 region among probable and definite primary open-angle glaucoma patients. Different degrees of shading represent haplotype sharing by subsets of patients. Data in bold are from definite POAG patients, data in regular font style are from probable POAG patients

[a] Position of marker in centimorgans from the top of the relevant chromosome on the Whitehead genetic map (www-genome.wi.mit.edu)

[b] Markers D2S2156 and D2S123 yielded high lodscores

[c] Numbers of individuals correspond to numbers in the pedigree (Figure 1 in chapter 5.1)

The lod score was 1.6 (λ = 1.0) in the group of only definite patients and 2.25 (λ = 0.8), when probably and definitely affected patients were included. Marker D12S97 on chromosome 12q at 161 cM yielded the second-highest lod score of 0.7 (λ = 0.5) under the narrow phenotype and 1.6 (λ = 0.8) under the broad phenotype. Further interesting lod scores were obtained for markers D4S3042 and D21S1908, with lod scores 1.25 (λ = 0.69) and 1.15 (λ = 1), respectively, under the broad phenotype.

Fine mapping and haplotype analysis in the regions with the highest lod scores

Around the two markers with the highest lod scores, D2S123 and D12S97, additional markers were screened (Fig. 2 and 3). In the D2S123 flanking region, D2S2156 also yielded a high lod score in the analysis of the definite POAG patient group (lod score = 1.53, λ = 1), but a low lod score in the group with definite and probable patients (lod score = 0.2, λ = 0.44). The other, additionally screened, markers resulted in lod scores = 0 with λ = 0. Haplotype analysis revealed that a region up to 3.1 cM between D2S2378 and D2Sm1 is most likely in LD with the disease-associated allele. This region is completely shared by 14 out of 26 definite and 15 probable patients and by eight out of 28 controls.

Among the markers screened in the D12S97 region, marker D12S343 also yielded high lod scores. Lod scores were 0.6 (λ = 0.45) under the narrowly defined phenotype and 1.25 (λ = 0.57) under the broadly defined phenotype. The other markers tested around D12S97 resulted in lod scores = 0 with λ = 0. A large region (11.3 cM) between D12S1609 and D12S343 appeared to be in LD and was shared among nine out of 26 patients and by two out of 28 controls.

Haplotypes in the D12S97 region among definite and probable primary open-angle glaucoma patients

	Marker (position in cM[a])						
	D12S395	D12S324	D12S1609	D12S367[b]	D12S97[b]	D12S343	D12S357
Individual[c]	(137.5)	(148.3)	(154.4)	(160.9)	(160.9)	(165.7)	(169.1)
3	**8**	**10**	**2**	**3**	**13**	**1**	**15**
19	7	10	2	3	13	1	15
4	**8**	**.**	**2**	**3**	**13**	**1**	**15**
25	7	10	4	3	13	1	15
11	9	10	1	3	13	1	15
7	7	12	2	3	13	3	.
2	8	10	2	3	13	3	13
10	7	13	6	3	13	5	16
21	**8**	**12**	**6**	**3**	**13**	**5**	**16**

Minimal region of linkage disequilibrium

Maximal region of linkage disequilibrium

Figure 2

Haplotype sharing among probable and definite primary open-angle glaucoma patients. Different degrees of shading represent haplotype sharing by subsets of patients. Data in bold are data from definite POAG patients, data in regular font style are probable POAG patients

[a] Position of the marker in centimorgans from the top of the relevant chromosome on the Whitehead genetic map (www-genome.wi.mit.edu).

[b] Markers D12S367 and D12S97 yielded high lodscores

[c] Numbers of individuals correspond to numbers in the pedigree (Figure 1 in chapter 5.1)

[d] Individuals 3 and 4 are siblings

Discussion

We performed a whole genome scan in 11 definite and 15 probable POAG patients from a genetically isolated population. Although based on subjective criteria, the type of POAG observed in our sample of patients could be described as moderate to severe, with a relatively late age of diagnosis, various levels of IOP, and the use of medical therapy in most patients, with a few surgical interventions. A combined test for linkage and LD yielded lod scores of > 1.5 in two regions on chromosome 2p21 and 12q24, and lod scores > 1.0 on chromosome 4q13 and 21q21. In the two loci most significantly associated with POAG, haplotype sharing was present among patients.

Suitability of study population

Our isolated population has proven successful in identifying genes for rare monogenic eye disorders.[25,28] However, the genetic heterogeneous character of POAG complicates genetic research. Although it is expected that this heterogeneity is reduced in a genetic isolate due to the limited number of founders, there is considerable debate whether such populations as ours are suitable to map common multifactorial diseases.[33,38,39] It has been suggested by simulation studies[40-42] and by observational studies[43] that the extent of LD is similar in older isolated populations and mixed populations. As a consequence, the former populations would not be more useful for mapping genes for complex diseases than the latter. Other studies provide evidence for an increased extent of LD in recently isolated populations.[44-46] Our population is relatively young, was founded by a few ancestors, and increased dramatically after 1830. Therefore, we expect that the extent of LD is considerable. Since, in a genome scan, the probability to detect LD is determined by the extent of LD, our genetic isolate is most suitable for finding potential gene loci. A disadvantage of this substantial LD may be the difficulty to narrow down the region of interest at a later stage of the study.

Patients

The lack of a conclusive and worldwide-applied clinical definition of POAG complicates research as well as valid comparison of results from different genetic POAG studies. The heterogenous molecular etiology of POAG necessitates an exact description of the phenotype. Unfortunately, in many genetic studies, the

phenotype is not reported in detail. The clinical definition we used was based on the Rotterdam Study,[4] a population-based prospective cohort study among persons 55 years and older. An elevated IOP was not part of the definition, since it is rather considered a risk factor for POAG than a criterion required for diagnosis by most studies.[6,47,48] Compared to other genetic studies, our definition was rather strict, because the cut-off points for a glaucomatous optic neuropathy were set at a high level, and because the IOP was not used as a criterion for POAG. For example, if Wirtz' criteria[15] would be applied to our data, 21 of our patients (probable or definite) would also be assigned as affected. However, five of our probable patients would not be assigned as affected because they had an IOP $\leq$ 21 mm Hg. In contrast, 18 additional individuals of our study would be classified as POAG, while by our criteria, they were classified as having ocular hypertension or unaffected (data not shown). A strict clinical definition of POAG decreases the number of patients. It may seem that this reduces the power of the analyses to yield an interesting lod score. However, the opposite may also occur if a strict definition reduces misclassification of POAG patients: in that case, the power of the analyses may increase strongly.

Statistical analysis

POAG frequently does not show a simple inheritance pattern, as is seen in classical Mendelian genetics. In contrast, incomplete penetrance, a variable phenotype, as well as phenocopies are observed with POAG. Since statistical analyses have to take these observations into account, simple Mendelian models are not appropriate. In a genetically isolated population, it is possible to perform a combined test for linkage and association, where λ allows the presence of genetic heterogeneity, phenocopies, and allelic heterogeneity.

The region around marker D2S123, which yielded our highest result (lod score = 2.25 with λ = 0.8), was recently also reported by Suriyapperuma[49] to be involved in POAG. This group found linkage with 2p14-p16 in a large British family. The affected individuals shared a haplotype from D2S123 to D2S329, a region of approximately 27 cM. Recombination occurred in individuals with ocular hypertension, potentially restricting this locus to less than 8 cM. Our interval extended from D2S2378 to D2Sm1 (3 cM) and possibly to D2S2251, since half of the patients shared an allele at this marker. Therefore, our interval overlaps the interval reported by Suriyapperuma only for the 70kb part between D2S123 and D2Sm1, possibly

extending 2.8 cM to D2S2251. Since the overlap between the two regions is rather small (70 kb) compared to their respective sizes, the most likely explanation of our results combined with those of the literature, is that there are two genes located on 2p14.

In our study, multiple markers with multiple alleles were tested, as it was not known a priori which allele of which marker would be in LD with the disease. This procedure will lead to an increased probability for false-positive findings.[24] Therefore, a higher level of significance would be required before definite evidence of linkage or association can be considered as definitive. As our genome scan findings are only used to give an initial indication for localization of disease susceptibility genes, we considered lod scores > 1 interesting for further exploration.

Candidate genes

In the 3.1 cM region between D2S2378 and D2Sm1, at least 15 genes and 18 EST's are located, such as the potassium channel (KCNK12), the gene similar to solute carrier family 13 (LOC115211), and the matrilin gene. It is not known whether KCNK12, LOC115211 or the matrilin genes are expressed in eye tissues.

Ion transporters are involved in the production of aqueous humor in the ciliary body. Therefore, although there is no conclusive clinical or scientifical evidence that POAG is caused by hypersecretion of aqueous humour, KCNK12 and LOC115211 are interesting candidate genes. The matrilin gene is a member of a family of proteins that are thought to be involved in the extracellular matrix of various tissues. Since it is hypothesized that extracellular deposits in the trabecular meshwork obstruct the outflow of aqueous humor, leading to POAG,[9] matrilin is an interesting candidate gene for POAG.

In the region between D12S1609 and D2S343 on chromosome 12, screening of UDB revealed the presence of the genes GOLGA3 and ZNF26, three EST's, and two cDNA clones, moderately similar to zinc finger protein 84. In addition, Map view showed also two Drosophila homologous genes (PIWI L1 and FZD10). However, none of these genes are obvious candidate genes for POAG.

Clinical evaluation of first- and second-degree relatives of patients may reveal additional affected individuals. Genealogical data may help in the formation of clusters based on the degree of kinship. In such clusters, conventional linkage analysis may be feasible, with the assumption of a reduced penetrance. Furthermore, clinical data in close relatives gives information on clinical variability within a

phenotype, because close relatives share many genes including the disease gene(s) identical by descent. The identification of additional distantly related patients in the population may be useful to narrow down the region of interest based on haplotype sharing. A larger sample size generally leads to higher power. In addition, a large sample size may enable additional sub-analyses after stratification according to specific clinical characteristics, such as IOP or age of onset of the disease.

Our findings provide an indication of two new POAG loci on chromosome 2p21 and 12q24. These regions contain several interesting candidate genes for POAG. Further molecular analysis of these candidate genes is needed to identify a susceptibility gene involved in POAG.

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6

GENERAL DISCUSSION

6

General discussion

The aim of this thesis was to investigate and identify determinants for primary open-angle glaucoma (POAG) in order to increase our knowledge of the etiology of this multifactorial disorder. Several approaches were used in different types of populations, including a genetically outbred one, families of probands derived from this outbred population, and a genetically isolated population.

In this chapter, the main findings of these studies together with their methodological considerations will be discussed. Furthermore, recommendations for future research will be provided.

Epidemiological studies in the Rotterdam Study

Epidemiologic studies were carried out in the genetically outbred Rotterdam population, representative of the general Dutch population, aged 55 years and over. In this cohort study, ophthalmological, cardiovascular, neurological, and locomotor diseases are studied, which enables us to evaluate new risk factors for POAG, and to analyse genetic and non-genetic risk factors that have been proposed before. The criteria we used to define POAG and the prevalence of POAG according to this definition in the Rotterdam Study was described in chapter 1.

We found that women with an early natural menopause had a 2.6 times increased risk of POAG (chapter 2.2). Furthermore, a high systolic blood pressure, a high systemic pulse pressure, a low diastolic blood and perfusion pressure, as well as arterial stiffness were associated with an increased risk of high-tension POAG, while no significant association was found between these factors and normal-tension POAG (chapter 2.3).

Selection bias occurs when the relation between determinant and outcome is different for responders and non-responders. The non-responders of the Rotterdam Study were older. Therefore, it is likely that they had more exposure to risk factors and more diseases than the responders. Since the prevalence of POAG increases with age, exclusion of an older set of individuals probably causes underestimation of the prevalence of the disease. However, since it is not likely that the

relation between any of the determinants studied (age at menopause, blood pressure, arterial stiffness) was different in responders and non-responders, we think non-response had no serious bias on the results of our study.

In the study into age at menopause (chapter 2.2), data on age and cause of menopause were self-reported. Misclassification could have occurred because of the time period between the occurrence of age at menopause and the reporting. However, the distribution of ages at menopause in our study was comparable to the distributions reported by two leading studies on age at menopause in the Netherlands.[1,2] Furthermore, since we have no reason to assume that women with POAG would have reported an earlier age at menopause, misclassification would have been non-differential, and this would have given an underestimation of the true effect.

The studies into age at menopause and blood pressure parameters described in this thesis were cross-sectional. All parameters of the outcome measure (POAG) and all determinants, except arterial stiffness, were assessed in the baseline phase of the Rotterdam Study. In general, a cause-effect relation is difficult to establish in a cross-sectional design. Bias may occur when the determinant changes over time and when the determinant is affected by the outcome. In case of POAG, one can imagine that glaucomatous loss of nerve tissue in the optic nerve and retina may have influence on local processes in the eye. However, it is unlikely in our studies that our results are explained by an influence of POAG on systemic factors such as age at menopause, systemic blood pressure, and arterial stiffness. Since we have no reason to assume a change of any of these determinants due to the occurrence of POAG, we think that such bias is minimal.

In our study of blood pressure parameters (chapter 2.3), we classified POAG cases into those with and without an IOP > 21 mm Hg (high-tension POAG and normal-tension POAG, respectively), despite the limited number of IOP measurements and the time span in which they were taken. The value of 21 mm Hg was chosen because this value represents the 97.5th centile in our population, and because it is generally used as cut-off point.[3] There is much debate about the question whether the two types of POAG should be considered separate entities. Opinions differ on whether they have a different clinical course and a different etiology. Although we realize that any cut-off point creates an artificial distinction, we chose to stratify our POAG cases according to IOP for the following reason: it is generally accepted that POAG is considered a multifactorial disease, where different combinations of factors are responsible for the different phenotypes of

the disease. Therefore, it is likely that normal tension POAG and high tension POAG are subtypes along the continuum of IOP. Stratification for IOP facilitates the search for other risk factors than IOP for POAG. Therefore, risk factors that are involved in a subset of the heterogeneous group of POAG may better be identified when analysing these subsets, instead of analysing the total group.

Each risk factor may in itself, at least partly, be influenced by genetic and non-genetic factors. In chapter 2.3, we hypothesized that blood pressure parameters and arterial stiffness may lead to POAG if the IOP is elevated (at the upper end of the IOP continuum). Likewise, mutations in any gene involved in blood pressure or arterial stiffness may lead to POAG. We did not find any association between the determinants we examined and normal tension POAG (at the lower end of the IOP continuum). Risk factors reported in the literature involved in normal tension POAG include vasospasmic disorders such as migraine and Raynaud disease.[4] Mutations in any gene involved in these disorders may lead to normal tension POAG.

Mutation analysis in a genetically outbred population

Myocilin (MYOC) is the first, and, so far, only gene found to be involved in POAG. Despite an immense increase in studies of MYOC since its discovery in 1997, the function in normal individuals and the mechanism by which this gene causes POAG remain largely unknown. MYOC is expressed in multiple eye tissues, including the trabecular meshwork,[5] ciliary body and sclera,[6] axons of ganglion cells and several parts of the optic nerve head,[7] as well as in many nonocular tissues.[6] It is hypothesized that the outflow of aqueous humour is obstructed.[8] The mutated protein may either disturb the normal cytoskeletal function,[9] or contribute to the extracellular matrix.[10] Increased MYOC expression in the trabecular meshwork was found in POAG and pseudoexfoliative glaucoma.[11]

It has been established that the contribution of the MYOC gene to adult onset POAG is 3-5% in large, clinic-based samples of patients.[8,12-14] We tested a population-based sample of 47 POAG cases from the Rotterdam Study for mutations in this gene (chapter 3). One disease-causing mutation (Asn480Lys) and six polymorphisms were found. Although our numbers are relatively small, this prevalence (1/47) was similar to the 3-5% found in clinic-based studies.

The number of patients included in our study was limited by the prevalence of the disease in the population and the rather strict criteria used to define definite

POAG. Both a glaucomatous optic neuropathy and a glaucomatous visual field defect were required for the definition of definite POAG, and the threshold values for a glaucomatous optic neuropathy were high, compared to other studies. An important advantage of our population-based approach was the minimalization of selection bias towards a specific type of POAG, such as that with an elevated IOP or progressed stages.

We examined the relatives of the POAG case in whom we identified the Asn480Lys mutation in MYOC, and identified one individual with probable and one with definite POAG, who carried the same mutation, while four unaffected relatives did not carry the mutation. The phenotypes of the three patients within this family varied widely, ranging from a glaucomatous optic neuropathy but no visual field damage in a 70-year old patient to a severely excavated optic disc and a remaining temporal rest in a 34-year old patient. Apparently, even in this family, where POAG is inherited as a simple autosomal dominant trait, other factors must be present to cause these differences in phenotypes.

The family derived from our population study demonstrates that knowledge of genetic factors may be useful for POAG patients and their relatives. By screening for the presence of this specific mutation, future relatives who carry the mutation may be put under more strict control of the ophthalmologist. At the same time, relatives negative for this mutation run no higher risk of POAG than an individual with the same age from the general population.

Family study in a population-based setting

A positive family history of POAG has been established as a major risk factor of POAG. This risk factor is often indicated as presence or absence of a positive family history for a given individual. In chapter 4, we applied a new statistical method to quantify the familial risk for an individual, by calculating her/his family score (FS) based on all data obtained by examination of their family. This FS accounted for the age, gender, number, kinship, and presence or absence of POAG in relatives. To test whether this FS could be used as a risk factor for POAG, we examined whether FS was associated with POAG. Case and control individuals were derived from the population-based Rotterdam Study and their relatives were examined. FS's were calculated from these relatives, and logistic regression was performed to examine whether FS was associated with POAG. We found that individuals with a high FS had an increased risk of POAG. This association was

independent of the presence of an elevated IOP.

Cases and controls, further noted as case and control probands to discern them from their relatives, were derived from the population-based Rotterdam Study. Relatives underwent the same standardized ophthalmological examination as the probands, and response rates were high. Features of POAG were assessed separately in a masked fashion to ensure an unbiased diagnosis. As a result, selection and ascertainment bias were minimized. Examining relatives instead of taking a family history from the probands reduced misclassification of disease status. Misclassification of POAG could otherwise easily occur due to the insidious course of the disease, lack of knowledge of the disease status in proband or relative, and different and changing definitions of POAG.[15]

A positive FS indicated that in a family, more cases were present than would be expected from the age- and gender-specific prevalence in the general population. A high FS therefore points to an increased genetic risk for individuals in that family and vice versa. An FS = 0 meant that the number of cases was equal to that expected from the age- and gender-specific prevalence in the general population. However, many probands had an FS of 0, because they had small and/or young families, which did not contain much information about the genetic risk. In individuals of these families, FS could not be used as a risk factor.

In larger families, the FS can be used as risk factor of POAG of an individual. It can be used by the clinician to identify individuals with a high familial risk for follow up and for further genetic analysis. For research purposes, the FS can be used to represent the genetic risk of an individual in multifactorial models of POAG.

Localization of genes in a genetically isolated population

In chapter 5, we aimed to localize one or more genetic susceptibility loci for POAG. We identified 11 definite and 15 probable POAG patients, and 23 individuals with ocular hypertension in a genetically isolated population in the North of the Netherlands. In chapter 5.1 we first excluded all candidate POAG regions known from literature, including MYOC (GLC1A),[12] regions on chromosome 2cen-q13 (GLC1B),[16] 3q21-q24 (GLC1C),[17] 8q23 (GLC1D),[18] 10p15-p14 (GLC1E),[19] and 7q35 (GLC1F).[20] In addition, we excluded five regions that were described in a genome scan in a large, heterogeneous sample of POAG families (chromosome 2p, 14p, 17p, 17q, and 19q).[21] In chapter 5.2, we present the results

of a whole genome scan in this sample. Lod scores > 1.5 were obtained in two regions on chromosome 2p21 and 12q24, and lod scores > 1.0 on chromosome 4q13 and 21q21. In the two loci most significantly associated with POAG, haplotype sharing was present among patients.

The POAG loci GLC1A to GLC1F were identified using linkage analysis in a subset of unrelated POAG families. In these families, POAG segregated as an autosomal dominant trait. However, in the majority of families, POAG does not follow a clear-cut Mendelian inheritance pattern and the inheritance pattern is unknown. Conventional linkage analysis has limited value in localizing loci and genes for POAG in the latter group of families, because of several reasons. The power of linkage analysis decreases rapidly if the mode of inheritance is not exactly known. Furthermore, POAG is genetically heterogeneous. It is hypothesized that many genes are involved in POAG, each contributing for a small part to the occurrence of the disease.[22] Linkage analysis has limited power to detect modest effects of susceptibility loci contributing to a complex disease. Finally, linkage analysis requires large families with a considerable number of affected individuals. Due to the relatively late age of onset of POAG, parents of patients are often diseased, while the children have not reached the age of onset yet, resulting in limited numbers of affected individuals per family. We chose a genetically isolated population to deal with these problems.

In a genetically isolated population, the current generation has arisen from a limited number of founders, which reduces genetic complexity. Therefore, it is plausible that many patients of the current generation share the same genetic factor, inherited from a common ancestor. Linkage disequilibrium (LD) mapping is based on the assumption that these patients also share a genomic region spanning this genetic factor. These chromosomal regions of LD may be used to localize a disease gene. These regions are detected by comparing allele and haplotype frequencies between patients and controls, and are confirmed by haplotyping of markers in the chromosomal region. In our studies, we used a combined test for linkage and LD. This test is appropriate to exclude or localize chromosomal regions of interest for multifactorial disorders in a genetically isolated population, where the inheritance pattern is not exactly known. Sampling from a genetic isolate enabled us to individually collect a considerable number of patients.[23] Genealogical research revealed that the majority of patients was related and a pedigree could be constructed.

The probability to detect LD is determined by the extent of LD. If the extent of

LD is small, and the distance between the markers used in the genome scan is large, the region of LD will be missed. The extent of LD decreases as the number of generations since the common ancestor increases. Isolated populations have often been proposed as suitable populations for LD mapping, because it was expected that they have larger regions of LD than mixed populations.[24,25] Recently, though, simulation[26-29] and observational studies[30-32] suggested that LD in older genetic isolates would not be larger than in outbred populations. However, it has been argued by others[33] that the assumptions used in these studies about the demographic history and the expansion of a population were not realistic. A continuous decrease of LD was assumed, while genomic regions of lower recombination and hotspots of recombination exist, as well as expansion, contraction, and bottlenecks in population growth. Finally, investigators provided empirical evidence for extensive LD in recently isolated populations, founded by a small number of individuals. LD would be especially large in populations, which had undergone rapid growth after a considerable period of constant size.[34-37]

Our studies into the identification of genes for monogenic eye disorders showed that the extent of LD was approximately 3-5 cM.[38,39] This size was expected from the history of the population, because it was relatively recently founded by only a few ancestors, and the population increased dramatically after the year 1830. As a consequence of the recent isolation, this population may resemble the general population more than older genetic isolates. The latter increases the probability that a POAG susceptibility gene identified in our population may also play a role in POAG in the general population.

In our study, multiple markers with multiple alleles were tested, because it was not known a priori which allele of which marker would be in LD with the disease. This increases the probability of false-positive findings. A higher level of significance is required before the results can be considered as conclusive evidence, but there is debate on the exact threshold that should be used in a genome wide LD screen for a complex disease. The highest lod scores reported in chapter 5.2 (2.25 and 1.6) were not corrected for multiple testing and correspond to uncorrected p-values of 0.006 and 0.02, respectively. However, the lod scores shown in chapter 5.2 should not be interpreted as conclusive evidence, but as an initial indication of regions interesting for further exploration.

Spouses of patients and elderly, unrelated, individuals without any signs of POAG were included as controls. The choice of a control group may generate false

positive findings if unaffected controls and affected individuals are chosen from different populations because of a potential difference in population allele frequencies. Our patients and controls were derived from the same population, which minimized selection bias. In the analyses, the disease status of the controls was assigned as unknown. A disease gene frequency of 1% accounted for a possible prevalence of POAG among the control individuals of 1%. This prevalence was based on the assumption that we have identified nearly all POAG patients in this community through general practitioners and ophthalmologists in the region, since response rates were high.

In our combined analysis of linkage and LD, λ (the proportion of disease chromosomes with the associated allele) allows the presence of a few phenocopies. In the exclusion analysis of POAG candidate genes, fixed λ's of 0.8 and 0.9 were used, allowing for 10 to 20% of phenocopies. If in the analyses of the genome scan, a few phenocopies are present among the patients, the power of analyses and λ are reduced, but this effect will be minimal.

Our criteria for the presence of definite POAG were strict, compared to other definitions of POAG from genetic studies. The cut-off points for a glaucomatous optic neuropathy were high, and the level of IOP was not used as a criterion for inclusion.[40] These strict criteria reduced the number of patients. What are the possible consequences of this reduction?

The consequences of reduction depend upon the question which individuals are excluded from the study by using a strict clinical definition. In general, decreasing the sample of patients lowers the overall power to yield positive results. The latter is the case, if the exclusion leads to lowering the number of patients, who share the same genetic cause. In contrast, if the exclusion decreases the number of individuals wrongly classified as POAG (misclassification) or if it decreases the number of phenocopies (diagnosis of POAG is right, but the disease results from a different genetic cause), the power of the analyses increases strongly. We excluded a considerable number of individuals, compared to, for example, Wirtz' study (see chapter 5.2). Using strict clinical criteria likely results in more homogeneity of the phenotype, and thus we think that the gain in power caused by decreasing misclassification and phenocopies is larger than the loss of power due to the reduction of patients, who shared the same genetic cause.

Strategies for future research

Epidemiologic studies

To evaluate a causal-effect relation of a determinant on the outcome, longitudinal studies are preferred. The determinant is then measured before occurrence of the outcome. The Rotterdam Study has a prospective cohort design. Soon, incidence data on POAG will become available from the third examination phase, which has taken place between 1997 and 1999, resulting in follow up time of about 7 years. Then, it will be possible to study the effect of determinants measured in the first phase on the occurrence of POAG, determined in the third phase.

For both etiologic and therapeutic reasons, further research into the effects of endogenous and exogenous exposure to female sex hormones would be of interest. The Rotterdam Study recently started a new study phase, including again individuals age 55 years and older. The use of hormone replacement therapy has increased largely in the last decade. Therefore, newly included women may have used hormonal replacement therapy more often than those from the original baseline cohort, leading to more power in analyses regarding hormone replacement therapy.

Myocilin mutation analysis in a genetically outbred population

Differences in phenotype among carriers of the same mutation in MYOC have been reported in several large families, including ours. It is unknown which factors influence the intrafamiliar differences in the phenotype. One patient may not have any damage, while another patient from the same family, carrying the same mutation, suffers from severe visual field loss. This leads to the conclusion that other factors must be present to explain these differences. Genotype-phenotype research in families with POAG caused by mutations in MYOC remains necessary to identify possible influencing factors. Furthermore, such studies may be helpful to elucidate the mechanism by which mutations in MYOC cause POAG. The Glu368Stop mutation leads to a considerably shorter protein, lacking a domain of MYOC that is hypothesized to be important for its function. It is the most prevalent (40%) of the approximately 30 mutations known at present. However, it is unknown why this mutation causes a much milder phenotype than mutations with a more subtle effect.[41] Furthermore, it is unknown why patients heterozygous for a mutation are affected, and patients homozygous for the same mutation do not display any signs of POAG.[41] Apart from these family studies, research in transgenic animal models

may aid in examining the function of normal and mutated MYOC. These studies may be useful to study genetics of individual glaucoma genes and to identify interaction between genes and thus help in the identification of modifier genes.[24]

Family study in a population-based setting

In a complex disease, the number of affected individuals per family is relatively small. This complicates segregation analysis. To further identify and quantify the attribution of genetic and environmental risk factors, families with high FS could be followed up, and examination of families could be extended to 2nd degree relatives and spouses.

Furthermore, the inheritance of features of POAG could be investigated separately. These can be quantitative measures, such as vertical cup-to-disc ratio and minimal neuroretinal rim, and categorical measures, such as the presence of a glaucomatous visual field defect. In addition, the FS could be used to examine more thoroughly the inheritance of IOP and its role in the inheritance of POAG.

Localization of genes in a genetically isolated population

Further clinical follow up of the patients from the genetically isolated population may be useful to define the phenotype more accurately. This follow up could include assessing the rate of excavation of the optic disk, visual field deterioration, and response to treatment.

Follow up of close relatives of previously identified patients may reveal additional affected individuals. A higher number of patients may increase the power of the analyses. A higher number of patients may also be useful to perform additional detailed analyses. Genealogical data may be used to form clusters based on the degree of kinship. In such clusters, conventional linkage analysis may be feasible, with the assumption of a reduced penetrance. Furthermore, clinical data in close relatives gives information on clinical variability within a phenotype, because close relatives share the disease gene identical by descent.

The identification of additional distantly related patients in the population may be useful to narrow down the region of interest based on haplotype sharing. A larger sample size generally also leads to higher power. In addition, a large sample size may enable additional analyses after stratification according to specific clinical characteristics, such as IOP or age of onset of the disease.

Candidate genes in the ultimately identified chromosomal regions may be

screened for mutations. Sequence alterations may be compared between cases and controls of the isolated population, and, subsequently, between cases and controls from the general population. Selection of candidate genes is based on several criteria. Genes may show homology with genes already known to be involved in POAG. Since MYOC is the only gene known for POAG, genes showing homology with MYOC may be interesting candidates. Furthermore, genes are selected because of the function of the protein they encode. For POAG, a large number of genes would be appropriate according to this criterion, because multiple mechanisms are thought to be involved in its pathogenesis. In addition, selection of genes is based on the expression of the gene. In case of POAG, many tissues in the eye are involved, including retinal ganglion cells and their axons, but also the lamina cribrosa, ciliary body, and trabecular meshwork. Finally, the phenotype may aid the selection of candidate genes. For example, if an elevated IOP is an important feature in the phenotype, genes involved in aqueous humor dynamics could be appropriate.

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7

SUMMARY

7.1

Summary

This thesis describes our research into risk factors of primary open-angle glaucoma (POAG). POAG is a clinically and genetically heterogeneous eye disorder, which mostly affects people aged 55 years and older. The clinical features are an excavation of the optic nerve, so called glaucomatous optic neuropathy (GON) and a glaucomatous visual field defect (GVFD). Important risk factors include age, an elevated intraocular pressure (IOP), black race, and the presence of POAG in relatives. The epidemiological part and one family study described in this thesis took place in the Rotterdam Study, a population-based cohort study of subjects aged 55 years and older. Data were derived from the baseline phase of this study (1990-1993), when 6,872 subjects participated in the eye examination. The other genetic studies described in this thesis were performed in a genetically isolated population in the Netherlands.

Chapter 1 describes the aim of our studies on POAG. A brief introduction is given of POAG, its risk factors, and the problems encountered when examining a complex disease such as POAG, using an epidemiologic and genetic approach.

In chapter 2.1, we present a proposal for an international classification system and we give the prevalence of POAG in the Rotterdam Study according to this system. POAG is classified in three categories: definite, probable, and possible. Definite POAG is defined as a probable or possible GON based on the 99.5th and 97.5th percentile, respectively for this population, together with a GVFD. Probable POAG is either a GVFD without GON or a probable GON without a GVFD or a visual field test. Possible POAG is defined as a possible GON without a GVFD or visual field test. The overall prevalence of definite POAG was 0.8% (50 cases). The prevalence of POAG in men was double that in women. When criteria used by other population-based studies were applied to data of the Rotterdam Study, the prevalence differed by a factor of 12.

We examined whether an early age at menopause was associated with POAG in 2,469 women who experienced a natural menopause and 609 women who experienced menopause after surgery or irradiation therapy (chapter 2.2). Definite

or probable POAG was diagnosed in 78 women with a natural menopause and 15 with an artificial menopause. Women who went through natural menopause before reaching the age of 45 had a 2.6 times higher risk of POAG than those with their menopause at or after age 50. The number of women with an artificial menopause and women who used hormonal replacement therapy was too small to draw conclusions.

In chapter 2.3, we studied the relation between blood pressure, arterial stiffness and the risk of POAG. We analyzed separately POAG cases with and without an elevated IOP (high-tension POAG and normal-tension POAG, respectively) and subjects with and without systemic antihypertensive therapy. Subjects with a high systolic blood pressure, a high difference between systolic and diastolic blood pressure, and a high arterial stiffness, had an increased risk of high-tension POAG, in the group without antihypertensive therapy. Subjects with a low diastolic blood pressure also had an increased risk of high-tension POAG, in those with treatment for systemic hypertension.

The prevalence of mutations in myocilin, the only gene known to be associated with POAG, were investigated in the population-based sample of 47 POAG cases from the Rotterdam Study (chapter 3). In one case, a disease-causing mutation was found, which prevalence was expected from the prevalence in clinic-based studies (2-4%). Upon investigating the family of this case, mutations were present in two affected, and absent in four unaffected relatives. The clinical features differed largely among the patients carrying the mutation, suggesting the presence of other factors influencing the expression of POAG.

In chapter 4, the family score method (FS), which is a new statistical method, is described to assess the genetic risk of an individual for POAG, based on the information in his/her relatives. The FS accounts not only for the disease status of relatives, but also for their total number, age, gender, and degree of kinship. An increase in FS was significantly associated with an increased risk of POAG in a given individual. The presence of an elevated IOP did not influence this association.

In chapter 5, a sample of 11 definite and 15 probable POAG patients is described, derived from a genetically isolated population. This population has arisen from a limited number of founders, and has been isolated during many generations for cultural and religious reasons. As a result, the genetic complexity of the population and the number of genes possibly involved in POAG is most likely

decreased, which is an advantage for genetic research into the genetic causes of POAG. In chapter 5.1, POAG candidate loci known from literature were excluded with a combined test for linkage and association. The test was based on the assumption that most patients identified in this population share the same genetic cause and a larger genomic region around it. Unrelated spouses and elderly subjects from the same population were included as controls.

Chapter 5.2 describes a genome scan performed in this sample of POAG patients, using 347 markers distributed over the total genome. Analyses were performed including both the group of definite and probable POAG patients, and the group of definite patients only. Two regions on chromosome 2 and 12, most likely involved in POAG, were further examined with additional markers. Haplotype sharing in these regions was observed between patients. Potential candidate genes, such as KCNK12, a gene for a potassium channel, and matrilin, an extracellular matrix protein, were discussed.

In chapter 6, the main findings of the studies described in this thesis are discussed, together with their methodological considerations. Furthermore, recommendations for future research are provided.

7.2

Samenvatting

Primair open kamer (angle) hoekglaucoom (POAG) is een oogziekte, die vooral bij ouderen voorkomt. De klinische verschijnselen zijn een glaucomateuze excavatie van de gezichtszenuw, ook glaucomateuze opticus neuropathie (GON) genoemd, en bijpassende gezichtsvelddefecten. Belangrijke risicofactoren voor het ontstaan van POAG zijn: leeftijd, een verhoogde oogdruk, bijziendheid, en negroïde ras. Naast deze factoren spelen genetische oorzaken een belangrijke rol. In dit proefschrift worden determinanten van POAG onderzocht met verschillende benaderingen. De studies vonden plaats in een algemene populatie, die genetisch gemengd is, in families van POAG patiënten en controles, afkomstig uit deze algemene populatie, en in een genetisch geïsoleerde populatie. Het Erasmus Gezondheid en Ouderen (ERGO) onderzoek werd uitgevoerd in een populatie die representatief was voor de algemene Nederlandse Caucasische bevolking. In de ERGO studie zijn 7983 personen van 55 jaar en ouder onderzocht op oogziekten, hart-en vaatziekten, aandoeningen van het bewegingsapparaat, en neurologische ziekten. De genetische studies werden verricht in een genetisch geïsoleerde bevolkingsgroep in Noord-Nederland.

Hoofdstuk 1 beschrijft het doel van de studies naar POAG. Hierin wordt een korte introductie gegeven over het klinische beeld en de risicofactoren van POAG. Verder worden de problemen die men tegenkomt bij het onderzoeken van POAG vanuit de verschillende benaderingen besproken, zoals het ontbreken van een wereldwijd toegepaste klinische definitie of classificatiesysteem, het feit dat er waarschijnlijk meerdere genen betrokken zijn bij POAG, de complexe manier van overerving, en de late leeftijd van ontstaan of manifest worden van POAG.

In hoofdstuk 2.1 doen wij een voorstel voor een internationale classificatie van POAG en beschrijven we de prevalentie van POAG in de ERGO populatie volgens dit classificatiesysteem. POAG wordt ingedeeld in drie categorieën: definitief, waarschijnlijk en mogelijk. Definitief POAG wordt gedefinieerd als een waarschijnlijke of mogelijke GON, gebaseerd op de 99.5 respectievelijk 97.5^{e} percentiel in deze populatie, samen met glaucomateus gezichtsveldverlies (GGV).

Waarschijnlijk POAG wordt gedefinieerd als GGV zonder GON of een waarschijnlijke GON zonder GGV of zonder gezichtsveldonderzoek. Mogelijk POAG tenslotte berust op een mogelijke GON zonder GGV of gezichtsveldonderzoek. De totale prevalentie van definitief POAG was 0.8% (50 personen). De prevalentie van POAG onder mannen was 2x zo hoog als die onder vrouwen. Wanneer de criteria uit andere populatiestudies werden toegepast op data uit het ERGO onderzoek, verschilde de prevalentie met factor 12.

In hoofdstuk 2.2 onderzochten we of er een verband bestond tussen het op jonge leeftijd doormaken van de menopauze en POAG in 2469 vrouwen met een spontane menopauze en 609 vrouwen die in de menopauze kwamen na een operatie of na bestraling (artificiële menopauze). POAG was aanwezig in 78 vrouwen met een spontane menopauze en 15 met een artificiële menopauze. Vrouwen die voor hun 45e spontaan in de menopauze geraakten, hadden een 2.6 zo hoog risico op POAG als degenen die na hun 50e menopauzaal werden. Analyses bij vrouwen met een artificiële menopauze en vrouwen die hormonale substitutie gebruikten wezen ook op een verhoogd risico voor hen die voor hun 45e in de menopauze kwamen, maar aantallen waren te laag om definitieve conclusies uit te trekken. Deze resultaten konden vanuit de literatuur verklaard worden door het wegvallen van een beschermend effect van vrouwelijke geslachtshormonen, zoals oestrogenen en progestagenen.

In hoofdstuk 2.3 bestudeerden we de relatie tussen bloeddruk, vaatwand-stijfheid en het risico van POAG. Eerdere studies rapporteerden verschillende resultaten met betrekking tot bloeddruk en POAG. Personen met POAG met en zonder verhoogde oogdruk (hoge drukglaucoom en lage drukglaucoom) en personen met en zonder bloeddrukverlagende therapie werden apart geanalyseerd. In de groep zonder bloeddrukverlagende therapie hadden individuen met een hoge systolische bloeddruk een hoger risico op hoge drukglaucoom. Dit gold eveneens voor individuen met een groot verschil tussen systolische en diastolische bloeddruk (polsdruk) en voor individuen met een hoge vaatwandstijfheid. De diastolische bloeddruk werd apart geanalyseerd in de groep die bloeddrukverlagende medicatie gebruikten. Individuen met een lage diastolische bloeddruk hadden een verhoogd risico op hoge drukglaucoom. Dit gold ook voor individuen met een laag verschil tussen diastolische bloeddruk en oogdruk. Deze resultaten zouden verklaard kunnen worden door een verminderde doorbloeding van het oog met schade van de gezichtszenuw als gevolg.

In hoofdstuk 3 wordt de frequentie van mutaties in het myociline gen in respondenten met POAG uit de ERGO studie beschreven. Het myociline gen is betrokken bij de meeste gevallen van POAG op kinder- en jong volwassen leeftijd, en bij 3-5% van de patiënten bij wie POAG zich manifesteert op volwassen leeftijd. De normale functie van het myociline gen en het mechanisme waarop mutaties in dit gen POAG kunnen veroorzaken, zijn nog onduidelijk. Een hypothese is, dat mutaties in het myociline gen kunnen leiden tot een verhoogde afzetting ervan in het trabekelsysteem, dat zorgt voor de afvoer van het kamerwater uit het oog. Als gevolg zou de afvoer verminderd worden, de oogdruk oplopen en zo POAG kunnen veroorzaken. Het vóórkomen van mutaties in myociline bij glaucoom-patiënten is uitgebreid onderzocht in grote aantallen patiënten die verzameld werden vanuit ziekenhuizen. Het was echter nooit onderzocht bij een groep personen met POAG die verzameld zijn vanuit een bevolkingsonderzoek. Wij onderzochten 47 personen met POAG afkomstig uit het ERGO onderzoek op mutaties in dit gen. In één van hen werd een ziekte-veroorzakende mutatie gevonden, een frequentie die verwacht was op basis van de klinische studies. Bij het nakijken van de familie van deze persoon, werd de mutatie aangetroffen in twee familieleden die eveneens POAG hadden en was de mutatie afwezig bij vier gezonde familieleden. Het klinisch beeld onder degenen met de mutatie varieerde sterk, van geen gezichtsveldverlies bij een 70-jarige man tot ernstige defecten bij een 28-jarige vrouw. Dit wijst op het bestaan van andere, nog onbekende, factoren voor de manifestatie van POAG. Dit kunnen zowel genetische als omgevingsfactoren zijn. Voor de familieleden van deze familie is het nuttig te weten of zij de mutatie hebben. Als blijkt dat zij die dragen, is frequente controle gewenst. Degenen die de mutatie niet hebben lopen geen hoger risico dan een willekeurig individu met dezelfde leeftijd uit de algemene populatie.

In hoofdstuk 4 wordt de familiescore (FS) beschreven, een statistisch nieuwe methode om het genetisch risico voor een individu op een ziekte te bepalen, op basis van informatie van familieleden. Deze FS houdt niet alleen rekening met de ziektestatus van familieleden, maar ook met het totaal aantal familieleden, hun leeftijd, geslacht, en graad van verwantschap. Wij pasten deze methode toe op de POAG data uit de familiestudie van ERGO. In deze studie zijn van de 50 individuen met definitief POAG en 150 personen zonder POAG de eerstegraadsfamilieleden oogheelkundig onderzocht. FS's werden berekend en getest werd of de verdeling van FS verschilde tussen de individuen met en zonder

POAG. FS bleek positief geassocieerd met POAG, wat inhield dat het risico van POAG hoger werd naarmate de FS steeg. Deze associatie tussen FS en POAG verschilde niet tussen POAG met een verhoogde oogdruk en POAG zonder verhoogde oogdruk. De FS methode is nuttig voor het selecteren van individuen met een verhoogd genetisch risico. Voor de onderzoeker is de FS een bruikbare maat voor genetisch risico in POAG modellen met meerdere factoren.

In hoofdstuk 5 wordt beschreven hoe 11 definitieve en 15 waarschijnlijke POAG patiënten uit een genetisch isolaat werden onderzocht. Deze populatie is afkomstig van een beperkt aantal voorouders en is gedurende vele generaties geïsoleerd door vanwege culturele en religieuze redenen. Daardoor is de genetische complexiteit en daarmee het aantal genen dat mogelijk betrokken is bij POAG gereduceerd, wat een voordeel is voor genetisch onderzoek naar POAG. De structuur van een genetisch isolaat biedt ook de mogelijkheid voor het uitvoeren van alternatieve analyses voor het lokaliseren van een gen. Conventionele koppelingsanalyse gaat uit van een duidelijke manier van overerving, wat bij de meeste POAG families niet het geval is. In een genetisch isolaat moet een ander type analyse worden gebruikt, dat veel minder afhankelijk is van aannames over de precieze overerving van POAG. Een laatste voordeel van een genetisch isolaat is dat niet hoeft te worden gezocht naar grote POAG families, wat moeilijk is gezien de late leeftijd van ontstaan van de ziekte. In deze populatie kunnen POAG patiënten individueel opgespoord worden omdat de kans groot is dat zij ver verwant blijken te zijn bij genealogisch onderzoek.

In hoofdstuk 5.1 zijn de 11 definitieve POAG patiënten onderzocht op kandidaat regio's voor POAG, bekend uit de literatuur. De gebruikte test gaat ervanuit dat de meeste POAG patiënten dezelfde genetische oorzaak (mutatie in een gen) hebben, geërfd van een gemeenschappelijke voorouder, met een groter gedeelte DNA eromheen. Getest wordt of zo'n deel DNA vaker voorkomt bij POAG patiënten dan bij controlepersonen uit dezelfde populatie. Geen van de onderzochte POAG kandidaatregio's bleek significant vaker voor te komen bij patiënten dan bij controles. Dit betekent dat er een ander gen kan zijn dat verantwoordelijk is voor POAG bij deze patiënten.

In hoofdstuk 5.2 wordt een volledige genoom scan beschreven met 347 merkers verdeeld over het gehele genoom. Deze scan is uitgevoerd bij 11 definitieve en 15 waarschijnlijke POAG patiënten, 38 familieleden en 28 controle personen. Voor de analyses werd dezelfde test gebruikt als in hoofdstuk 5.1. Analyses werden gedaan

met definitieve en waarschijnlijke patiënten samen, en met alleen definitieve patiënten. Lod scores (waarschijnlijkheidsquotiënt) werden berekend voor elke merker. De hoge lod scores op chromosoom 2 and 12 wijzen erop dat het zeer waarschijnlijk is dat er genen voor POAG op deze chromosoomgedeelten liggen. Dus werden er in deze regio's extra merkers getest. Het bleek dat de meeste patiënten een identiek stuk DNA, afkomstig van een verre voorouder, gezamenlijk bezaten. Dit is een aanwijzing dat het ziekte-gen hierop zou kunnen liggen. Mogelijke kandidaatgenen voor POAG in die regio's werden bediscussieerd, zoals KCNK12, een gen voor een kalium kanaal, evenals matriline, een extracellulair matrix eiwit.

In hoofdstuk 6 worden de belangrijkste resultaten van alle studies kort besproken samen met enkele voor- en nadelen van de toegepaste benaderingen. Daarna worden aanbevelingen voor vervolgonderzoek besproken.

7.2

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About the author

Caroline Hulsman was born on December 5, 1970 in Leeuwarden. She graduated in 1989 (Gymnasium β) from the Stedelijk Gymnasium in Haarlem. From 1989 to 1990, she studied Spanish at the university of in Granada in Spain. She started her medical studies at the University of Amsterdam in 1990. During her studies, she cooperated in a research project on the efficacy of Oral Rehydration Solution therapy at the pediatric Hospital Manuel de Jesus Rivera in Managua, Nicaragua. She obtained her medical degree in November 1997, and started the studies described in this thesis at the Department of Ophthalmogenetics (head: Dr. AAB Bergen) of the Netherlands Ophthalmic Research Institute (head: Prof.dr. PTVM de Jong) in Amsterdam in collaboration with the Department of Epidemiology and Biostatistics (head: Prof.dr. A Hofman) at the Erasmus University in Rotterdam. On March 16, 2002, she will start her residency in Ophthalmology at the Department of Ophthalmology, Academic Medical Center, Amsterdam (head: Prof.dr. MD de Smet).