Determinants of glioma
An epidemiological and genetic study

M.P.W.A. Houben

Acknowledgements

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Determinants of glioma
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Een epidemiologisch en genetisch onderzoek

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Contents

This thesis is based on the following publications and manuscripts

Chapter 1.1 & 3.1

Osborne RH, Houben MPWA, Tijssen CC, Coebergh JWW, van Duijn CM. The genetic epidemiology of glioma. Neurology 2001;57:1751-1755.

Chapter 2.1

Houben MPWA, Aben KKH, Teepen JLJM, Schouten-van Meeteren AYN, Tijssen CC, van Duijn CM, Coebergh JWW. Stable incidence of childhood and adult glioma in the Netherlands, 1989-2003. Acta Oncol; *Accepted*.

Chapter 2.2

Houben MPWA, Louwman WJ, Tijssen CC, Teepen JLJM, van Duijn CM, Coebergh JWW. Hypertension as a risk factor for glioma? Evidence from a population-based study of comorbidity in glioma patients. Ann Oncol 2004;15:1256-1260.

Chapter 2.3

Houben MPWA, Coebergh JWW, Herings RMC, Casparie MK, Tijssen CC, van Duijn CM, Stricker BHCh. The association between antihypertensive drugs and glioma. *Submitted*.

Chapter 2.4

Houben MPWA, Coebergh JWW, Birch JM, Tijssen CC, van Duijn CM, McNally RJQ. Space-time clustering patterns of gliomas in the Netherlands suggest an infectious aetiology. Eur J Cancer 2005;41:2917-2923.

Chapter 2.5

Houben MPWA, Coebergh JWW, Birch JM, Tijssen CC, van Duijn CM, McNally RJQ. Space-time clustering of gliomas cannot be attributed to specific histological subgroups. *Submitted*.

Chapter 3.3

Houben MPWA, Leenstra S, Aulchenko YS, Tijssen CC, Coebergh JWW, Oostra BA, Baas F, van Duijn CM, Hulsebos TJM. Homozygosity mapping suggests a novel glioma susceptibility locus at 11p13. *Submitted*.

Chapter 3.4

Houben MPWA, Ammerlaan ACJ, Teepen JLJM, Coebergh JWW, van Duijn CM, Tijssen CC, Hulsebos TJM. Secondary meningioma in a patient with a genetic predisposition for ependymoma. *Manuscript in preparation*.

Chapter 5.1

Houben MPWA, van Duijn CM, Coebergh JWW, Tijssen CC. Gliomen: de rol van omgevingsfactoren en genetische predispositie. Ned Tijdschr Geneeskd 2005;149:2268-2272.

Chapter 1
Introduction

Chapter 1.1

General introduction and review of the literature

Pathological, clinical and epidemiological aspects of glioma

About one thousand primary tumours of the brain are diagnosed each year in the Netherlands.[1] Most of these tumours are gliomas of neuroglial origin,[2] that are among the most rapidly fatal of all human malignancies. Half of the patients is still alive after one year, and no major improvements in survival have been noted over the past decades.[3]

Glioma classification

Gliomas comprise nearly half of all primary intracranial tumours, and can be classified according to the presumed cell of origin. Neurons in the central nervous system are supported by several types of neuroglial cells. Astrocytes provide structural support for neurons and maintain electrolyte and neurotransmitter homeostasis in the brain. Oligodendrocytes produce and maintain myelin in the central nervous system, and ependymal cells form the endothelium that lines the ventricles of the brain and the central canal of the spinal cord. Astrocytes and ependymal cells also play a role in the physical and chemical integrity of the blood-brain barrier. Astrocytomas and glioblastomas are the predominant type of glioma, followed by oligodendrogliomas, mixed oligoastrocytomas and ependymomas (figure 1).

Glial tumours can also be divided into malignancy grades. Nowadays, the World Health Organisation (WHO) classification of brain tumours is widely used,[4] which is based on several pathological criteria: increased cellularity, nuclear atypia, mitosis, microvascular proliferation and necrosis. Gliomas usually show an ongoing dedifferentiation into higher-grade malignancies.

In astrocytomas, three malignancy grades are recognised according to the WHO: grade II astrocytoma, grade III anaplastic astrocytoma and grade IV glioblastoma multiforme. Another frequently used classification is that of low-grade (WHO grade II) and high-grade (WHO grade III and IV) tumours. Increased cellularity

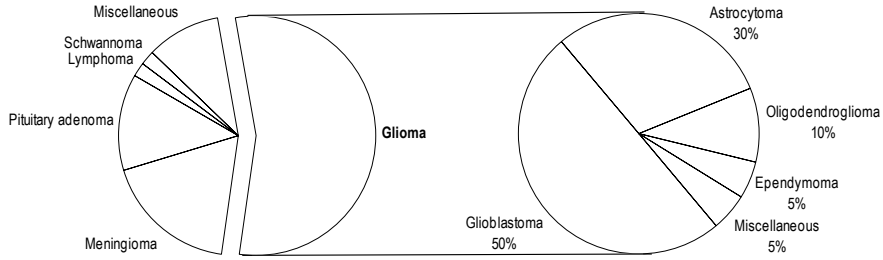

Figure 1. Diagram showing the distribution of adult primary intracranial neoplasms (left), and the distribution of histological subtypes of glioma (right) (modified from Kros, 1996).[207]

and occasionally nuclear atypia are histopathological features of astrocytomas. Anaplastic astrocytomas also show nuclear atypia and mitotic activity, and glioblastomas in addition have characteristics of necrosis and microvascular proliferation. Glioblastomas may develop by ongoing dedifferentiation of lower-grade lesions (secondary glioblastoma), but they can also arise de novo without any evidence of a less malignant precursor (primary glioblastoma).[5] There are also special types of indolent astrocytomas which are therefore graded as grade I. These pilocytic astrocytomas must be considered a separate entity since these childhood tumours are characterised by a distinct histology, location in the cerebrum, cerebellum and optic nerve, benign behaviour and an excellent prognosis.[4,6,7]

Only two malignancy grades are distinguished in oligodendroglioma, WHO grade II oligodendrogliomas and grade III anaplastic oligodendrogliomas. Also mixed oligoastrocytic gliomas exist, but the separation from pure oligodendroglial or astrocytic neoplasms is often unclear and has been inconsistent in the past.[4] Malignant progression towards glioblastoma is common (glioblastoma with oligodendrocytic component), but less frequent than in diffuse astrocytomas. The same five histopathological criteria as for astrocytic tumours apply, and significant mitotic activity, prominent microvascular proliferation and necrosis indicate progression to anaplastic oligodendroglioma.

Ependymomas are separated into WHO grade II ependymomas and grade III anaplastic ependymomas. Also myxopapillary ependymomas and subependymomas (WHO grade I) exist that generally have a favourable prognosis. Compared with astrocytic and oligodendrocytic gliomas, ependymomas have a greater tendency to metastasise to other parts of the central nervous system by flow of the cerebrospinal fluid.

The origin of glioma

Traditionally, gliomas are thought to be of neuroepithelial origin with astrocytomas arising from astrocytes or their immediate precursors, oligodendrogliomas from oligodendrocytes et cetera. Indeed, forced overexpression of oncogenes in glial cells in mice produces a variety of tumours similar to human gliomas.[8-17] However, the concept of dedifferentiation of mature glia is questionable. Adult glial cells generally have no potential for cell division which is required for developing cancer, and no significant reactive proliferation with associated neoplastic events is known to occur following appropriate stimuli. The discovery of neuroectodermal stem cells raised the possibility of a new mechanism of gliomagenesis. Many gliomas arise from the ventricular zone were a layer of mitotically competent glial progenitors is located.[18] These neuroectodermal stem cells, capable of generating both neurons and glial cells, are present throughout life and maintain proliferative and migratory potential.[19] Stem cells can actually be found in multiple regions of the adult brain including the subventricular zone, the lining of the lateral ventricles, the dentate gyrus and the subcortical white matter.[18,20-22] Glioma cells have a remarkable capability of migration,[23] and the location of a glioma does not have to correlate with the locations of the stem cells. It is also postulated that neuroectodermal cells may evolve from systemic precursor cells like bone marrow,[24] that undergo neoplastic transformation elsewhere before migrating into the brain to further develop into glial malignancies. However, the role of stem cells in glioma is probably more complex than currently acknowledged,[20,25] and the precise origin of glioma remains uncertain.

Clinical presentation and diagnosis

Brain tumours can cause either focal or generalised neurological symptoms. Focal symptoms and signs such as hemiparesis and aphasia reflect the intracranial location of the tumour whereas generalised symptoms are the result of increased intracranial pressure: headache, nausea, vomiting or visual complaints. High-grade gliomas often present with hemiparesis or mental abnormalities, whilst an epileptic seizure is the most common presenting symptom in low-grade gliomas.[26] Symptoms are usually progressive, and together with the side-effects of treatment they seriously affect cognition and quality of life.[27-29]

Gliomas infiltrate diffusely centimetres beyond the primary lesion, often also into the contralateral hemisphere, but their extent often escapes detection, even by modern neuroimaging techniques.[30] Diagnosis relies on neuroimaging (magnetic resonance imaging (MRI) with gadolinium enhancement) followed by biopsy or surgical decompression to obtain tissue for histopathological diagnosis. However, gliomas show regional heterogeneity in morphology and malignancy grade.[31-36]

The accuracy of glioma classification and grading therefore highly depends on the extent of sampling. Sampling error in biopsy can lead to an underestimation of the malignancy grade, and information from neuroimaging, clinical behaviour and histopathology must be combined into a definite diagnosis.

Prognosis

The prognosis of glioma is largely determined by the malignancy grade. Age and performance status of the patient (as measured by the Karnofsky scale and Mini Mental State Examination) are independent prognostic indicators, the extent of surgical resection is an independent prognostic factor.[37-40] Despite recent advances in diagnosis and treatment, only modest improvements in survival are evident in people under 65 years of age.[3] The median survival of patients with low-grade glioma is 5-9 years and patients usually die after progression of their disease into a high-grade tumour. Despite aggressive treatment, the median survival is 3 years for anaplastic astrocytoma and less than 1 year for glioblastoma.[41] Oligodendrogliomas and astrocytic tumours with an oligodendroglial component have a better prognosis than astrocytomas.[42] The median survival for low-grade oligodendrogliomas can be up to 10-16 years.[43]

Incidence and incidence trends

Approximately 85% of all histologically verified primary central nervous system tumours are gliomas.[2] Although gliomas are the predominant type of primary brain tumour they are still relatively rare. In the Netherlands, low-grade astrocytomas show a more or less constant incidence of 1 per 100,000 person-years, and if a peak incidence exists, it is in the third and fourth decades of life. The anaplastic astrocytoma and glioblastoma multiforme are the most common glial tumours with an incidence of 3-4 per 100,000 person-years. The incidence of high-grade glioma increases with age and peaks between 50 and 70 years (9 per 100,000 person-years). The decrease after age 70 years is probably artificial, caused by a strong increase in clinically diagnosed tumours without pathological verification at older ages.[2] Gliomas show a male predominance with a male/female ratio of 1.5-1.8,[2] which is consistently observed in different countries.[44] Compared with other cancers, little geographical variation in incidence exists. In general, incidence rates tend to be higher in Nordic countries. Other European countries and North America have a higher incidence than Africa, Asia and South America.[45] These differences can be explained, at least partly, by differences in access to medical care and in registration and classification of these tumours.

Glioma incidence has been remarkably stable over the past decades in Europe and the United States. In the late 1970s and the early 1980s, a temporal increase

in incidence has been observed, particularly in childhood tumours and in patients over 65 years. In more recent years, incidence is stabilising or slightly decreasing in almost every age group.[3,46-49] This suggests that the observed increase in incidence was artifactual, probably owing to the introduction of computed tomography (CT) and MRI in this period. Indeed, incidence trends followed utilisation trends for CT and MRI.[3,50] In the most recent years, increasing glioma incidence is particularly apparent among the elderly, which is probably the result of increasing efforts to obtain histopathological diagnosis.[46,47,51] A simultaneous decrease in clinically diagnosed tumours and in tumours 'not otherwise specified' is compatible with this view.[48,52] In addition, physicians treating elderly patients are increasingly willing to use more diagnostics in the elderly, revealing malignancies that otherwise would have gone undetected.[3,47,51] An observed increase in high-grade gliomas and simultaneous decrease in low-grade tumours is probably caused by improving techniques in neuroimaging and neurosurgery resulting in less sampling error and better characterisation of malignancy grade.[3,48,51] The excess of male patients remained stable over the past decades and tumour location showed no remarkable changes.[48] The slight increase in tumours of the cerebellum and the brain stem can also be explained by improved neuroimaging techniques.[3,48]

Some authors argue that not all of the observed increases in incidence can be explained by better detection.[53-56] There was no increase in the incidence of other malignancies that depend on imaging techniques, suggesting that the increase in incidence is independent of increased case ascertainment associated with the introduction of CT scanning.[56] Trends of increasing incidence of childhood astrocytoma were largely confined to girls, and a lack of increase for PNET/medulloblastoma and ependymoma would make the influence of diagnostic bias unlikely.[54] Also for ependymoma, an observed increase in incidence could not be explained by diagnostic practice.[52] However, good explanations for these trends cannot be offered.

In conclusion, only minor trends in incidence are observed in Europe and the United States, and almost all variation can be explained by better detection. Incidence rates have to be interpreted with care: they are sensitive to changes in diagnostic procedures, access to medical investigations and availability of health care technology. Comparison of incidence rates over time is also complicated by histopathological classifications that have changed repeatedly.[57]

Environmental risk factors for glioma

Examination of the variation in incidence between countries reveals small differences compared with other cancers, no cultural or regional patterns, and no obvious patterns of change in migrant groups after adaptation to the country of arrival in the next generation.[58] In general, incidence rates tend to be somewhat higher in highly developed countries. The variations that exist may also be related to access to medical investigations and availability of health care technology, differences in socio-economic status and the local system of registration and classification.[59,60] This suggests that environmental causes are few or ubiquitous, or that the brain is largely protected by the blood-brain barrier.

The low incidence and histological heterogeneity of glioma has frustrated epidemiological attempts to precisely define the level of risk of putative risk factors.[2,4,26,57] This has prompted international population-based collaborations;[61,62] however these studies have also led to rather inconclusive results. An overview of the many risk factors that have been considered to play a role in glioma aetiology is given in table 1, the evidence for and against putative risk factors is reviewed elsewhere.[44,58,59,63] The only established risk factor is exposure to therapeutic doses of ionising radiation,[64,65] and explains the occurrence of glioma in only a small minority of patients owing to low exposure rates. Other types of electromagnetic radiation, e.g. from mobile phones, do not appear to be a risk factor.[66-69] There are no other proven causes of glioma, nor have any major environmental or lifestyle factors been identified that are amenable to public health or lifestyle interventions.

Glioma molecular genetics

Genetics of cancer in short

Gliomas, as well as other cancers, result from a stepwise accumulation of deleterious genetic mutations leading to tumour formation and progression.[70,71] Also epigenetic mechanisms in which gene function is modulated, e.g. by aberrant DNA methylation, can lead to carcinogenic genetic alterations.[72] Some of these mutations are constitutional: heritable germline mutations that can be transmitted from one generation to the next.

Many genes involved in the malignant transformation of normal cells play a role in cell proliferation, cell differentiation or apoptosis. These genes can be classified into three different groups: proto-oncogenes, tumour suppressor genes and DNA repair genes.[73] Proto-oncogenes have a stimulating effect on

cell growth. By an activating mutation, proto-oncogenes are transformed into oncogenes that stimulate uncontrolled cell growth. Oncogenes act in a dominant manner; a mutation in one of both gene copies suffices for tumour induction. Tumour suppressor genes have an inhibitory effect on cell growth which is lost after inactivating mutations. Tumour suppressor genes are recessive; both copies of the gene have to be functionally incapacitated or lost before normal function is affected, according to Knudson's two-hit model.[74] DNA repair genes prevent tumour formation by repairing DNA damage caused by chemical or physical mutagenic agents, or mismatches that occur during DNA replication. Defects in these genes result in an accumulation of DNA mutations and an increased cancer susceptibility. DNA repair genes also act in a recessive manner.

Table 1. Putative environmental risk factors that have been considered to play a role in glioma aetiology (modified from Wrensch et al., 2002).[44] Only for therapeutic doses of ionising radiation has an aetiological role been established

- **Ionising radiation**: therapeutic, diagnostic and other sources
- **Infectious agents or immunologic response**: viruses (common colds, influenza, varicella zoster virus, BK virus, JC virus, SV40, others), tuberculosis, Toxoplasma gondii
- **Allergies**
- **Head trauma**
- **Epilepsy, seizures or convulsions**
- **Drugs and medications**
- **Diet and vitamins**: nitrosamine/nitrosamide/nitrate/nitrite-consumption, calcium, food frequency, fruits and vegetables, cured foods
- **Alcohol**
- **Tobacco smoke exposures**
- **Hair dyes and sprays**
- **Traffic-related air pollution**
- **Occupations and industries**: synthetic rubber manufacturing, petroleum refining/production work, licensed pesticide applicators, agricultural work, fire-fighters, electrical workers, scientists and biomedical/health professionals, others
- **Chemicals**: lead, polycyclic aromatic hydrocarbons, formaldehyde, vinyl chloride, arsenic, mercury, others
- **Parental workplace exposures**
- **Sociodemographic indicators of affluence and education**
- **Reproductive and hormonal factors**
- **Electromagnetic radiation**: cellular telephones, other radiofrequency exposures, power frequency electromagnetic fields, others

Molecular genetic mechanisms in the pathogenesis of glioma

Most molecular genetic investigations of sporadic (non-familial and non-syndromic) glioma have focused on somatic mutations, genetic alterations in tumour material such as loss of function of tumour suppressor genes or overexpression of proto-

oncogenes. Studies suggest that several genes are involved, and that a cascade of genetic events takes place prior to clinical manifestation of a tumour.

Detailed descriptions of the molecular genetics of glioma can be found elsewhere.[4,5,75-80] In short: several common genetic alterations at the chromosomal level have been identified in tumour material obtained from sporadic astrocytic gliomas: loss of *17p* (including *p53*), *9p*, *10*, *11p*, *13q*, *19q*, *22q* (suggesting the presence of tumour suppressor genes), and amplification of *7* and *12q* (suggesting the presence of proto-oncogenes). These alterations lead to changes in the expression of several genes: phosphatase and tensin homolog (*PTEN*), deleted-in-colon carcinoma (*DCC*), epidermal growth factor receptor (*EGFR*), platelet-derived growth factor receptor (*PDGFR*), mouse double minute 2 (*MDM2*), glioma-associated oncogene homolog (*GLI*), cyclic AMP-dependent kinase number 2 A/B (*CDKN2A/B*), cyclin-dependent kinase 4 and 6 (*CDK4/6*) et cetera. Two possible molecular genetic pathways have been proposed that link clinical observations with molecular genetic evidence (figure 2). It is thought that a *p53* mutation plus a platelet derived growth factor (*PDGF*) mutation, *PDGFR* overexpression and loss of a gene in *22p* are responsible for the formation of astrocytoma. From there, the mutation of retinoblastoma (*Rb1*) gene on *13q* and loss of tumour suppressor genes on *9p* and *19q* would lead to anaplastic astrocytoma. Further aberrations in chromosome 10 (loss of tumour suppressor genes on *10p* and *10q*), in addition to amplification of *PDGFR*, are responsible for the transformation of anaplastic astrocytoma to glioblastoma.[81,82]

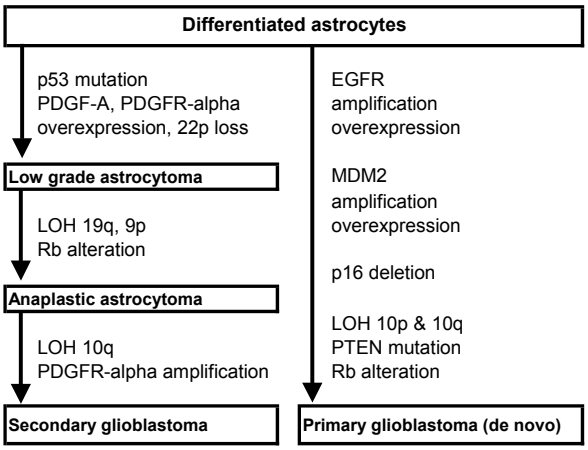

Figure 2. Summary of major genetic alterations observed at stages of glioblastoma development (modified from Kleihues and Ohgaki, 1999).[208] *PDGFA*: platelet-derived growth factor A; *PDGFR*-alpha: platelet-derived growth factor receptor-alpha; *EGFR*: epidermal growth factor receptor; LOH: loss of heterozygosity; *Rb*: retinoblastoma; *MDM2*: mouse double minute 2; *PTEN*: phosphatase and tensin homologue.

These hypothetical steps in the development of secondary glioblastoma have been proposed after observing accumulated genetic defects through increasing grades of tumours, particularly where a resected low-grade glioma was followed by a later diagnosis of a more malignant tumour. Nonetheless, many sporadic high-grade gliomas are diagnosed with no constructible genetic history and have therefore been termed 'primary glioblastoma'. A number of genetic aberrations appear to be common to these gliomas:[83] amplification or overexpression of *EGFR* and *MDM2*, *p16* deletion, loss of chromosome 10, and mutations in *PTEN* and *Rb1* are all frequently observed within primary glioblastomas (figure 2). However, the sequence of these defects has not been established, nor that a sequence is important.

The situation is different for oligodendroglioma. Frequent genetic alterations in oligodendrogliomas are losses of chromosomes 1p and 19q,[77,84,85] increased expression of *EGFR* and deletion of *p16*. In the more malignant anaplastic oligodendrogliomas, also gain of chromosome 7 and loss of *10q* can be found.[86] Unlike the profile of astrocytic tumours, mutations of *PTEN* and *p53* are uncommon in oligodendrogliomas. Similar to astrocytic gliomas, an accumulation of genetic defects leading to progressive stages of dedifferentiation can be recognised.

The genetic profile of ependymomas is clearly different from astrocytomas and oligodendrogliomas.[84,87-89] Losses of *6q* and *22q* are the most frequent events in these tumours.

The many molecular and genetic changes found in gliomas however do not explain why a healthy glial cell initially transforms into a neoplastic one. Although the systematic study of proto-oncogenes and tumour suppressor genes in tumour material is promising for the discovery of causative genetic lesions, the results so far have been somewhat disappointing. In studies comparing tumour material with constitutional DNA, it is difficult to ascertain what the causative lesion(s) is (are), and which lesions are the result of the chaotic cellular processes that occur within neoplastic cells.

Familial cancer syndromes and diseases associated with glioma

Familial cancer syndromes

Clearly defined familial tumour syndromes exist in which a single gene defect is transmitted from generation to generation. In some of these syndromes, carriers have an increased risk of glioma together with a variety of other cancers.[90,91] These hereditary syndromes can explain approximately 1-5% of all glioma cases.[92-94] Most genes so far associated with predisposition to central nervous system

tumours appear to act as tumour suppressor genes, displaying an autosomal dominant mode of inheritance.

The syndromes that predispose to glioma include neurofibromatosis type 1 and 2,[95-99] Li-Fraumeni syndrome,[100-102] tuberous sclerosis,[103,104] Turcot syndrome,[105-107] Gorlin syndrome,[108-111] and melanoma-astrocytoma syndrome.[112-117] The clinical manifestations and the genes responsible for these conditions are summarised in table 2. Since these syndromes display a clear pattern of cancer in families over multiple generations, they have attracted attention of clinicians and researchers. The syndromes were easily recognised as monogenetic diseases with a mendelian pattern of inheritance, and genetic research resulted in the elucidation of the gene defects underlying these syndromes, and a well described phenotype. Although familial tumour syndromes are rare, knowledge of the causative genetic defects can provide insights in the pathogenesis of sporadic gliomas. Genes predisposing to familial tumours may also be involved in their sporadic counterparts, as demonstrated by the Li-Fraumeni syndrome: mutations in the *TP53* gene found in this syndrome are also recognised as an early genetic aberration in sporadic low-grade astrocytomas.[77,101,118]

Several genes have been examined in families with indications of a mendelian pattern of inheritance, but without signs of one of the known tumour syndromes. The most frequently examined is the tumour suppressor gene *TP53* which plays an important role in controlling the cell cycle. In a Swiss family with four brain tumours in two generations, a germline deletion in the *TP53* gene was the underlying cause in all affected members.[119] This specific mutation had not been previously described and was the only evidence of a specific causal factor for glioma. Other studies report inconsistent findings of searches for mutations in *TP53*.[120-125] The same is true for mutations in other genes such as *PTEN, p14, p15*, *p16* and *CDK4*,[125-129] suggesting that germline mutations in these genes account for no more than a small subset of familial glioma cases.

Cancer and other diseases associated with glioma

Many different cancers have been reported to occur in higher frequencies in glioma patients or their relatives than expected from population-based incidence rates. Malmer et al. observed a modest increased risk of melanoma among first-degree relatives of high-grade glioma patients (standardised incidence ratio (SIR) 1.37; 95% confidence interval (CI) 1.09-1.71).[130] Associations between astrocytoma and melanoma were also reported in other studies, as well as associations between glioma and cancers of endometrium, rectum, thyroid gland and prostate.[131-133] Conversely, persons with a first-degree relative with stomach, colon or prostate cancer or Hodgkin's disease had 1.4-3.4-fold increased risks of

Table 2. Hereditary tumour syndromes with gliomas (modified from Kleihues and Cavanee, 2000) [4]

Syndrome	Locus	Gene	Nervous system tumours	Other
Neurofibromatosis 1	17q11.2	*NF1*	Neurofibroma, optic glioma, neurofibrosarcoma, astrocytoma, plexiform neurofibroma, meningioma	Café-au-lait spots, axillary freckling, iris hamartomas (Lisch noduli), osseous lesions, leukaemia, phaeochromocytoma, mental retardation
Neurofibromatosis 2	22q12.2	*NF2*	Vestibular and peripheral schwannoma, meningioma, spinal ependymoma, astrocytoma	Retinal hamartoma, posterior lens opacities
Li-Fraumeni	17p13.1 22q12.1	*TP53* *CHK2*	Astrocytoma, glioblastoma multiforme, primitive neuroectodermal tumours	Breast carcinoma, bone- and soft tissue sarcoma, adrenocortical carcinoma, leukaemia
Tuberous sclerosis	9q34 16p13.3	*TSC1* *TSC2*	Subependymal noduli, subependymal giant cell astrocytoma, ependymoma, cortical tubers	Cutaneous angiofibroma, peau chagrin, subungual fibromas, cardiac rhabdomyomas, lymphangioleiomyomatosis, renal angiomyolipoma, polyps of small intestine, cysts of lung and kidney, epilepsy, mental retardation
Turcot	5q21-22 3p21.3-23 7p22.2	*APC* *MLH1* *PMS2*	Medulloblastoma, astrocytoma, ependymoma, glioblastoma multiforme	Café-au-lait spots, colorectal polyps, ovarian carcinoma
Gorlin	9q22.3	*PTCH*	Medulloblastoma, meningioma, astrocytoma	Basal cell carcinomas, palmar and plantar pits, ovarian fibromas, skeletal abnormalities
Retinoblastoma	13q14.2	*RB1*	Retinoblastoma, glioma, pineoblastoma	Osteosarcoma
Melanoma-astrocytoma	9p21.3	*CDKN2A*	Astrocytoma, meningioma, schwannoma	Melanoma

glioma.[134] Associations found in other studies could not be confirmed.[134]

In some studies, no increased incidence of other cancers could be shown,[93,135-137] and a decreased risk of breast cancer and colon cancer could not be confirmed in a subsequent study by the same authors.[138,139] Paunu et al. reported that the overall cancer risk is significantly decreased (SIR 0.6; 95% CI 0.4-0.9) in families with juvenile onset gliomas (under 20 years of age, half of them pilocytic astrocytomas).[133] On the other hand, in families with adult onset glioma, the overall cancer risk was equal to that of the reference population, whereas the risk of skin melanoma and meningioma was significantly increased (SIR 4.0; 95% CI 1.5-8.8 and SIR 5.5; 95% CI 1.1-16 respectively).[133]

Aggregation of multiple cancers in glioma patients has also been observed. It has been suggested that in brain cancer patients, carcinogenic side-effects of the treatment is the major factor underlying positive associations with primary cancers of other sites, with a lesser contribution from genetic susceptibility and increased medical surveillance.[140] Altogether, studies reporting on risks of cancers other than glioma are not consistent. It remains unclear whether an association exists, and for which specific types of cancer.

Other diseases than cancer have been suspected to be associated with glioma, but again, most of these associations are not well established. Gliomas possibly occur less frequently in diabetic patients,[61,141-144] in patients with a history of varicella zoster virus infection,[145] and in patients with self-reported allergic conditions, asthma or autoimmune disease in both case-control and cohort study designs.[61,141,146-149] A significant inverse dose-response relationship between glioma risk and the number of allergens,[146] and the absence of an association between allergies and meningiomas and acoustic neuromas,[141] further suggests an influence of immunological factors on the development of gliomas. More evidence for an association between allergic conditions and glioblastoma comes from a study investigating genetic polymorphisms associated with asthma, that are inversely associated with glioblastoma multiforme.[149]

Seizures have been frequently linked to an increased risk of glioma.[93,142,144,147] The risk, however, decreases with time since diagnosis of epilepsy and with total duration of medication use. It is therefore more likely that seizures are the early consequence of glioma, not the cause.[144,150-152] A history of meningitis and viral encephalitis was also positively associated with glioma, but only in the 2-3 years before glioma diagnosis.[61,142,144] This suggests that patients with a glioma probably have a higher risk of developing meningitis and encephalitis. Associations with glioma were also suggested for the rare metabolic encephalopathy L-2-hydroxyglutaric aciduria,[153,154] Fanconi's anaemia,[155] and cerebral palsy.[156] No or inconsistent associations were found for a variety of other medical conditions

including stroke,[93,142] heart disease, psychiatric disease, thyroid disease,[93] and multiple sclerosis.[157-162]

Aggregation of gliomas in families without established cancer syndromes

Glioma families

Familial aggregation has been described for almost all human cancers, and it is estimated that first-degree family members have a two- or threefold increased risk of developing the same tumours.[163] Occasionally, familial clustering of two or more gliomas occurs without the clinical signs of a well-recognised hereditary tumour syndrome, and without a clear mendelian pattern of inheritance. In most patients with 'familial glioma' no known germline mutations can be found. In the vast majority of gliomas, no other family members are affected.

Information on the familial aggregation of glioma is sketchy. Some remarkable families have been recorded at the Johns Hopkins National Familial Brain Tumor Registry where 72 families with 154 affected individuals have been studied.[164] Other earlier reports include a study of 19 families with 45 affected relatives,[165] a study describing three families identified in a chemotherapy trial where each case had one affected first-degree relative,[166] and two studies of multiple families in which more than one member had glioma.[167,168] Other reports include families in which there were three or four cases of glioma within one generation,[121,169-171] or several affected members over two or three generations.[172,173] The overall familial pattern is somewhat atypical for hereditary cancers: cases did not always occur over multiple generations, early onset was not apparent and in cases with parent-child pairs, the child was often diagnosed before the parent.[164] Glioma families remain infrequent and certainly do not have the high 'cancer density' seen in families with dominant forms of breast and colorectal cancer.

Glioma risk in relatives of patients

Many authors have investigated the risk of glioma for relatives of glioma patients. In three studies, patients and control subjects had a similar proportion of first-degree relatives with adult glioma.[134,137,143] In a study from Iceland, comparing incidence in relatives of patients with incidence rates of the total population, no increased risk of central nervous system tumours was found either.[136] However, Wrensch et al. found a higher proportion of relatives with glioma when the sample was restricted to verified cases only (odds ratio (OR) 2.3; 95% CI 1.0-5.8).[93] In a Swedish population-based study in which all cases of astrocytoma occurring between 1985 and 1993 (n=432) were identified, a threefold increased incidence

of astrocytoma was seen among the 1,890 first-degree relatives (SIR 3.12; 95% CI 1.42-5.92). Most of this risk was restricted to the younger cohort (OR 4.71; 95% CI 1.52-10.99) suggesting that inherited factors may contribute to the occurrence of astrocytoma.[139] The same authors also compared first-degree relatives with spouses of glioma patients.[130] No increased risk was found for spouses, but the relatives had a two- to fourfold increased risk of glioma. Furthermore, first-degree relatives of glioma patients were divided into two cohorts of high-grade glioma and low-grade glioma probands.[174] The risk of low-grade glioma among relatives of low-grade glioma patients was increased (SIR 3.65; 95% CI 2.31-5.47), and was higher in siblings (SIR 7.00; 95% CI 3.35-12.87), especially in siblings under 40 years of age (SIR 9.01; 95% CI 4.31-16.57). The risk of high-grade glioma was generally twofold increased in both the low-grade and high-grade cohorts. The authors concluded that low-grade glioma families apparently have features manifesting a distinct pedigree pattern with sibpairs affected at a young age.[174]

Although estimates are inconsistent, it seems that the incidence of glioma in first-degree relatives of patients with the disease is elevated two- to ninefold, and that this increased risk is mostly attributable to low-grade astrocytoma.[130,132,139,174,175] Familial aggregation of glioma suggests that yet unknown inheritable genetic factors may be involved in the susceptibility to glioma. It is also possible that (part of) the observed familial aggregation is caused by exposure to shared environmental risk factors,[137,164] although this idea was rejected by others.[130] Spouses of patients do not appear to have an increased risk although they share the same environment.[130]

Segregation analyses

Segregation analysis aims to determine the transmission pattern of a disease in families, by calculating the likelihood of an observed distribution of disease given several specific modes of inheritance. A study by Malmer et al., based on first-degree relatives of adult glioma patients, suggested that familial glioma occurs in about 5% of all glioma cases and that 1% may have an autosomal dominant mode of inheritance with reduced penetrance.[135] In segregation analysis, an autosomal recessive gene model provided the best fit, which could possibly explain 2% of all glioma cases. A multifactorial model was not clearly rejected.[135] De Andrade et al. included both first-degree and selected second-degree relatives of glioma probands and analysed for the segregation of cancer overall. A multifactorial mendelian model provided the best explanation of cancer occurrence, and it was suggested that brain tumours are probably the result of a multigenic action with involvement of unknown environmental exposures.[176] A model postulating a purely environmental cause of brain cancer was rejected.[176] Bondy et al. carried

out a segregation analysis on families with various childhood brain tumours which also favoured a multifactorial model.[92] In a multifactorial model, glioma can be considered the result of environmental risk factors operating on a genetic background. The genetically determined susceptibility to environmental risk factors is often the result of, for example, common genetic polymorphisms.

Genetic polymorphisms and risk of glioma

Polymorphisms are genes with more than one variant (multiple alleles) prevalent in the population, that affect the function and efficiency of the gene products. These genes are involved in processes like the detoxification of carcinogens, DNA stability and repair, the oxidative metabolism or immune response. Each polymorphism has a (usually small) contribution to glioma risk and adds to the total genetic susceptibility.

The best studied polymorphisms in glioma are those in the carcinogen-metabolising enzymes glutathione *S*-transferase (*GST*). Four main families of *GST* enzymes have been identified: α (*GSTA*), μ (*GSTM*), π (*GSTP*) and θ (*GSTT*).[177] The genes encoding *GSTM1*, *GSTT1* and *GSTP1* are polymorphic (*GSTM1* wildtype/null, *GSTT1* wildtype/null, *GSTP1* Ile105Val and *GSTP1* Ala114Val).[178] An overview of eight studies involving *GST* polymorphisms is given in table 3.[179-186] Three studies are population-based and were conducted within the San Francisco Bay Area Adult Glioma Study,[179,180,185] the others are hospital-based.[181-184,186]

The *GSTT1* null genotype was significantly associated with high-grade astrocytoma and astrocytoma overall,[183] although others found an association with oligodendroglioma only,[185] or no associations.[179,181,182,184,186] The finding from Kelsey et al. that oligodendroglioma was associated with *GSTT1* null genotype,[185] could not be replicated in two consecutive studies from the same research group (partly studying the same cases as in the first study) due to changes in histopathological classification of glioma.[179,180] In seven studies, no associations were found either for the *GSTM1* null genotype,[179-184,186] although *GSTM1* deletion may be associated with earlier age at onset among women.[180] For the *GSTP1* Ile105Val polymorphism, significantly more functional Ile alleles were found in patients with adult astrocytic glioma compared with the general population.[182] In a population-based study carried out in the San Francisco Bay area, a reduced risk was found for patients over 60 years of age with 105Val/Val genotype only.[179] In a third study however, the 105Val/Val genotype was associated with increased glioma incidence, with a dose-effect relation between the number of variant Val alleles and glioma risk.[181] Another hospital-based study with only 31 glioma patients failed to find any association.[186] Finally, the Ala114Val variant of *GSTP1*

Table 3. Overview of case-control studies for polymorphisms in glutathione *S*-transferase (*GST*) enzymes and risk of adult glioma

			Glutathione *S*-transferase polymorphism			
	Ascertainment of patients	**Number of cases/ controls**	**GSTT1 Wt/null**	**GSTM1 Wt/null**	**GSTP1 Ile105Val**	**GSTP1 Ala114Val**
Elexpuru-Camiruaga, 1995 [183]	Hospital-based	112/577	Null: total astrocytoma vs co, OR 2.09; 95% CI 1.28-3.39 Null: HG astrocytoma vs co, OR 2.36; 95% CI 1.41-3.94	No association		
Kelsey, 1997 [185]	Population-based [a]	160/159 [b]	Null: oligodendroglioma vs co, OR 3.2; 95% CI 1.1-9.2			
Wiencke, 1997 [180]	Population-based [a]	158/157 [b]		No association		
Trizna, 1998 [184]	Hospital-based	90/90	No association	No association		
Ezer, 2002 [182]	Hospital-based	141/- [c]	Not significantly different [c]	Not significantly different [c]	Excess Ile alleles in all adult glioma combined	Not significantly different [c]
De Roos, 2003 [181]	Hospital-based	422/604	No association	No association	Val/Val: glioma vs co, OR 1.8; 95% CI 1.2-2.7	No association
Wrensch, 2004 [179]	Population-based [a]	367/428 [b]	No association	No association	Val/Val: glioma >60y vs co, OR 0.38; 95% CI 0.15-0.93	No association
Pinarbasi, 2005 [186]	Hospital-based	31/153 [d]	No association	No association	No association	

Wt: wildtype; HG: high-grade; OR: odds ratio; CI: confidence interval; co: control; y: years.
[a] San Francisco Bay Area Adult Glioma Study. [b] Cases from the studies of Kelsey et al. and Wiencke et al. were also included in the study of Wrensch et al. In total, there were 367 unique cases. [c] Allele frequencies in glioma patients were compared with frequencies in different populations. [d] Turkish population.

was not associated with adult glioma.[179,181,182] Only one hospital-based study examined paediatric glioma and showed a significant increase in the frequency of functional *GSTM1* alleles in high-grade paediatric astrocytomas ($p<0.002$), a significant increase in the frequency of *GSTP1* 114Val/Val genotype in paediatric astrocytomas ($p<0.002$), but no significant differences for *GSTT1* or *GSTP1* Ile105Val variants.[182] In conclusion, there are no overall main effects of these *GST* polymorphisms on glioma risk. A recent meta-analysis found no association between any of the *GST* variants and the risk of glioma or histopathological subgroups.[187] Results of all the studies published thus far include too few subjects to reach definitive conclusions on the association between *GST* variants and glioma subgroups.

CYP genes are also involved in carcinogen metabolism and detoxification. Poor metabolising variants of *CYP2D6* were associated with both low- and high-grade glioma when corrected for other variables (OR 4.17; 95% CI 1.57-11.09),[183] although this association could not be confirmed in a population-based case-control study.[185] Risk of glioma was neither significantly associated with *CYP2E1* Rsa1 and Ins96 variant genotypes,[181] nor with *CYP1A1* Val/Val genotype.[184] Probably no single polymorphism in a metabolising enzyme contributes sufficiently to the risk of developing glioma, but the interaction between different enzymes may play a crucial role. Supermultiplicativity was already shown for the joint effect of *GSTP1* Ile105Val and *CYP2E1* Rsa1 variants.[181]

Rare alleles of the *HRAS* proto-oncogene were already known to increase the risk of a variety of malignancies including leukaemia and carcinomas of the lung, testes, breast, colorectum and urinary bladder.[188] Likewise, the risk of glioma was elevated for carriers of at least one rare *HRAS* allele (OR 2.72; 95% CI 1.17-6.32).[189] In an earlier study by Diedrich et al., patients with glioma were shown to have a higher incidence (10.5%) of rare *HRAS* alleles than controls (3%).[190] A population-based case-control study however was unable to confirm an excess of rare *HRAS* alleles among adult glioma patients.[191]

Capability of DNA repair is related to cellular sensitivity to radiation and cancer. A measurable DNA repair defect in lymphocytes of glioma patients was found, suggesting the importance of constitutional DNA repair defects in the formation of gliomas.[192,193] In a population-based study, homozygosity for a polymorphism in *ERCC1* (A8092C), a subunit of the nucleotide excision repair complex, was significantly associated with oligoastrocytoma (OR 4.6; 95% CI 1.6-13.2), but not with other histologies or all gliomas combined.[194] Glioma patients were also more likely to be homozygous for the AA variant in codon 156 of the *ERCC2* gene (OR 2.3; 95% CI 1.3-4.2).[195] This association was strongest (OR 3.2) for oligoastrocytoma. Since this variant is a silent polymorphism, another

gene linked to *ERCC2* might be involved. In a study investigating six single nucleotide polymorphisms (SNPs) on the long arm of chromosome 19, two SNPs in *ERCC2* and *GLTSCR1* were associated with oligodendroglioma risk.[196] Wang et al. investigated polymorphisms in several other DNA repair genes and found an increased risk of glioma for the *XRCC7* G6721T variant only.[197] The combined T variant genotype was associated with a 1.82-fold increased risk (95% CI 1.13-2.93). An overview of the role of these and other polymorphisms in DNA repair genes and the risk of various cancers can be found elsewhere.[198]

As explained previously, a reduced risk of glioma is observed among people reporting asthma and allergic conditions. Schwartzbaum et al. have therefore conducted a case-control study to determine whether polymorphisms associated with asthma are inversely related to glioblastoma multiforme risk.[149] Polymorphisms in the genes interleukin-4RA and interleukin-13 were associated with glioblastoma in the opposite direction of a corresponding polymorphism-asthma association. It remains to be determined whether allergic conditions per se or their related cytokines affect glioblastoma risk.

Other polymorphisms that have been studied include variants in carcinogen metabolising enzymes NAD(P)H dehydrogenase quinone 1 (*NQO1*) and N-acetyltransferase (*NAT*).[184,199] Both were not significantly associated with adult glioma risk. A positive association between PPARγ sequence variants and glioblastoma was found, but only for American and not for German patients, suggesting that another locus in linkage disequilibrium with PPARγ might be responsible for glioma risk.[200] The C3435T polymorphism in the multidrug resistance-1 (MDR1) gene is possibly associated with glioblastoma among men only.[201] An Arg/Pro polymorphism in codon 72 of the TP53 gene is probably associated with susceptibility to brain tumours, particularly high-grade astrocytomas, with the Arg/Pro heterozygous genotype being the risk genotype.[202] In a group of 43 gliomas, one rare c-mos allele was present compared with none in 50 controls.[190]

Overall, many genetic polymorphisms have been studied, but for most of them the role in glioma risk remains vague and controversial. Many studies have shortcomings: combined analyses for histological subtypes of glioma, insufficient power, no correction for multiple testing,[203] inappropriate statistical analyses and other flaws in study design. These and other problems lead to false-positive associations that cannot be replicated in other studies.[204]

Linkage studies

In linkage analysis, the cosegregation of genetic markers with disease is studied to determine the location of disease-causing mutations on the genome. To date,

two linkage studies were published. Paunu et al. performed a genome-wide linkage analysis in four families from a limited geographical area in Finland, followed by association analyses (haplotype pattern mining) and the transmission disequilibrium test (TDT) in fifteen families.[205] A novel low-penetrance locus for familial glioma at 15q23-q26.3 was suggested. This large area of 40 cM contains several potential candidate genes, but none of them has been previously associated with hereditary brain tumours.

Malmer et al. performed non-parametric linkage analysis and homozygosity mapping in three distantly related glioma families in Sweden.[206] Previous segregation analysis of these families supported an autosomal recessive gene.[135] Homozygosity mapping with 811 markers did not reveal any allele homozygous in five affected persons from these related families. Non-parametric linkage analysis showed a maximum allele-sharing LOD-score of 1.05 at chromosome 1q21-q25, consistent with a low-penetrant dominant gene.

Conclusion

When evaluating all available evidence about risk factors for glioma, the conclusions are disappointing. Little is certain about glioma aetiology. Exposure to ionising radiation is the only established environmental risk factor, and a few monogenetic tumour syndromes can explain only <5% of all gliomas. The evidence for many other proposed risk factors is inconclusive. Although glioma aetiology is believed to be multifactorial, the precise factors are unknown. Continuing effort is needed to gain progress in this complex field of research.

Chapter 1.2

Objective and outline of the thesis

The aetiology of glioma remains puzzling. Despite all efforts from scientists in diverse fields of research, our understanding of this complex disease is still limited. The objective of the research presented in this thesis is twofold: in the first part, the aim is to obtain additional insights in the aetiology of, and risk factors for glioma. By the use of classical and novel methods, hypotheses are formulated about mechanisms that possibly play a role in the development of glioma. The second part of this thesis aims at the genetic basis of the disease by studying both families and more distantly related patients from genetically isolated communities.

In **chapter 2.1**, we evaluate trends in the incidence of glioma and the possible explanations for changes in the incidence since 1989. Comorbidity in glioma patients is described and compared with other cancer patients in **chapter 2.2**. We also formulate an aetiological hypothesis about an association between glioma and hypertension. This association is further explored in **chapter 2.3**, where we examine the effect of antihypertensive medication use on the risk of glioma. In **chapter 2.4** and **chapter 2.5**, we study the putative role of infections in glioma aetiology, by investigating the geographical and temporal clustering of glioma patients.

Chapter 3.1 describes the difficulties in genetic epidemiological research of glioma, and shows the results of a pilot study for a new genetic epidemiological research design that can be useful in genetically isolated populations. This chapter is followed by a report of the systematic collection and genealogical research of glioma patients for genetic epidemiological studies, together with recommendations for the future (**chapter 3.2**). In **chapter 3.3**, we report on a study of seven distantly related patients from a genetically isolated population in the Netherlands. We analyse the results of a genome-wide search using homozygosity mapping and present novel loci for familial glioma. **Chapter 3.4** focuses on a family in which two brothers each had two sons diagnosed with an anaplastic ependymoma. The possible mechanism underlying a new meningioma in one of the patients, twenty years after radiotherapy, is discussed here.

Finally, in **chapter 4**, we summarise our findings and discuss some methodological issues of the studies. This chapter ends with suggestions for future research.

Chapter 2

Environmental risk factors

Chapter 2.1

Stable incidence of childhood and adult glioma in the Netherlands, 1989-2003

Summary

Time trends in the incidence of glioma may reflect changes in the prevalence of environmental risk factors for glioma. We therefore investigated trends in the incidence of childhood and adult glioma in the Netherlands from 1989 to 2003. We used population-based incidence data from the Netherlands Cancer Registry. We calculated European standardised incidence rates for glioma, and stratified for age, gender and glioma subgroups. Changes in the incidence were estimated by calculating the Estimated Annual Percentage Change. We compared these with trends in Europe and the United States. Similar to other countries, the overall incidence of glioma was fairly stable in the Netherlands during the period 1989 to 2003, for both children and adults. In adult astrocytic glioma, a significantly increasing incidence of high-grade astrocytoma was balanced by simultaneous decreases of low-grade astrocytoma, astrocytoma with unknown malignancy grade and glioma of uncertain histology. Most time trends can be explained by improving detection and diagnostic precision. Stable incidence rates of adult and childhood glioma suggest that no major changes in environmental risk factors have occurred, which influenced the incidence of glioma in the studied period.

Introduction

Approximately 85% of all histologically verified primary cancers of the central nervous system (CNS) are gliomas,[2] malignant brain tumours of neuroepithelial origin.[4] Although gliomas are the most common type of primary brain tumours they are still relatively rare. In the Netherlands, world-standardised incidence rates of glioma are 6.5 per 100,000 person-years for males and 4.4 for females with a male/female ratio of 1.5-1.8.[2] The male predominance, which is consistently observed in different countries, remains yet unexplained.[44] Several authors

reported that the incidence of glioma is stable or slightly decreasing in almost every age group since the late 1980s.[3,46-48] In the most recent years, an increase in incidence is particularly apparent among the elderly in western countries,[3,46-48] which is probably the result of better detection.

Many environmental risk factors for glioma have been studied. Only for ionising radiation has an aetiological role been established,[64,65] and no other major risk factors have been identified.[44] Temporal trends can therefore not be explained on the basis of known environmental risk factors. However, the monitoring of time trends and the early detection of changing patterns in the incidence of glioma may reveal changes in the prevalence of environmental risk factors and provide new hypotheses for glioma aetiology. For example, attempts have been made to link trends in the incidence of glioma to the increasing use of mobile phones.[48]

In this study we investigated trends in the incidence of childhood and adult glioma and of glioma subgroups in the Netherlands, in the period 1989 to 2003. We compared these with the observed trends in Europe and the United States.

Methods

All epidemiological data were obtained from the Netherlands Cancer Registry (NCR) from the Association of Comprehensive Cancer Centres (ACCC). This population-based nationwide cancer registry records data of all malignant neoplasms. Information is available on date of incidence, histology, topography, invasiveness, grade, stage and basis of diagnosis.[209] Histology is coded according to the International Classification of Diseases for Oncology (ICD-O), first edition (until 1992), second edition (1993-2000) and third edition (since 2001).[210-212] Pathological diagnoses were derived from the nationwide network and registry of histo- and cytopathology (PALGA) containing data of all histological, cytological and autopsy examinations in the Netherlands. Diagnoses were also derived from the Dutch Medical Register (LMR) that comprises data from all hospital admissions in the Netherlands, including discharge diagnoses and performed medical procedures. Since 1989, all hospitals in the Netherlands are linked to one of the regional cancer registries that submit their data to the NCR. During the study period there were no major changes in health care facilities in the Netherlands, nor major improvements in diagnostic methods. Neurosurgery, neuroradiology and neuropathology are concentrated in large centres with close links to radiotherapy departments. Computed tomography (CT), magnetic resonance imaging (MRI) and stereotactic biopsies were introduced and already widely available before 1989.[2]

Table 1. International Classification of Diseases for Oncology (ICD-O) codes for the analysed glioma groups

Main group	Subgroup	ICD-O morphology code		ICD-O grading
Astrocytic glioma	Low-grade	9400, 9410, 9411, 9420	Astrocytoma	1, 2
		9421	Pilocytic astrocytoma	1, 2, 9
		9424	Pleomorphic xanthoastrocytoma	1, 2
	High-grade	9400, 9410, 9411, 9420	Astrocytoma	3, 4
		9401	Anaplastic astrocytoma	3, 4
		9424	Pleomorphic xanthoastrocytoma	
		9440, 9441, 9442, 9481	Glioblastoma	
	Grade unknown	9400, 9410, 9411, 9420	Astrocytoma	9
		9424	Pleomorphic xanthoastrocytoma	9
Oligodendroglial/ mixed glioma		9382	Mixed glioma (oligoastrocytoma)	1-4, 9
		9450	Oligodendroglioma	1-4, 9
		9451	Anaplastic oligodendroglioma	
Ependymal glioma		9391	Ependymoma	1-4, 9
		9392	Anaplastic ependymoma	
Uncertain histology		-	No microscopic verification	
		9380	Glioma, not specified	1-4, 9

Six histological groups were defined for analysis following a cluster scheme for CNS tumours.[57] Astrocytic gliomas were divided in low-grade tumours (ICD-O grade 1, 2), high-grade tumours (ICD-O grade 3, 4), and tumours with unspecified malignancy grade (ICD-O grade 9). Oligodendrogliomas and ependymal gliomas were analysed without considering the malignancy grade, as numbers in these groups were considered too small for subgroup analysis. A sixth group (glioma with uncertain histology) consisted of clinically diagnosed CNS tumours without histopathological confirmation. We also classified 'glioma not otherwise specified' (glioma NOS) into this group. ICD-O codes for the six groups are given in table 1. Tumours with codes 9383 (subependymal glioma), 9384 (subependymal giant cell astrocytoma), 9393 (papillary ependymoma) and 9394 (myxopapillary ependymoma) were only registered since 1999 and were therefore excluded from the analyses.

Annual incidence rates were calculated per 100,000 person-years, using the average annual population as obtained from Statistics Netherlands. Rates were age-adjusted by standardisation to the European standard population (European Standardised Rates, ESR) and calculated as 3-year moving averages. Trends were estimated by calculating the Estimated Annual Percentage Change (EAPC). A regression line was fitted to the natural logarithm of the rates using calendar

year as a regressor variable, i.e. y=mx+b where y=ln(rate) and x=calendar year. Then the EAPC=100*(e^m-1). Testing the hypothesis that the EAPC is equal to zero is equivalent to testing the hypothesis that the slope of the line in the above equation is equal to zero. The latter hypothesis was tested using the t-distribution of m/SE_m, while the number of degrees of freedom equals the number of calendar years minus two. The standard error of m, SE_m, was obtained from the fit of the regression line.[213] This calculation assumes that the rates increase or decrease at a constant rate over the entire period.

Incidence rates and trends were calculated for males and females separately. Age was stratified into four groups: 0-14 years, 15-44 years, 45-64 years and 65+ years. Analyses were performed using Statistical Analysis System version 8.2 and Microsoft Excel 2003. All data were analysed respecting the privacy legislation that applies in the Netherlands.

Results

All glioma

Between 1989 and 2003, 9,290 newly diagnosed gliomas were registered in the Netherlands, 5,402 in males and 3,888 in females. An additional 1,312 males and 1,210 females were registered with a clinically diagnosed tumour or a glioma NOS. The numbers of registered glioma patients per diagnosis group and age category are shown in table 2. Age-adjusted incidence rates for all glioma combined and including gliomas of uncertain histology were stable between 1989 and 2003, for males (EAPC -0.2%, p=0.57) and females (EAPC 0.3%, p=0.49) (figure 1). Within age categories, small and non-significant trends in the incidence of all glioma could be seen (figure 2). Incidence rates in the elderly (aged 65+ years)

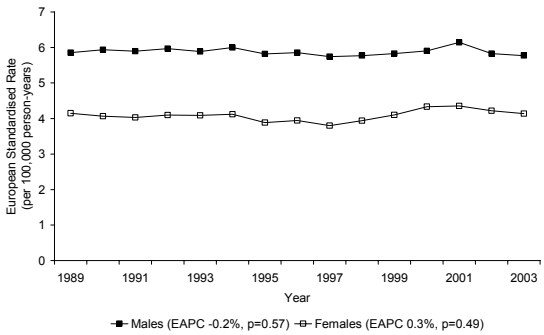

Figure 1. Incidence of all glioma according to gender, European Standardised Rates, 3-year moving average, with Estimated Annual Percentage Change (EAPC).

Table 2. Total number of registered glioma patients in the Netherlands during the period 1989-2003, for diagnosis groups and age categories

Diagnosis and age (years)	Number of patients, 1989-2003	
	Males	Females
Astrocytic glioma, low-grade		
0-14	187	193
15-44	467	332
45-64	220	135
65+	71	49
Astrocytic glioma, high-grade		
0-14	30	28
15-44	559	367
45-64	1,533	985
65+	1,015	772
Astrocytic glioma, grade unknown		
0-14	23	21
15-44	131	83
45-64	95	79
65+	73	68
Oligodendroglial/mixed glioma		
0-14	23	23
15-44	346	265
45-64	300	249
65+	105	83
Ependymal glioma		
0-14	74	47
15-44	61	54
45-64	65	38
65+	24	17
Uncertain histology [a]		
0-14	64	48
15-44	145	106
45-64	382	259
65+	721	797
Total		
0-14	401	360
15-44	1,709	1,207
45-64	2,595	1,745
65+	2,009	1,786
All ages	6,714	5,098

[a] This category comprises clinically diagnosed tumours without histopathological confirmation (87%) and glioma not otherwise specified (13%).

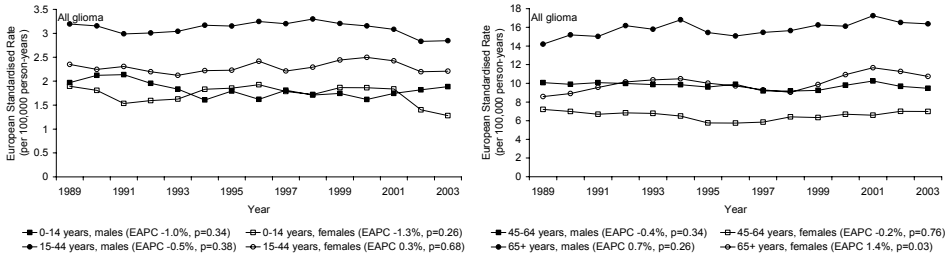

Figure 2. Incidence of all glioma according to gender and age, European Standardised Rates, 3-year moving average, with Estimated Annual Percentage Change (EAPC).

showed a small increase (EAPC 0.7%, p=0.26 for males and EAPC 1.4%, p=0.03 for females), whereas in children, incidence rates were slightly decreasing (EAPC –1.0%, p=0.34 for boys and EAPC –1.3%, p=0.26 for girls).

Astrocytoma

In an all ages analysis, age-adjusted incidence rates of high-grade astrocytoma increased significantly for males (EAPC 1.5%, p=0.005), accompanied by a significantly decreasing trend in low-grade astrocytoma (EAPC -1.9%, p=0.02), in astrocytoma with unknown malignancy grade (EAPC -12.5%, p<0.001) and in glioma of uncertain histology (EAPC –1.7%, p=0.06) (figure 3). The incidence of astrocytic glioma in females showed a similar pattern although the accompanying decrease in low-grade astrocytoma was less pronounced (EAPC -0.8%, p=0.46) (figure 3). Within age categories, a significantly rising incidence of high-grade astrocytoma was seen in young adults and elderly, but not in adults aged 45-64 years (figure 4). The incidence of low-grade astrocytoma showed no consistent pattern. A marked decrease was seen in adult astrocytoma with unknown malignancy grade (EAPC -7.0% to -16%). The incidence of glioma of uncertain histology showed a modest and mostly borderline significant decreasing trend in all adult age categories (figure 4).

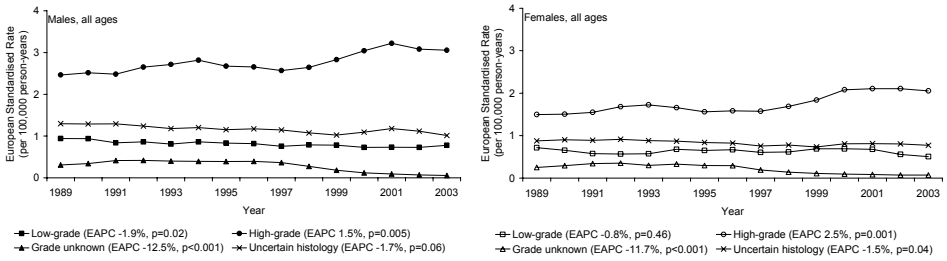

Figure 3. Incidence of astrocytic glioma and glioma of uncertain histology according to gender, European Standardised Rates, 3-year moving average, with Estimated Annual Percentage Change (EAPC).

In children aged 0-14 years, decreasing incidence was seen for all astrocytoma groups (figure 4). This was balanced by glioma of uncertain histology (EAPC 8.0%, p=0.05 for boys and EAPC 3.5%, p=0.44 for girls). None of these trends were statistically significant.

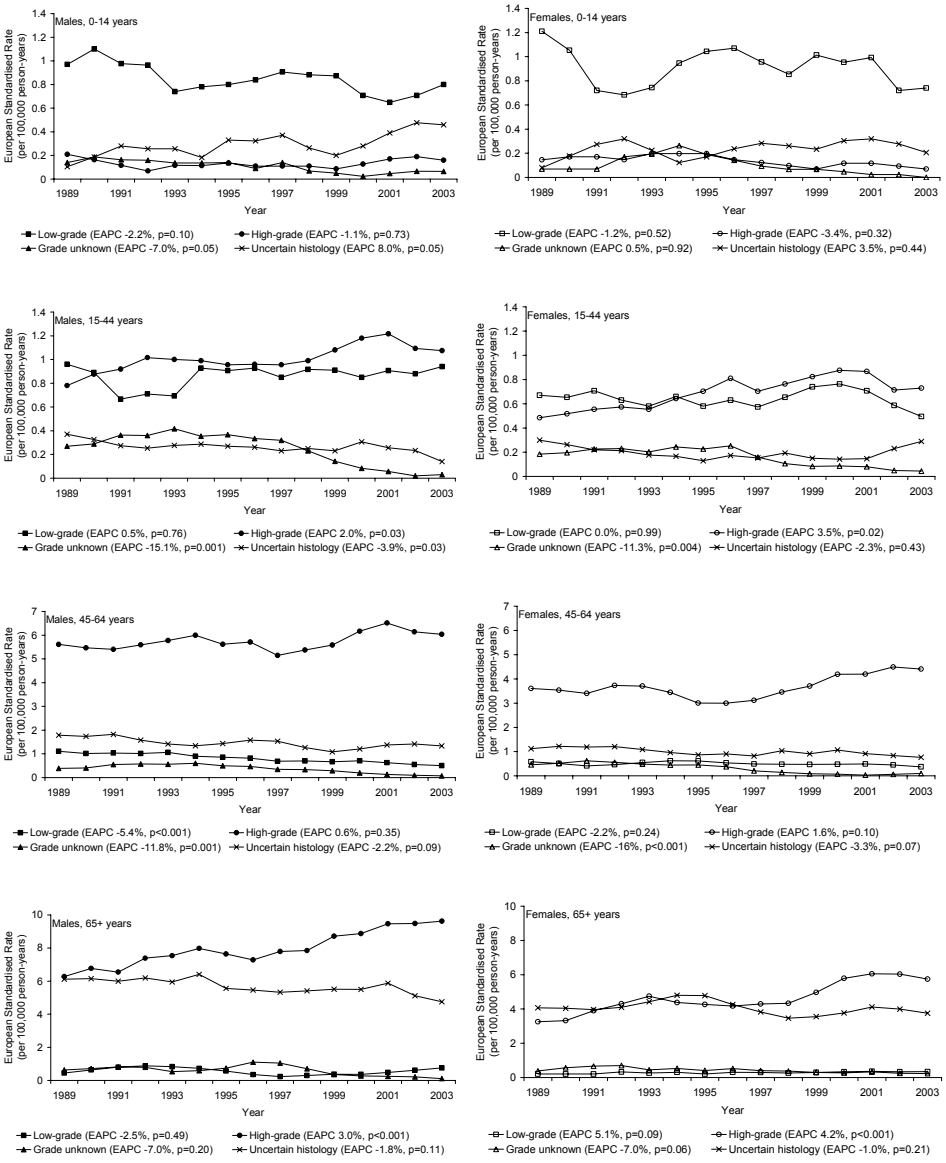

Figure 4. Incidence of astrocytic glioma and glioma of uncertain histology according to gender and age, European Standardised Rates, 3-year moving average, with Estimated Annual Percentage Change (EAPC).

Oligodendroglioma and ependymoma

The overall incidence of oligodendroglioma was stable for males (EAPC 0.4%, p=0.71) and females (EAPC -0.2%, p=0.84) (figure 5). Also within age categories, no clear trends in the incidence could be seen. The overall incidence of ependymoma showed a small but non-significant increase between 1989 and 2003 (EAPC 1.5%, p=0.53 for males and EAPC 3.4%, p=0.12 for females). In general, incidence rates were decreasing in children and increasing in adults. None of these trends however were statistically significant and rates varied widely over the studied period, which is probably caused by the small numbers in each age category (table 2).

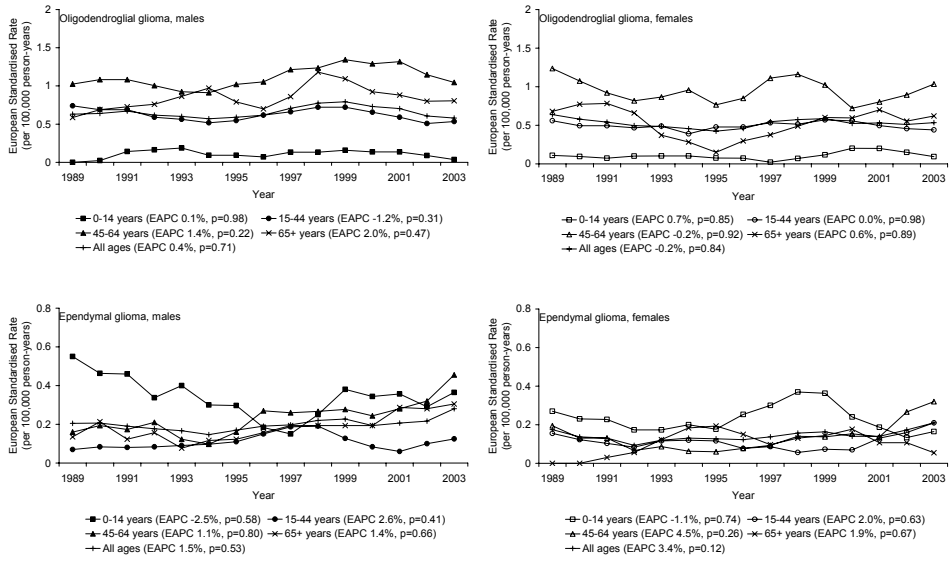

Figure 5. Incidence of oligodendroglial/mixed glioma and ependymal glioma according to gender and age, European Standardised Rates, 3-year moving average, with Estimated Annual Percentage Change (EAPC).

Male/female ratios

The male/female ratio remained constant with an average of 1.44 for all glioma, 1.41 for ependymal glioma, 1.25 for oligodendroglial glioma and 1.49 for astrocytic glioma (including low-grade astrocytoma, high-grade astrocytoma and astrocytoma with unknown malignancy grade) (not shown). Within age groups of all glioma and astrocytic glioma, the sex-ratios were fairly constant as well. Other analyses within age groups were not performed because of small numbers of cases.

Discussion

In general, minor trends in the incidence of glioma were observed in the Netherlands during the period 1989 to 2003. Age-adjusted incidence rates were stable for all glioma combined and for oligodendroglioma/mixed glioma. In adult astrocytic glioma, a significant increase in high-grade astrocytoma was accompanied by a simultaneous decrease in low-grade astrocytoma, astrocytoma with unknown malignancy grade and glioma of uncertain histology. The incidence of astrocytoma and ependymoma in children showed a decreasing trend, which was accompanied by an increasing incidence of gliomas of uncertain histology. The overall incidence of glioma in children was stable.

Methodological considerations

The NCR is characterised by high quality incidence data and near complete ascertainment with less than 2% underregistration.[214,215] Completeness of registration is achieved by combining data from different sources, including PALGA and the LMR. In the studied period, access to medical care in the Netherlands was good and less than 1% of the population was uninsured.[209] We also verified registration procedures for different areas in the Netherlands which did not change since 1989. However, in some of the studied glioma subgroups the numbers of cases are small and trend estimation may be difficult. We therefore evaluated the general pattern of incidence trends.

Evaluation of incidence rates over time is complicated by changes in histopathological classifications.[57] We used a uniform cluster scheme for ICD-O coded primary CNS tumours.[57] In this system, primary CNS tumours are clustered as clinically relevant entities, based on the second edition of the World Health Organisation (WHO) classification of CNS tumours.[216] In our opinion, no essential changes in pathological practice have occurred in the studied period that could have greatly influenced the incidence statistics. Changes in classifications may have occurred within the analysed groups, most likely in the low-grade astrocytoma group. Pilocytic astrocytomas, for example, are characterised by a very distinct histology and prognosis.[6,7] The recognition and classification of these tumours is improving,[6] resulting in more pilocytic astrocytomas that were separately classified from other low-grade astrocytomas (data not shown). However, these shifts have occurred within the group of low-grade astrocytoma and it is therefore unlikely that this has influenced incidence rates in this study. It is possible that some of the clinically diagnosed gliomas without histopathological confirmation will in fact be a different type of tumour. This may particularly be a problem for the primary central nervous system lymphomas (PCNSL) which can be difficult

to distinguish from glioma, based on neuroimaging only. The world standardised incidence of PCNSL however is less than 0.3 per 100,000 person-years.[217,218]

Comparison with other countries

The incidence of glioma has been remarkably stable over the past decades in Europe and the United States. A temporal increase in incidence has been observed in the late 1970s and the early 1980s, but incidence was stabilising or slightly decreasing in almost every age group in more recent years.[3,46-48] This suggests that these increases in incidence were artifactual, probably owing to the introduction of CT and MRI in this period.[3,50] In the most recent years, increasing incidence of glioma was particularly apparent among the elderly.[3,46-48] This was probably the result of increasing efforts to obtain histopathological diagnosis. A simultaneous decrease in clinically diagnosed tumours and in glioma NOS is compatible with this view.[48,52] In addition, physicians treating elderly patients are increasingly willing to use more diagnostics in the elderly, revealing malignancies that otherwise would have gone undetected.[3,47,51] An observed increase in high-grade gliomas and simultaneous decrease in low-grade tumours was probably caused by improving techniques in neuroimaging and neurosurgery resulting in less sampling error and better characterisation of malignancy grade.[3,48,51]

In the Netherlands, similar patterns were seen as those described in the literature. Not only was the incidence of glioma stable, also similarly occurring shifts in astrocytoma subgroups suggest improving diagnostic methods and better detection. The small increase in incidence of glioma in patients aged over 65 years is most likely the result of a changing attitude towards the elderly in which diagnosis and therapy were more persistently pursued.[3,47,219] This assumption is supported by a decreasing incidence of glioma with unknown malignancy grade and clinically diagnosed tumours. However, these trends were most marked in adults aged 15-64 years, suggesting that patients in this age category may benefit most from new techniques. The observed male/female ratios also correspond with figures previously reported in the literature.

Some authors argue that not all of the observed increases in incidence can be explained by better detection.[53-56] For example, increasing trends in the incidence of childhood astrocytoma were largely confined to girls,[54] and an observed increase in the incidence of ependymoma could not be explained by diagnostic practice.[52] However, good explanations for these trends cannot be offered. In the present study we noticed a decreasing instead of increasing trend of childhood astrocytoma. Ependymoma incidence possibly showed a modest increase in adults but this increase was not statistically significant and numbers were small (figure 5).

In conclusion, the incidence of glioma in the Netherlands shows only minor trends between 1989 and 2003. Most variation can be explained by better detection, improving diagnostic precision and changing attitude towards the elderly. Stable incidence rates suggest that no major changes in environmental risk factors have occurred which influenced the incidence of glioma in the studied period.

Acknowledgements

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Chapter 2.2

Summary

Little is known about the aetiology of glioma. Research is often hampered by the low incidence and high mortality of the disease. Concomitant diseases in glioma patients may indicate possible aetiological pathways. We therefore studied comorbidity in glioma patients. We performed a case-control study using population-based data from the Eindhoven Cancer Registry. We compared prevalences of concomitant diseases in 510 glioma patients with two reference cancer populations from the same registry. Compared with all other cancer patients, a significantly higher prevalence of hypertension was found in glioma patients for age categories 60-74 years (odds ratio (OR) 1.37; 95% confidence interval (CI) 1.02-1.84) and 75+ years (OR 2.37; 95% CI 1.34-4.21). The association was most pronounced in elderly men and in astrocytic glioma, with a maximum in age category 75+ years (OR 5.86; 95% CI 2.20-15.7). The prevalence of cerebrovascular disease was higher in glioma patients >45 years old (OR 1.67; 95% CI 1.12-2.47), whereas the prevalence of other cancers was lower (OR 0.64; 95% CI 0.48-0.87). No consistent associations were detected for several other concomitant diseases. Our data suggest an association between hypertension and glioma, although questions remain about causality and the possible mechanisms. We hypothesise that this association is mediated through potentially neurocarcinogenic effects of antihypertensive medication.

Introduction

Gliomas are primary brain tumours of neuroepithelial origin and are among the most malignant cancers.[26] Owing to the low incidence, high mortality and histological heterogeneity of glioma,[2,26] little is known about the causes of the disease. A few genetic syndromes exist that can explain less than 5% of glioma cases, and the search continues for other genetic risk factors.[90,220] Ionising radiation is the only established environmental risk factor and explains the occurrence of

glioma in only a small minority of patients, owing to low exposure rates.[64,65] The evidence for many other proposed aetiological factors is inconclusive.[44]

Additional clues about glioma aetiology may come from concomitant diseases in glioma patients. Concomitant disease can be an indication of aetiological mechanisms. Exposure to one risk factor may cause more diseases, as illustrated by smoking, lung cancer and emphysema cases in lung cancer patients. Given a known relation between smoking and emphysema, detection of excess emphysema in lung cancer patients could identify smoking as an aetiological factor for cancer. Cancer can also be the result of a disease-related exposure whereas the disease itself is not causally related with cancer. An example of this is the former use of glioma-inducing radiation therapy for tinea capitis.[65]

In this study we examined potential risk factors for glioma by studying comorbidity in glioma patients.

Methods

The Eindhoven Cancer Registry, within the framework of the Comprehensive Cancer Centre South, registered all glioma patients newly diagnosed between 1993 and 2000 in the southeast of the Netherlands. This population-based registry covers an area with over two million inhabitants.[221] Health care in this area is provided by sixteen general hospitals, two radiotherapy institutes and a large neurosurgical centre. Within six months after registration, information on clinically relevant concomitant diseases was additionally collected from medical records, medical correspondence and current medication use, according to a slightly amended version of the list of Charlson.[222-224] Only concomitant diseases pre-existing at time of diagnosis of the cancer or before were registered. Diabetes mellitus, hypertension and chronic obstructive pulmonary disease were only registered if they were also considered to be current problems, i.e. requiring medication.

Age-specific prevalences of concomitant diseases in glioma patients were compared with ratios in two reference cancer populations without glioma from the same registry, diagnosed between 1993 and 2000, in a case-control study design. The first reference population comprised all patients with invasive cancer (n=52,063); in the second population, the tobacco-related cancers were excluded (lung, head and neck, bladder) (n=39,626). Because of the low frequency of comorbidity in younger patients, the analyses were restricted to age over 45 years. Categories of comorbidity with sufficient numbers of patients were considered in the analyses: diabetes mellitus, hypertension, pulmonary disease, heart disease,

peripheral vascular disease, other cancer (non-glioma cancer diagnosed before the glioma) and cerebrovascular disease (cerebral haemorrhage or infarction, hemiparesis and cerebral vascular diseases or carotid artery surgery). For each concomitant disease, crude odds ratios (ORs) were calculated with 95% confidence intervals (CIs). Age was stratified into three categories (45-59, 60-74, 75+ years) and pooled ORs were calculated with the Mantel-Haenszel method (OR_{MH}). Analyses were performed using SPSS for Windows version 11.0.1.

Results

Information on comorbidity was collected for 510 of 672 registered glioma patients between 1993 and 2000 (61% males; median age at diagnosis 62 years, range 45-93). Comorbidity was unknown for 162 patients, mainly owing to first registration by adjacent cancer registries that do not actively collect comorbidity data. The primary cancer registration is largely determined by the geographical location of the patient, not by disease-related characteristics. Comparison of patient characteristics from patients with known and unknown comorbidity revealed no relevant differences between these two groups (not shown).

The main diagnoses were astrocytic glioma (75%), oligodendroglioma (7.3%) and clinically diagnosed tumours without histological confirmation (10%). Of the 510 glioma patients, 44% were diagnosed with at least one and 14% with more than one concomitant disease.

There were no significant differences between glioma patients and the reference cancer populations for the prevalence of diabetes mellitus and heart disease (table 1). When compared with all cancer patients, the significantly lower prevalence of peripheral vascular disease (OR_{MH} 0.41; 95% CI 0.20-0.82) and pulmonary disease (OR_{MH} 0.51; 95% CI 0.35-0.75) disappeared after exclusion of tobacco-related cancers, although a nearly significant association remained for peripheral vascular disease (OR_{MH} 0.52; 95% CI 0.26-1.04). Compared with both reference cancer populations, a significantly lower prevalence of other cancers in glioma patients was observed. The prevalence of cerebrovascular disease was higher (OR_{MH} 1.67; 95% CI 1.12-2.47 when comparing with all cancer patients; OR_{MH} 1.80; 95% CI 1.21-2.68 after exclusion of tobacco-related cancers) (table 1).

Compared with all other cancer patients, a significantly higher prevalence of hypertension was found in glioma patients for age categories 60-74 years (OR 1.37; 95% CI 1.02-1.84) and 75+ years (OR 2.37; 95% CI 1.34-4.21) (table 2). This association persisted after exclusion of tobacco-related cancers (table 1). The association was most pronounced in elderly with an astrocytic glioma, with a

Table 1. Prevalences and crude odds-ratios for concomitant diseases in glioma patients over 45 years of age, compared with other cancer patients in the southeastern Netherlands, 1993-2000

Concomitant disease	Prevalence n, (%)		Pooled OR [a] (95% CI)	p-value
	Glioma	Controls		
Diabetes mellitus	33 (6.4)	5,055 (9.7)	0.82 (0.57-1.17)	0.27
Heart disease	48 (9.4)	7,589 (15)	0.80 (0.59-1.09)	0.16
Peripheral vascular disease	8 (1.6)	2,400 (4.6)	0.41 (0.20-0.82)	0.01
Pulmonary disease	29 (5.7)	6,319 (12)	0.51 (0.35-0.75)	<0.001
Other cancer	48 (9.4)	8,223 (16)	0.64 (0.48-0.87)	0.004
Cerebrovascular disease	27 (5.3)	2,262 (4.3)	1.67 (1.12-2.47)	0.01
Hypertension	106 (21)	9,037 (17)	1.46 (1.18-1.82)	<0.001
	Glioma	**Controls excluding tobacco-related**		
Diabetes mellitus	33 (6.4)	4,008 (10)	0.80 (0.56-1.14)	0.62
Heart disease	48 (9.4)	5,388 (14)	0.90 (0.67-1.22)	0.51
Peripheral vascular disease	8 (1.6)	1,479 (3.7)	0.52 (0.26-1.04)	0.06
Pulmonary disease	29 (5.7)	3,506 (8.8)	0.76 (0.52-1.11)	0.15
Other cancer	48 (9.4)	5,972 (15)	0.69 (0.51-0.93)	0.01
Cerebrovascular disease	27 (5.3)	1,652 (4.2)	1.80 (1.21-2.68)	0.003
Hypertension	106 (21)	7,346 (19)	1.36 (1.09-1.69)	0.006

OR: odds ratio; CI: confidence interval.

[a] Pooled odds ratio over three age categories using the Mantel-Haenszel method.

Table 2. Age- and gender-specific prevalences and crude odds-ratios for hypertension in glioma patients, compared with all other cancer patients in the southeastern Netherlands, 1993-2000

	Age (years)	Prevalence n, (%)		OR (95% CI)	p-value
		Glioma	Controls		
All glioma					
Men + women	45-59	28 (13)	1,251 (9.8)	1.32 (0.89-1.97)	
	60-74	59 (25)	4,764 (19)	1.37 (1.02-1.84)	
	75+	19 (38)	3,022 (21)	2.37 (1.34-4.21)	
	Total [a]			1.46 (1.18-1.82)	<0.001
Men	45-59	17 (12)	550 (10)	1.24 (0.74-2.07)	
	60-74	31 (22)	2,346 (16)	1.45 (0.97-2.16)	
	75+	12 (39)	1,141 (15)	3.70 (1.79-7.65)	
	Total [a]			1.56 (1.17-2.08)	0.002
Women	45-59	11 (13)	701 (9.6)	1.42 (0.75-2.69)	
	60-74	28 (29)	2,418 (24)	1.30 (0.84-2.03)	
	75+	7 (37)	1,881 (27)	1.56 (0.61-3.96)	
	Total [a]			1.37 (0.97-1.92)	0.07
Astrocytic glioma					
Men + women	45-59	25 (14)	1,251 (9.8)	1.54 (1.00-2.36)	
	60-74	47 (25)	4,764 (19)	1.42 (1.02-1.99)	
	75+	11 (44)	3,022 (21)	3.04 (1.38-6.71)	
	Total [a]			1.57 (1.22-2.01)	<0.001
Men	45-59	16 (14)	550 (10)	1.47 (0.86-2.51)	
	60-74	24 (22)	2,346 (16)	1.53 (0.97-2.41)	
	75+	8 (50)	1,141 (15)	5.86 (2.20-15.7)	
	Total [a]			1.71 (1.24-2.37)	<0.001
Women	45-59	9 (15)	701 (9.6)	1.60 (0.79-3.26)	
	60-74	23 (29)	2,418 (24)	1.30 (0.80-2.13)	
	75+	3 (33)	1,881 (27)	1.33 (0.33-5.34)	
	Total [a]			1.38 (0.94-2.04)	0.10

OR: odds ratio; CI: confidence interval.

[a] Pooled odds ratio using the Mantel-Haenszel method.

maximum in age category 75+ years (OR 5.86; 95% CI 2.20-15.7) (table 2). Other glioma subgroups were not evaluated because of small numbers of patients.

Discussion

We showed that, compared with all cancer patients, the prevalence of hypertension and cerebrovascular disease is higher in glioma patients over 45 years of age. The prevalence of other cancers and peripheral vascular disease was lower in glioma patients.

The lower prevalence of other cancers in glioma patients could be the result of ubiquitous risk factors causing multiple cancers but not gliomas, e.g. alcohol and smoking. The higher prevalence of cerebrovascular disease in glioma patients could be explained by the high vascularity of gliomas owing to angioneogenesis and vascular remodelling, particularly in high-grade gliomas.[4] A bleeding glioma can easily be missed on a brain computed tomography (CT) scan owing to the haemorrhage. Another source of misdiagnosis is the resemblance of low-grade gliomas to cerebral infarction, on a brain CT and also by clinical symptoms, which can be very abrupt at onset.[225] In this study, no significant association was found between glioma and diabetes mellitus, although a trend for a lower prevalence of diabetes was observed, which is consistent with previous studies assessing this relationship.[61,141]

Hypertension

The prevalence of hypertension in our reference groups was low compared with the screened general population of the Rotterdam Study, a population-based cohort of elderly people in which 22-52% of women and 22-39% of men aged over 55 years are hypertensive.[226] Our method of data collection without measuring actual blood pressures hampers meaningful comparison. In the Rotterdam study, 35% of participants were either unaware of their hypertension and/or were not treated for it.[226] We therefore assume that the lower prevalence in our study largely reflects the data collection procedure. Hypertension has been underestimated, but probably equally for cases and controls.

Several hypotheses may explain a higher prevalence of hypertension in glioma patients. Raised intracranial pressure or brainstem compression can cause systemic hypertension. However, hypertension is usually diagnosed after sustained high blood pressure over a period of weeks or months, as recommended in widely used Dutch guidelines for general practitioners and medical specialists. If already causing high intracranial pressure or brainstem compression, a glioma will

therefore usually be diagnosed before the hypertension. It is also known that (high-grade) gliomas produce cytokines and vasoactive substances involved in angioneogenesis and vascular remodelling.[4] Although these are believed to act in a paracrine manner, a systemic influence on blood pressure cannot be excluded. Finally, unknown exposures could increase the risk of both hypertension and glioma. The more pronounced associations in men would suggest lifestyle or occupation-related exposures.

The prevalence of hypertension could have been equal or even higher in the non-glioma cancer patients, but could have decreased owing to systemic progression and associated weight loss of more advanced cancers. This rarely occurs in glioma patients as glioma does not metastasise. We excluded this possibility by considering the relationship between the various stages in colorectal cancer and the prevalence of hypertension, adjusted for age and gender. We did not find any significant decreases in hypertension, nor trends (data not shown).

Glioma and antihypertensive drugs

Could there be a relation between glioma and antihypertensive drugs (AHD)? Treatment with AHD decreases morbidity and mortality from cardiovascular disease, but does not consistently lower all-cause mortality,[227-229] owing to an increased mortality from other diseases. In two meta-analyses, hypertension was shown to be associated with an increased cancer mortality.[230] This association was strongest for renal cell carcinoma (OR 1.75) and attributable to the use of diuretics (OR 1.54).[231] Batty et al.[232] found no convincing associations for systolic and diastolic blood pressure and cancer. However, for brain tumours, no distinction was made between different types of brain tumour, and the effect of AHD could not be studied because of insufficient data. Thiazides and loop diuretics contain amines and amides, precursors of *N*-nitroso compounds that are potent nervous system carcinogens.[233] Maternal use of diuretics during pregnancy was shown to increase the risk of childhood brain tumours,[234] but this was not confirmed by subsequent studies,[235-237] nor could an increased risk be shown for adults.[147] Even though associations between AHD and other cancers besides renal cell carcinoma are less evident and an effect of *N*-nitroso compounds is questionable, a glioma-inducing effect of certain AHD may exist.

Study design

There are more suitable designs to study the association between comorbidity and cancer, particularly cohort studies. For glioma, many of these designs are of limited value owing to the low incidence of these tumours. Existing data with sufficient numbers of glioma patients, e.g. from cancer registries, do not always provide

all the necessary information. The data used in our study are population-based and prospectively collected with almost complete ascertainment, regardless of the cancer diagnosis. The reference groups received similar treatment modalities and medical attention to the glioma patients. These characteristics should have reduced possible information and selection bias to a great extent. Only comorbidity pre-existing at time of cancer diagnosis or before was registered. It is therefore unlikely that the associations were confounded by cancer-specific interventions such as preoperative assessments or therapy. Confounding might also result because some of the cancers in our comparison groups are associated with comorbidity, like hypertension and renal cell carcinoma,[231] or diabetes mellitus and pancreatic cancer.[238] This effect is likely to be cancelled out by the wide range of cancers in the comparison groups, of which most are unrelated to the exposures under study. We did use a second reference group without tobacco-related cancers. These cancers were excluded because smoking affects the pattern of comorbidity but has no known association with, amongst others, hypertension and glioma. Residual confounding will probably lead to an underestimate of risk and therefore not to false associations. However, we cannot make unequivocal conclusions about causality of the detected associations, and also have to consider the possibility of unknown confounding factors.

In conclusion, our data suggest an association between hypertension and glioma, although questions remain about possible mechanisms and causality. We hypothesise that one possible mechanism through which hypertension might cause glioma is through potentially neurocarcinogenic effects of AHD or their metabolites.

Chapter 2.3

The association between antihypertensive drugs and glioma

Summary

In an earlier study, we found an association between hypertension and glioma. Therefore, we investigated whether the use of antihypertensive drugs (AHD) is associated with an increased risk of glioma. We conducted a population-based nested case-control study with data from the PHARMO database. This database links dispensing records of prescription drugs to hospital discharge data on an individual basis. Pathological data were derived from the Dutch nationwide registry of histo- and cytopathology. Cases were defined as subjects over 30 years of age without a previous history of cancer and with an incident glioma between 1997 and 2003. Three hundred-six cases were matched to 1,108 controls for year of birth, sex, geographical region and duration of follow-up. Exposure was defined as cumulative duration of AHD use and, in an alternative analysis, as cumulative dose. We estimated the magnitude of the association with conditional logistic regression analysis. Cumulative use of any AHD for more than 6 months was associated with an increased risk of glioma (OR 1.56; 95% CI 1.07-2.27). Positive associations for the use of beta-blockers (OR 2.37; 95% CI 1.57-3.58) and miscellaneous AHD (OR 3.07; 95% CI 1.05-9.01) were only found for a duration of use of <2 years. After subtracting a latency period of 3 years before the date of diagnosis, no associations were found. In conclusion, the use of AHD seems to be associated with an increased risk of glioma, but this is probably not causal. Although we cannot exclude that AHD modify the presentation of glioma, the most likely explanation is that AHD are prescribed during the prodromal phase of this disease.

Introduction

Gliomas are malignant brain tumours of neuroepithelial origin.[26] Although gliomas are the most common type of primary brain tumour, they are nevertheless relatively rare. In the Netherlands, world-standardised incidence rates of glioma are 6.5 per 100,000 person-years for males and 4.4 for females.[2] The aetiology of glioma remains largely unclear. Ionising radiation is the only established environmental risk factor but the exposure rate is low.[64,65] Some rare genetic disorders exist in which patients have an increased risk of glioma and a variety of other cancers.[90,91] No other major factors in the aetiology of glioma have been identified thus far.[44]

Previously, we found a significantly higher prevalence of hypertension in glioma patients, in a population-based case-control study in the Eindhoven Cancer Registry.[239] We hypothesised that one possible mechanism through which hypertension might be associated with glioma is through potentially neurocarcinogenic effects of antihypertensive drugs (AHD) or their metabolites.

To test the hypothesis of a glioma-inducing effect of AHD, we conducted a population-based nested case-control study with prospectively collected automated pharmacy data, linked to morbidity and pathology data.

Methods

Setting

We used data from the PHARMO record linkage system, a database that links dispensing records of prescription drugs to hospital discharge data on an individual basis.[240] Since 1985, drug dispensing pharmacy data are collected from a representative sample of Dutch community pharmacies. These pharmacies are scattered over the Netherlands and currently cover data of more than two million residents, corresponding with 12% of the Dutch population. Participants in the PHARMO population enter the database when their first prescription is filled in a PHARMO community pharmacy, and they are followed until their last prescription is filled. The recorded information of each dispensed drug includes the Anatomical Therapeutic Chemical (ATC) code,[241] the dispensing date, the quantity of the dispensed drug, the prescribed daily dose, and the estimated duration of use. Because almost all persons designate a single pharmacy to fill their drug prescriptions, dispensing histories are virtually complete.[242]

Drug dispensing histories are linked to hospital discharge records from the Dutch Medical Register (LMR), using a validated and reliable probabilistic algorithm.[240] The LMR comprises all hospital admissions in the Netherlands and

includes, among others, information about dates of admission and discharge, discharge diagnoses, comorbidity and performed medical procedures. All diagnoses are coded according to the International Classification of Diseases, ninth edition clinical modification (ICD-9-CM). Since 1991, the primary diagnosis is determined by the specialist who treated the patient. Other sources of morbidity and drug exposure include hospital pharmacies, clinical laboratories and general practitioners.

Study population and validation

From the LMR database we obtained all patients with a brain tumour (ICD-9-CM code 191). As glioma is not a separate code in the ICD-9-CM, these potential cases were linked to the Dutch nationwide network and registry of histo- and cytopathology (PALGA) containing data of histological, cytological and autopsy examinations of all 16 million inhabitants in the Netherlands. We included all incident gliomas from January 1, 1997 to December 31, 2003 to include at least 12 years of potential medication histories since 1985. The index date was defined as the first admission date because of a brain tumour which was (later) confirmed to be a glioma by clinical pathology. All patients with a previous history of non-glioma cancer before the index date were excluded, identified by a history of cancer (ICD-9-CM codes 140-208), a history of radiotherapy or use of anti-cancer medications (ATC codes L01, L02, L03). For each case we took 3-4 controls from the PHARMO database, matched on geographical region, date of birth (5-year intervals), gender and duration of follow-up in the PHARMO database (3-month intervals). Duration of follow-up was defined as the difference between the date of entry in PHARMO and the index date. Controls were assigned the same index date as the cases and were part of the PHARMO population on this date. To be certain that controls were free of (yet undetected) cancer at the index date, controls with a history of cancer up to two years after the index date were excluded. A history of cancer in controls was identified similarly as in the cases.

Exposure definition

In the Netherlands, all AHD are prescription-only drugs. For all cases and controls, the complete history of filled prescriptions before the index date was obtained. The following antihypertensive drugs were analysed: ATC codes C02 (miscellaneous AHD), C03 (diuretics), C07 (beta-blockers), C08 (calcium antagonists) and C09 (ACE inhibitors and ATII antagonists). Exposure was calculated as the duration of use before the index date in number of days, and as the sum of dispensed defined daily doses (DDD). The DDD is the recommended standard dose for the main indication in adults, as defined by the World Health Organisation. As it is

highly unlikely that occasional short-term use causes glioma, we defined a prior cut-off point of cumulative use of 6 months.

Statistical analysis

We restricted the analyses to patients aged 30 years and over. First, we assessed ever use of AHD before the index date as a potential risk factor for glioma. We then extended the analyses for categories of cumulative duration of use and cumulative dose. Diuretics, beta-blockers, calcium antagonists, drugs acting on the renin-angiotensin system (ACE inhibitors, ATII antagonists) and miscellaneous AHD were analysed separately, adjusted for the other ATC classes. We also stratified for gender, age and histological subgroups of glioma.

To exclude the possibility that use of AHD was started because of symptoms in the prodromal phase of glioma, and to take into account the delay between tumour induction and diagnosis, we performed sensitivity analyses by subtracting a lag time of 3 and 5 years from the index date as performed before.[243] Chi-square statistics were used to compare proportions. Conditional logistic regression was used to estimate the association between AHD and the risk of glioma, expressed as odds ratios (ORs) and 95% confidence intervals (CI). Analyses were adjusted for residual variation in age and duration of follow-up. Since the cumulative dose can be the result of prolonged use of low-dose AHD, or of short-term high-dose AHD, we also adjusted for duration of use in the analyses for cumulative dose. In the analyses for cumulative duration of use, we additionally adjusted for mean DDD per day of use. All statistical tests where performed two-sided and statistical significance was indicated if $p<0.05$. Analyses were performed using SPSS for Windows version 12.0.1 and Statistical Analysis System version 8.2.

Results

The baseline characteristics of 306 glioma patients and 1,108 matched controls aged over 30 years are shown in table 1. The most frequent diagnosis was astrocytic glioma (82.3%), followed by oligodendroglioma (10.5%) and mixed glioma (4.9%). The distribution of diagnoses and the excess of male patients (60.1%) are comparable with figures that were previously reported in the literature.[2,4] The median follow-up time in PHARMO was 5.4 years for patients and 5.2 years for controls. The cumulative duration of AHD use was longer in patients than in controls ($p=0.03$).

In the conditional logistic regression analyses, an increased risk of glioma was found for users of any AHD. There was no difference in risk for patients who

Table 1. Characteristics of glioma cases and controls

Characteristic	Patients (n=306)		Controls (n=1,108)		p-value
	n	(%) [a]	n	(%) [a]	
Age, years					
30-44	66	(21.6)	227	(20.5)	
45-59	115	(37.6)	440	(39.7)	
60-74	100	(32.7)	363	(32.8)	
>=75	25	(8.2)	78	(7.0)	0.84
Median (25th-75th percentile)	56.6	(47.3-67.5)	56.2	(48.0-66.4)	
Sex					
Men	184	(60.1)	669	(60.4)	
Women	122	(39.9)	439	(39.6)	0.94
Follow-up time in PHARMO, years					
0 to <1	43	(14.1)	164	(14.8)	
1 to <3	53	(17.3)	204	(18.4)	
3 to <5	50	(16.3)	167	(15.1)	
5 to <7	37	(12.1)	130	(11.7)	
7 to <9	28	(9.2)	104	(9.4)	
9 to <11	36	(11.8)	117	(10.6)	
>=11	59	(19.3)	222	(20.0)	0.99
Median (25th-75th percentile)	5.4	(2.1-10.1)	5.2	(2.0-10.1)	
Diagnosis					
Astrocytoma, glioblastoma multiforme	252	(82.3)			
Oligodendroglioma	32	(10.5)			
Ependymoma	3	(1.0)			
Mixed glioma	15	(4.9)			
Glioma not otherwise specified	4	(1.3)			
Cumulative duration of AHD use					
No use	197	(64.4)	804	(72.6)	
<6 months	35	(11.4)	95	(8.6)	
6 months to 4 years	40	(13.1)	98	(8.8)	
>=4 years	34	(11.1)	111	(10.0)	0.03

AHD: antihypertensive drugs; SD: standard deviation.

[a] Percentages may deviate from 100% due to rounding.

used AHD for less than 2 years (OR 1.56; 95% CI 1.07-2.27) and for patients who used AHD for more than 2 years (OR 1.52; 95% CI 0.99-2.32), with almost identical odds ratios (table 2). For cumulative dose, a slightly higher risk was found for users who received 1-730 DDD (OR 1.54; 95% CI 1.00-2.35) compared with users who received more than 730 DDD (OR 1.30; 95% CI 0.95-1.78).

After stratification for ATC classes, a higher risk of glioma was found for patients who used beta-blockers for less than 2 years (OR 2.37; 95% CI 1.57-3.58), but not for patients using these AHD for a longer period (OR 0.96; 95% CI 0.48-1.91). The same pattern of risk for users of beta-blockers was seen when exposure was measured as cumulative dose (not shown). Also for miscellaneous AHD, a higher risk was found for the lower category of cumulative duration of use (OR 3.07; 95% CI 1.05-9.01). However, when exposure was measured as cumulative dose, the risk for users of miscellaneous AHD was found to be comparable for both exposure categories (OR 2.59; 95% CI 0.90-7.49 for 1-730 DDD, compared with OR 2.71; 95% CI 0.50-14.8 for >=730 DDD). None of the other risk estimates reached statistical significance, although numbers were small in some of the exposure categories. These results did not change when the analyses for cumulative duration of use were adjusted for the mean DDD per day of use (not shown).

In the sensitivity analyses, a lag time was considered by subtracting 3 and 5 years from the index date. Consequently, cumulative exposure during this lag time was not included. A lag time of 3 years already moved the risk estimates toward unity (table 3). Furthermore, many potential associations disappeared with a lag time of 5 years instead of 3 years (results not shown).

We also stratified for diagnosis (astrocytoma only, oligodendroglioma only), gender and age, and analysed for the use of any AHD. This did not change the results (not shown). Particularly, no association was found for the category of elderly men, that has the highest prevalence of hypertension.[239]

Table 2. Associations between the duration of use of antihypertensive drugs and glioma, without considering a lag period of exposure

Exposure	Patients		Controls		Adjusted OR [a] (95% CI)	Adjusted OR [b] (95% CI)
	n	(%)	n	(%)		
Any AHD						
No use [c]	197		804		reference	
<2 years	55	(18.0)	156	(14.1)	1.56 (1.07-2.27)	
>=2 years	54	(17.6)	148	(13.4)	1.52 (0.99-2.32)	
Diuretics						
No use [c]	265		965		reference	reference
<2 years	29	(9.5)	97	(8.8)	1.09 (0.69-1.72)	0.84 (0.51-1.38)
>=2 years	12	(3.9)	46	(4.2)	0.93 (0.46-1.89)	0.64 (0.31-1.35)
Beta-blockers						
No use [c]	230		922		reference	reference
<2 years	58	(19.0)	109	(9.8)	2.28 (1.57-3.32)	2.37 (1.57-3.58)
>=2 years	18	(5.9)	77	(6.9)	0.98 (0.54-1.79)	0.96 (0.48-1.91)
Calcium antagonists						
No use [c]	281		1,016		reference	reference
<2 years	15	(4.9)	53	(4.8)	0.95 (0.52-1.73)	0.68 (0.36-1.28)
>=2 years	10	(3.3)	39	(3.5)	0.85 (0.40-1.78)	0.51 (0.23-1.13)
Ace inhibitors, ATII antagonists						
No use [c]	261		991		reference	reference
<2 years	25	(8.2)	62	(5.6)	1.42 (0.86-2.37)	1.12 (0.64-1.98)
>=2 years	20	(6.5)	50	(4.5)	1.62 (0.92-2.85)	1.59 (0.87-2.93)
Miscellaneous AHD						
No use [c]	296		1,094		reference	reference
<2 years	7	(2.3)	8	(0.7)	3.25 (1.17-9.00)	3.07 (1.05-9.01)
>=2 years	3	(1.0)	6	(0.5)	1.72 (0.43-6.93)	1.39 (0.34-5.67)

AHD: antihypertensive drugs; OR: odds ratio; CI: confidence interval.

[a] Adjusted for age, gender and duration of follow-up. [b] Adjusted for age, gender, duration of follow-up and for use of other types of antihypertensive drugs (ever vs never use) [c] No use is defined as a cumulative use of <6 months.

Table 3. Associations between the duration of use of antihypertensive drugs and glioma, considering a lag period of exposure of 3 years

Exposure [a]	Patients		Controls		Adjusted OR [b] (95% CI)	Adjusted OR [c] (95% CI)
	n	(%)	n	(%)		
Any AHD						
No use [d]	153		552		reference	
<2 years	32	(17.3)	97	(14.9)	1.29 (0.80-2.06)	
>=2 years	25	(14.0)	91	(14.2)	0.89 (0.51-1.57)	
Diuretics						
No use [d]	190		653		reference	reference
<2 years	11	(5.2)	61	(8.2)	0.63 (0.31-1.28)	0.58 (0.28-1.21)
>=2 years	9	(1.8)	26	(3.5)	1.15 (0.50-2.62)	0.90 (0.37-2.21)
Beta-blockers						
No use [d]	178		623		reference	reference
<2 years	22	(10.5)	70	(9.5)	1.22 (0.72-2.06)	1.21 (0.68-2.17)
>=2 years	10	(4.8)	47	(6.4)	0.68 (0.32-1.43)	0.55 (0.23-1.30)
Calcium antagonists						
No use [d]	197		688		reference	reference
<2 years	8	(3.8)	36	(4.9)	0.77 (0.35-1.70)	0.70 (0.29-1.66)
>=2 years	5	(2.4)	16	(2.2)	1.03 (0.35-2.98)	1.06 (0.34-3.28)
Ace inhibitors, ATII antagonists						
No use [d]	187		675		reference	reference
<2 years	15	(7.1)	37	(5.0)	1.52 (0.79-2.93)	1.77 (0.86-3.64)
>=2 years	8	(3.8)	28	(3.8)	1.03 (0.45-2.38)	1.05 (0.43-2.60)
Miscellaneous AHD						
No use [d]	204		730		reference	reference
<2 years	3	(1.4)	4	(0.5)	2.81 (0.62-12.7)	3.00 (0.64-14.1)
>=2 years	3	(1.4)	6	(0.8)	1.80 (0.45-7.27)	1.76 (0.43-7.29)

AHD: antihypertensive drugs; OR: odds ratio; CI: confidence interval.

[a] A lag period of exposure was considered by subtracting 3 years from the index date. [b] Adjusted for age, gender and duration of follow-up. [c] Adjusted for age, gender, duration of follow-up and for use of other types of antihypertensive drugs (ever vs never use) [d] No use is defined as a cumulative use of <6 months.

Discussion

To our knowledge, we are the first to study the effect of AHD use on the risk of glioma in detail, using prospectively collected automated pharmacy data. The higher risk of glioma for users of any AHD, beta-blockers and miscellaneous AHD disappeared when a lag time of at least 3 years was considered. None of the risk estimates for the different classes of AHD reached statistical significance, and no patterns of dose-response relationship could be demonstrated.

The association between the use of AHD and the risk of malignancies is controversial. There is evidence for some cancers to be associated with the use of AHD, e.g. diuretics and renal cell carcinoma,[231] although these findings are still under debate.[244,245] For gliomas, results are contradictory and mainly concern childhood tumours. Prenatal exposure to diuretics was associated with an increased risk of childhood brain tumours,[234] which could not be confirmed by subsequent studies.[235-237] The risk of exposure to AHD in adulthood is not well known although in one publication, no increased risk could be shown for adults who used diuretics.[147] One study was published in which no association was found between systolic and diastolic blood pressure and brain tumours, but numbers of cases were small, the effect of AHD was not investigated, and gliomas were not studied as a separate group.[232]

We previously found a higher prevalence of hypertension in glioma patients.[239] Since glioma is not known to induce clinically relevant hypertension and because hypertension is not a known risk factor for glioma, we hypothesised that the use of AHD might be the link in the observed association. The results of this study do not support such an association. However, if hypertension is part of the prodromal signs of glioma, the use of AHD would be expected to be highest in the period shortly before diagnosis of glioma. Indeed, associations between AHD and the risk of glioma were mainly found in the lower exposure categories and disappeared in the sensitivity analyses. This strongly suggests protopathic bias.

Major strengths of this study are the population-based and prospectively collected data with detailed information about drug exposure and pathology. Pharmacy records are more complete and more reliable than medical records or patient interviews, thereby avoiding recall bias.[246] It has been shown that computerised pharmacy records are a reliable source of true current drug exposure,[242] and that any misclassification is non-differential, leading to underestimation of the true effect in pharmaco-epidemiological studies rather than overestimation.[242] We considered information about glioma diagnosis to be very reliable since several sources were combined, including PALGA which is not only used for research purposes but also for daily patient care. Detection bias seems unlikely as gliomas

will almost always become symptomatic, regardless of medical surveillance. We therefore did not adjust for the effect of medical attention due to comorbidity. We were able to adjust for gender, age, duration of follow-up and geographical region. Because the PHARMO database does not contain information on lifestyle variables, we were not able to adjust for lifestyle factors such as obesity, smoking and alcohol use. We do not expect this to be a problem since none of these factors have been associated with glioma before.[44] Also, we are not aware of other known factors associated with glioma that might have confounded the analyses.

The proportion of clinically diagnosed central nervous system tumours without histopathological confirmation increases with age, particularly after age 65 years.[2] We did not include these tumours in the present analyses since they are not reliably recorded in the available registries. This may have led to a different distribution of glioma subtypes in this study, and might have had an effect on the risk estimate. The distribution of diagnoses, however, is comparable with previous studies.[4]

A potential problem is that the duration of observation might have been too short to study the influence of AHD on glioma risk, possibly leading to an underestimation of risk. Furthermore, we studied five categories of AHD, based on the main mode of action. Within these categories however, drugs can have different modes of action, and this might have consequences for the pathogenesis of glioma. For instance, ACE inhibitors and angiotensin II inhibitors are both classified in ATC group C09 but might differ in their ability to induce or promote glioma. The numbers of cases were too small to analyse these AHD separately.

In conclusion, a causal association between AHD and glioma seems unlikely, although we cannot exclude the possibility that AHD modify the occurrence of glioma. Both overall exposure and exposure to subgroups of AHD could not be clearly related to a higher risk of glioma. Some associations were mainly found in the low cumulative exposure categories and disappeared in the sensitivity analyses, indicating that hypertension might be a prodromal sign of glioma. However, the precise factors underlying a higher prevalence of hypertension in glioma patients, as reported before, remain to be clarified.

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Chapter 2.4

Space-time clustering patterns of gliomas in the Netherlands suggest an infectious aetiology

Summary

To test the hypothesis that infectious exposures may be involved in glioma aetiology, we have analysed space-time clustering and seasonal variation using population-based data from the south of the Netherlands between 1983 and 2001. Knox tests for space-time interactions between cases were applied, with spatial coordinates of the addresses at time of diagnosis, and with distance to the Nth nearest neighbour. Data were also analysed by a second order procedure based on K-functions. Tests for heterogeneity and Edwards' test for sinusoidal variation were applied to examine seasonal variation of incidence. There was statistically significant space-time clustering in the eastern, but not in the western part of the region. Clustering was only present in adults, particularly in less densely populated areas. There was no evidence for seasonal variation. The results support a role for infectious exposures in glioma aetiology that may act preferentially in certain geographical areas.

Introduction

Gliomas are the most common primary brain tumours in children and adults. Thus far only ionising radiation has been established as an aetiological factor,[64,65] and few genetic syndromes exist which predispose to glioma.[90,91] These factors however can only explain a small minority of cases, whilst the evidence for many other proposed risk factors is inconclusive.[44] A role for infections in the aetiology of glioma has been suggested. Certain viruses, including polyomaviruses JC virus, BK virus and simian virus 40 (SV40) have been considered as possible aetiological agents but the findings have been inconsistent.[247,248] If infectious exposures are involved, the distribution of cases may exhibit space-time clustering. This

would happen if an aetiologically linked infectious exposure occurred in 'mini-epidemics' and would be detected when the lag time from exposure to diagnosis is short or relatively constant.

Space-time clustering is said to occur when excess numbers of cases are observed within small geographical locations at limited periods of time that cannot be explained in terms of general excesses in those locations or at those times. The presence of seasonal variation would also provide indirect evidence for an aetiology involving infections that exhibited seasonal epidemicity. Examples of infections that display such epidemicity include the common cold, influenza and measles. Space-time clustering has been examined previously for childhood brain tumours using data from the Manchester Children's Tumour Registry (MCTR). Statistically significant evidence for space-time clustering was found, particularly for astrocytoma and ependymoma, with an excess of patients born in Autumn or Winter.[249] However, in a study investigating childhood astrocytoma in Sweden, space-time clustering could not be shown.[54] To date, studies examining space-time clustering in adult glioma have not been published.

In the present study, we investigated space-time clustering and seasonal variation in adult and childhood glioma to assess the possibility of an infectious aetiology, using population-based data from cancer registries in the south of the Netherlands.

Methods

The Eindhoven Cancer Registry, within the framework of the Comprehensive Cancer Centre South, and the Cancer Registry of Rotterdam registered all glioma patients in North Brabant. This province in the south of the Netherlands has 2.3 million inhabitants and covers an area of nearly 5,000 km^2. The cancer registries in the Netherlands are characterised by high quality incidence data and near complete ascertainment.[2,221] Data were available for 1983-2001 for the eastern part and 1989-2001 for the western part of the province (the eastern and western parts are contiguous). To avoid any methodological bias, the eastern and western areas were analysed separately. All cases diagnosed with a central nervous system glioma were analysed.

For each case of glioma, geographical coordinates were allocated to the postcode of the address at the time of diagnosis. The geographical coordinates were obtained using the Dutch Triangular System (Rijksdriehoeksmeting; http://www.rdnap.nl), the most widely used geographical reference system in the Netherlands. This enabled spatial referencing of the easting and northing

coordinates to within 0.1 km of the actual address. For 7% of the cases in the total area of North Brabant and for all of those in a small region in the most western part of the western area, only partial postcodes (first four digits) were available, locating cases to the level of neighbourhoods and small municipalities. For these cases a random coordinate was used within the specified area. Sensitivity analyses were performed by repeating the analyses with another two different random coordinates. This created three data sets for analysis.

The following aetiological hypotheses were tested: (i) a primary factor influencing geographical or temporal heterogeneity of incidence of gliomas is related to exposure to an infectious or other similarly occurring environmental agent relatively close to disease onset; and (ii) geographical or temporal heterogeneity of incidence of gliomas is modulated by differences in patterns of exposure related to level of population density. Space-time interactions based on time and place of diagnosis were tested.

Knox space-time clustering tests were applied to the data with thresholds fixed, a priori, as: close in space, less than 5 km; and close in time, less than 1 year apart.[250] These limits are arbitrary but have been used in a number of studies of space-time clustering of childhood cancers from northwest England.[249,251-254] Furthermore, this problem is overcome by using the K-function method (see below). In the Knox test, a pair of cases is regarded as being in 'close proximity' if they are both diagnosed at addresses that are simultaneously close in space and at times that are close. The number of pairs of cases observed to be in close proximity was obtained (O) and the number of pairs of cases expected to be in close proximity was calculated (E). If O exceeded E there was space-time clustering and statistical tests were used to determine whether this excess was statistically significant. The magnitude of the excess (or deficit) was estimated by calculating strength S=((O-E)/E)*100. To adjust for the effect of different population densities, the tests were repeated replacing geographical distance thresholds by distance to the Nth nearest neighbour, using all locations of all the cases in the data set. N was chosen such that the mean distance was 5 km and was found to be 30.

Two problems are apparent with the Knox test. First, boundary problems may be important since it can be impossible, or less probable, for some cases to be close in one dimension to other cases. The second problem concerns the arbitrariness of the thresholds chosen. A simplification of a second order procedure based on K-functions was used in the present analyses to overcome the problem of arbitrary boundaries.[255] This procedure involved a set of 225 Knox-type calculations where the boundaries changed over a pre-specified set of values (for close times, t=0.1, 0.2,...,1.5 years and for close in space, s=0.5,

1, 1.5,...,7.5 km). Statistical significance was assessed by simulation. Nearest neighbour (NN) approaches were also used (analogous to those described in relation to classical Knox tests).

Two age groups were studied: 0-14 and 15+ years. These age groups were selected to attempt to differentiate between the potential effect of infectious exposures for children and older cases. For younger cases, genetic predisposition would be predicted to be an important component of aetiology in combination with the triggering event of an infectious exposure, whilst for older cases the main aetiological factor would be predicted to be the infectious exposure that precipitates the onset of the tumour.

To test the effect of the opportunity for exposure to infectious agents via closer person to person contact, analyses were performed for two levels of population density. Addresses were classified as being located in a more densely populated area, or being located in a less densely populated area. For addresses at time of diagnosis the median distance to the 30th nearest neighbour was found. Diagnosis locations, whose 30th nearest neighbour was less than the median distance, were classified in the 'more densely populated' category. Diagnosis locations, whose 30th nearest neighbour was greater than the median distance, were classified in the 'less densely populated' category. Analysis was undertaken by considering pairs of cases including at least one case from the 'more densely populated' category and pairs of cases including at least one case from the 'less densely populated' category. The observed and expected numbers of pairs of cases were calculated where: (i) both cases came from a 'more densely populated' area; (ii) both cases came from a 'less densely populated' area; and (iii) one case came from a 'more densely populated area' and the other case came from a 'less densely populated' area. It should be noted that these analyses (especially the analyses of clustering pairs including at least one case from the 'less densely populated' category) are potentially subject to a strong diluting influence from edge effects since neither the 'more densely populated' areas nor the 'less densely populated' areas form a single spatially contiguous zone.

Of the three data sets, for all the analyses, the most conservative results in terms of p-value and for the Knox test, strength (S) within p-value are presented in the tables. Statistical significance was indicated if $p<0.05$, using at least two of the four methods (the geographical or NN versions of the Knox test and the K-function method), and including a NN threshold version.

To examine seasonal variation the cases were examined for monthly variation in dates of birth and diagnosis using: (i) a chi-squared test for heterogeneity, and (ii) Edwards' test for sinusoidal variation.[256] The overall distribution of months of birth and diagnosis of all cancer patients registered by the Eindhoven Cancer

Registry were used to correct the underlying variation in birth and diagnosis dates. All data were analysed respecting the privacy legislation that applies in the Netherlands.

Results

In the province of North Brabant there were 1,545 cases of glioma diagnosed between 1983 and 2001 (59.5% males; median age at diagnosis 52 years, range 0-92). There were 37 cases of pilocytic astrocytoma, 1,064 cases of other astrocytoma, 131 cases of oligodendroglioma, 79 cases of ependymoma and 234 cases of other glioma including glioma not otherwise specified (NOS) and clinically diagnosed tumours without histopathological confirmation. There were 124 cases of glioma in the most western part of the western area with only partial postcodes available.

Table 1. Space-time clustering tests for glioma cases (all ages) in the south of the Netherlands and diagnosed during the period 1983-2001, analysed by area and time period

Area and time period (n)	Knox test (observed space-time pairs [a]; expected space-time pairs; strength [b]; p-value [c])		K-function analysis [f] (p-value [g])	
	Geographical distance [d]	NN threshold [e]	Geographical distance [h]	NN threshold [j]
East, 1983-2001 (752)	O=2,550; E=2,483 S=2.7%; p=0.09	O=1,659; E=1,555 S=6.7%; p=0.005	p=0.14	p=0.01
West, 1989-2001 (793)	O=2,851; E=2,785 S=2.4%; p=0.11	O=2,411; E=2,366 S=1.9%; p=0.18	p=0.34	p=0.32

NN: nearest neighbour; O: observed; E: expected; S: strength.

[a] Cases are close in time if dates of diagnosis differ by less than 1 year. [b] Strength=((observed-expected)/expected)*100 counts of pairs which are close in time and space. [c] One-sided p-value derived from the Poisson distribution. [d] When using geographical distance cases are close in space if their locations are <5 km apart. [e] When using nearest neighbour thresholds cases are close in space if the locations of one (or both) is nearer than the other's 30th nearest neighbour in the total data set. [f] Cases are close in time if dates differ by <t where t is in the range 1-18 months. [g] p-value obtained by simulation (999 runs) with dates of diagnosis randomly re-allocated to the cases in the analysis. [h] When using geographical distance cases are close in space if distances between their locations differ by <s where s is in the range 0.5-7.5 km. [j] When using nearest neighbour thresholds cases are close in space if either is within the distance to the Nth nearest neighbour of the other (in the total data set) where N is in the range 23-37.

There was statistically significant space-time clustering for cases from the eastern, but not for cases from the western part of the province ($p<0.05$ using at

least two methods and including a NN threshold version) (table 1). Statistically significant space-time clustering was found for cases of glioma aged over 15 years ($p<0.05$ using at least two methods and including a NN threshold version), but not for children aged 0-14 years. Again this was apparent for cases from the east but not the west (table 2). There was also no cross-clustering between the older (aged 15+ years) and younger cases (aged 0-14 years). When testing for population density, there was statistically significant space-time clustering involving cases from 'less densely populated' areas in the east but not the west (table 3). Finally, there was no evidence of seasonal variation within both age groups using either the chi-squared test for heterogeneity or Edwards' test for sinusoidal variation (data not shown).

Table 2. Space-time clustering tests for glioma cases in the south of the Netherlands and diagnosed during the period 1983-2001, analysed by area, time period and age group

Area, time period and age group (n)		Knox test (observed space-time pairs [a]; expected space-time pairs; strength [b]; p-value [c])		K-function analysis [f] (p-value [g])	
		Geographical distance [d]	NN threshold [e]	Geographical distance [h]	NN threshold [j]
East, 1983-2001	Age 0-14 (56)	O=8; E=10.5 S=-23.5%; p=0.72	O=6; E=8.6 S=-29.9%; p=0.75	p=0.94	p=0.89
	Age 15+ (696)	O=2,239; E=2,161 S=3.6%; p=0.05	O=1,440; E=1,336 S=7.8%; p=0.003	p=0.06	p=0.003
West, 1989-2001	Age 0-14 (45)	O=9; E=10 S=-9.6%; p=0.54	O=4; E=7.8 S=-48.5%; p=0.89	p=0.52	p=0.79
	Age 15+ (748)	O=2,543; E=2,494 S=2.0%; p=0.16	O=2,162; E=2,120 S=2.0%; p=0.19	p=0.44	p=0.33

NN: nearest neighbour; O: observed; E: expected; S: strength.

[a] Cases are close in time if dates of diagnosis differ by less than 1 year. [b] Strength=((observed-expected)/expected)*100 counts of pairs which are close in time and space. [c] One-sided p-value derived from the Poisson distribution. [d] When using geographical distance cases are close in space if their locations are <5 km apart. [e] When using nearest neighbour thresholds cases are close in space if the locations of one (or both) is nearer than the other's 30th nearest neighbour in the total data set. [f] Cases are close in time if dates differ by <t where t is in the range 1-18 months. [g] p-value obtained by simulation (999 runs) with dates of diagnosis randomly re-allocated to the cases in the analysis. [h] When using geographical distance cases are close in space if distances between their locations differ by <s where s is in the range 0.5-7.5 km. [j] When using nearest neighbour thresholds cases are close in space if either is within the distance to the Nth nearest neighbour of the other (in the total data set) where N is in the range 23-37.

Table 3. Space-time clustering tests for glioma cases in the south of the Netherlands and diagnosed during the period 1983-2001, analysed by area, time period and population density

Area, time period and population density		Knox test (observed space-time pairs [a]; expected space-time pairs; strength [b]; p-value [c])		K-function analysis [f] (p-value [g])	
		Geographical distance [d]	NN threshold [e]	Geographical distance [h]	NN threshold [j]
East, 1983-2001	MDP [k]	O=2,221; E=2,172 S=2.2%; p=0.15	O=1,039; E=1,003 S=3.6%; p=0.13	p=0.19	p=0.14
	LDP [l]	O=463; E=444 S=4.2%; p=0.19	O=823; E=751 S=9.6%; p=0.005	p=0.25	p=0.02
West, 1989-2001	MDP [k]	O=2,385; E=2,336 S=2.1%; p=0.16	O=1,486; E=1,459 S=1.8%; p=0.24	p=0.37	p=0.49
	LDP [l]	O=791; E=786 S=0.6%; p=0.43	O=1,370; E=1,363 S=0.5%; p=0.43	p=0.57	p=0.52

NN: nearest neighbour; O: observed; E: expected; S: strength; MDP: more densely populated; LDP: less densely populated. [a] Cases are close in time if dates of diagnosis differ by less than 1 year. [b] Strength=((observed-expected)/expected)*100 counts of pairs which are close in time and space. [c] One-sided p-value derived from the Poisson distribution. [d] When using geographical distance cases are close in space if their locations are <5 km apart. [e] When using nearest neighbour thresholds cases are close in space if the locations of one (or both) is nearer than the other's 30th nearest neighbour in the total data set. [f] Cases are close in time if dates differ by <t where t is in the range 1-18 months. [g] p-value obtained by simulation (999 runs) with dates of diagnosis randomly re-allocated to the cases in the analysis. [h] When using geographical distance cases are close in space if distances between their locations differ by <s where s is in the range 0.5-7.5 km. [j] When using nearest neighbour thresholds cases are close in space if either is within the distance to the Nth nearest neighbour of the other (in the total data set) where N is in the range 23-37. [k] >=1 case from a more densely populated area. [l] >=1 case from a less densely populated area.

Discussion

To our knowledge, we are the first to apply formal statistical methods on population-based incidence data to study space-time clustering in adult glioma. Space-time clustering based on time and place of diagnosis was found. Clustering was only present in adults (aged 15+ years) from the eastern part of the province, particularly in less densely populated areas. Conversely, there was no evidence for space-time clustering amongst children (aged 0-14 years). Seasonal variation in incidence of glioma could not be shown.

The cancer incidence data from the Comprehensive Cancer Centres in the Netherlands are characterised by high quality and near complete ascertainment.[2,221] Pathological diagnoses were derived from different sources including the Dutch

computerised nationwide registry of histo- and cytopathology (PALGA) and the Dutch Medical Register (LMR), a hospital discharge registry. Methods for data collection were the same in the western and the eastern areas and have not changed since 1983. For all cases the address at diagnosis was recorded, as well as the last known address which is regularly updated using municipal records. For 24 cases only, these addresses were different indicating a low possibility of bias due to migration. Otherwise, migration may lead to either an underestimation or overestimation of the strength of clustering.

Unfortunately it was not possible to analyse the data as one entity because cases were not consistently available for the entire study area since 1983. Thus separate analyses were undertaken. However, comparison between the east and the west was possible. There is no method for combining the results of these separate analyses as the effect of time and space boundaries would invalidate such an attempt.

The problem of cases with only partially known postcodes was solved by sensitivity analyses using different data sets with random coordinates within the specified area. The analyses were performed using rigorous statistical methods. The many tests involved in this study raised the possibility of a multiple testing problem. Although analyses were performed following prior hypotheses and although only the most conservative results were used, the results still have to be interpreted with care. The number of cases in the younger age group (0-14 years) was small compared with the study from northwest England,[249] so there was much less power in the present study to be able to detect clustering in this age group.

It is possible that the methodology may be biased if there are certain differential population changes during the time period, especially when the population grows or declines at different rates in different areas of the study region. A method to deal with this particular type of problem has been proposed,[257] but it would not be possible to implement this procedure on the current data set, because it requires small area population data by month that are not available. However, it must be stressed that the current analyses provide a description of the space-time clustering patterns in the data, whether real or artifactual. Additionally, variations in population growth are not thought to be important in the current data set.

The pattern of space-time clustering found in this study is consistent with an exposure occurring at a relatively short time period before onset of the disease. It is likely that this exposure is more important among those aged over 15 years. The nature of space-time interaction implies an exposure emerging at many points in both place and time. Therefore, more sustained exposures which are geographically fixed and present for long periods of time (e.g. power lines,

environmental pollution or industry) can be excluded. The pattern is however more consistent with an infectious agent. Since there was only space-time clustering in the eastern part of the province, this agent is likely to act in limited geographical areas without spreading to other regions. This would imply that this agent does not have the capability for rapid spreading, or that it is linked to, e.g., industries or environments that are more common in the east. The more marked clustering in less densely populated areas might indicate that the aetiological agent is more prevalent in these environments. We however do not know of any common industry or environment that is typical for the eastern part of the province of North Brabant.

Evidence for the involvement of infections in the aetiology of glioma comes primarily from studies in experimental animals and from the isolation of several viruses from human tumour material. The importance of these findings to glioma aetiology is uncertain. Few epidemiological studies addressing the role of infections have been published, which may also indicate unpublished negative results. For adult glioma, antibody titres to Toxoplasma gondii were linked to astrocytoma,[258] although an association could not be confirmed by others.[259] For childhood glioma, four epidemiological studies suggested an infectious component to aetiology,[260-263] whilst another case-control study found no such relations.[264]

No studies concerning space-time clustering in adults have been published thus far. Therefore comparisons can only be made for childhood brain tumours. In the present study, no clustering was detected for the youngest age category. Also, no space-time clustering was found in childhood astrocytoma in Sweden.[54] Space-time clustering was however reported for childhood brain tumours using population-based data from the Manchester Children's Tumour Registry (MCTR).[249] Strong evidence for space-time clustering was found for astrocytoma, ependymoma and all glioma combined. The present study contained far fewer cases of childhood glioma than the MCTR study, whilst the Swedish study used a different methodology. It is possible that the lack of space-time clustering in the present study is due to insufficient power to detect such an effect.

We found no evidence for seasonal variation in glioma incidence. In earlier studies however, seasonal variation was observed for childhood astrocytoma and ependymoma,[249] for all childhood brain tumours,[265] and for adult glioma.[266] All studies reported excesses in incidence for late Autumn and Winter births. The first two studies concerned childhood glioma and brain tumours only, probably explaining most of the discrepancies with the present study in which there was insufficient power owing to a lack of childhood cases. The third study investigating adult glioma used a different methodology. Furthermore, we used a

robust method of adjusting variations in birth and diagnosis date with the overall distribution of months of birth and diagnosis for all cancer patients registered by the cancer registry.

In summary, space-time clustering was found for cases of glioma from the eastern part of the province, but only for adults aged >15 years. The results are consistent with an infectious agent, mainly acting in limited, less densely populated geographical areas without spreading to other regions. It is difficult to draw any firm conclusions concerning the childhood cases (aged 0-14 years) due to small numbers.

It is not clear whether there are one or more candidate infections or whether infectious agents in general act as a tumour promoter. Further research should include both epidemiological and laboratory investigations. An ecological investigation could relate incidence rates to levels of deprivation and studies of spatial clustering could determine if there are small areas with sustained high incidence. Laboratory studies might examine differences in the occurrence of specific putative agents between 'clustering' and 'non-clustering' cases.

Chapter 2.5

Space-time clustering of gliomas cannot be attributed to specific histological subgroups

Summary

We previously showed that infectious exposures may be involved in the aetiology of adult glioma, by analysing for space-time clustering using population-based data from the south of the Netherlands. Here, we extended these analyses and describe in detail the space-time clustering patterns in glioma subgroups, gender and age categories. Knox tests for space-time interactions between cases were applied, with spatial coordinates of the addresses at time of diagnosis. Tests were repeated replacing geographical distance with distance to the Nth nearest neighbour. Data were also analysed by a second order procedure based on K-functions. There was only statistically significant space-time clustering for oligodendroglioma. Clustering was present for adults aged 30-54 years and was more pronounced among males. Given the low prior probability of an infectious aetiology for this specific subgroup, these results should probably be interpreted as false-positive. We conclude that space-time clustering of glioma cannot be attributed to a specific glioma subgroup. The observed clustering in our previous study is therefore probably an overall effect within and between glioma subgroups.

Introduction

Gliomas are malignant primary brain tumours of neuroepithelial origin. The aetiology of glioma remains puzzling. Many environmental risk factors have been considered,[44] but only for ionising radiation has an aetiological role been established.[64,65] It has been suggested that certain viruses might induce gliomas, but attempts to verify such an effect have been inconsistent.[247,248,267]

If infectious agents are involved, the distribution of cases may exhibit space-time clustering. Space-time clustering is said to occur when excess numbers

of cases are observed within small geographical locations at limited periods of time. This would happen if an aetiologically linked infectious exposure occurred in 'mini-epidemics'. We previously showed that space-time clustering exists between adult glioma patients, in an all-glioma analysis using population-based data from the south of the Netherlands (chapter 2.4).[268] This was the first time that space-time clustering analyses were applied to adult glioma. Space-time clustering was only evident in the eastern part of the examined area, suggesting that an aetiological agent was acting in this area during the studied period.

Gliomas comprise a group of distinct tumours which vary in their cells of origin, locations within the central nervous system (CNS), morphological appearance and prognosis. Different factors may be involved in the aetiology of the various tumours. Furthermore, gender could be related either to susceptibility to infectious agents, or to exposure rates when the infectious agent is related to occupation or lifestyle. We therefore extended our analyses in the south of the Netherlands, but only for the eastern area where space-time clustering was found, and tried to identify glioma subgroups for which an infectious aetiology might be involved. We describe the space-time clustering patterns for the various types of glioma, gender and age categories.

Methods

All glioma patients in the southeastern area of the Netherlands, located in the province of North Brabant, were registered by the Eindhoven Cancer Registry within the framework of the Comprehensive Cancer Centre South.[221] Data were available for 1983-2001. Cases were classified into diagnostic groups according to the World Health Organisation (WHO) classification of CNS tumours.[269] Five diagnostic groups were specified a priori for analysis: (i) astrocytoma WHO grade II; (ii) astrocytoma WHO grade III-IV; (iii) all astrocytoma; (iv) oligodendroglioma, and (v) ependymoma.

For each case of glioma, geographical coordinates were allocated to the postcode of the address at the time of diagnosis. The geographical coordinates were obtained using the Dutch Triangular System (Rijksdriehoeksmeting; http://www.rdnap.nl). This enabled spatial referencing of the easting and northing coordinates to within 0.1 km of the actual address. For 7% of the cases only partial postcodes (first four digits) were available, locating cases to the level of neighbourhoods and small municipalities. For these cases a random coordinate was used within the specified area. Sensitivity analyses were performed by repeating the analyses with another two different random coordinates. This created three data sets for analysis.

Space-time interactions based on time and place of diagnosis were tested. Knox space-time clustering tests were applied to the data with thresholds fixed, a priori, as: close in space, less than 5 km, and close in time, less than 1 year apart.[250] In the Knox test, a pair of cases is regarded as being in 'close proximity' if they are both diagnosed at addresses that are simultaneously close in space and at times that are close. The number of pairs of cases observed to be in close proximity was obtained (O) and the number of pairs of cases expected to be in close proximity was calculated (E). If O exceeded E there was space-time clustering. The magnitude of the excess (or deficit) was estimated by calculating strength S=((O-E)/E)*100. To adjust for the effect of different population densities, the tests were repeated replacing geographical distance thresholds by distance to the Nth nearest neighbour, using all locations of all the cases in the data set. N was chosen such that the mean distance was 5 km and was found to be 30.

Two problems are apparent with the Knox test. First, boundary problems may be important since it can be impossible, or less probable, for some cases to be close in one dimension to other cases. The second problem concerns the arbitrariness of the thresholds chosen. A simplification of a second order procedure based on K-functions was used in the present analyses to overcome the problem of arbitrary boundaries.[255] This procedure involved a set of 225 Knox-type calculations where the boundaries changed over a pre-specified set of values (for close times, t=0.1, 0.2,...,1.5 years and for close in space, s=0.5, 1, 1.5,...,7.5 km). Statistical significance was assessed by simulation. Nearest neighbour (NN) approaches were also used, analogous to those described in relation to classical Knox tests.

The primary analysis was restricted to the a priori specified diagnostic groups. Statistical significance was indicated if $p<0.05$, using at least two of the four methods (the geographical or NN versions of the Knox test and the K-function method) and including a NN threshold version. If a group showed such statistically significant space-time clustering then it was analysed further. Analysis was then extended within age and gender subgroups. Three age groups were studied: 15-29 years, 30-54 years, and 55+ years. These age groups, whilst somewhat arbitrary, were selected to attempt to differentiate between the potential effect of infectious exposures for younger and older cases. If there is a difference in susceptibility or exposure between males and females, the strength and statistical significance of space-time clustering would differ by gender. To test this prediction, first space-time clustering analyses were performed for clustering pairs involving at least one male case and secondly for pairs involving at least one female case.

Of the three data sets, for all the analyses the most conservative results in terms of p-value and, for the Knox test, strength (S) within p-value are presented in

the tables. Statistical significance was indicated if $p<0.05$, using at least two of the four methods (the geographical or NN versions of the Knox test and the K-function method) and including a NN threshold version. All data were analysed respecting the privacy legislation that applies in the Netherlands.

Results

In the southeastern Netherlands there were 738 cases of adult glioma diagnosed between 1983 and 2001 comprising 46 cases of astrocytoma WHO grade II, 449 cases of astrocytoma WHO grade III-IV, 495 cases of all astrocytoma, 62 cases of

Table 1. Space-time clustering tests for glioma subgroups in the southeastern Netherlands and diagnosed during the period 1983-2001

Disease group (n)	Knox test [a] (strength [b]; p-value [c])		K-function analysis [f] (p-value [g])	
	Geographical distance [d]	NN threshold [e]	Geographical distance [h]	NN threshold [j]
Astrocytoma grade II (46)	S=26.7% p=0.20	S=25.6% p=0.19	p=0.11	p=0.08
Astrocytoma grade III-IV (449)	S=1.6% p=0.31	S=-1.2% p=0.61	p=0.58	p=0.63
All astrocytoma (495)	S=1.0% p=0.36	S=0.5% p=0.45	p=0.53	p=0.45
Oligodendroglioma (62)	S=43.7% p=0.04	S=73.9% p=0.01	p=0.01	p=0.004
Ependymoma (44)	S=10.3% p=0.41	S=17.9% p=0.38	p=0.55	p=0.25

NN: nearest neighbour; S: strength.

[a] Cases are close in time if dates of diagnosis differ by less than 1 year. [b] Strength=((observed-expected)/expected)*100 counts of pairs which are close in time and space. [c] One-sided p-value derived from the Poisson distribution. [d] When using geographical distance cases are close in space if their locations are <5 km apart. [e] When using nearest neighbour thresholds cases are close in space if the locations of one (or both) is nearer than the other's 30th nearest neighbour in the total data set. [f] Cases are close in time if dates differ by <t where t is in the range 1-18 months. [g] p-value obtained by simulation (999 runs) with dates of diagnosis randomly re-allocated to the cases in the analysis. [h] When using geographical distance cases are close in space if distances between their locations differ by <s where s is in the range 0.5-7.5 km. [j] When using nearest neighbour thresholds cases are close in space if either is within the distance to the Nth nearest neighbour of the other (in the total data set) where N is in the range 23-37.

oligodendroglioma and 44 cases of ependymoma. A further 137 cases of other glioma and glioma not otherwise specified were excluded from the analyses.

There was only statistically significant space-time clustering for oligodendroglioma ($p<0.05$ using at least two of the four methods and including a NN threshold version). There was no evidence for statistically significant space-time clustering in astrocytoma or ependymoma (table 1).

Table 2. Space-time clustering tests by age group and gender, for oligodendroglioma in the southeastern Netherlands and diagnosed during the period 1983-2001

Age group and clustering pairs (n)	Knox test [a] (strength [b]; p-value [c])		K-function analysis [f] (p-value [g])	
	Geographical distance [d]	NN threshold [e]	Geographical distance [h]	NN threshold [j]
Oligodendroglioma				
Age 15-29 (7)	S=-100% p=1.0	N/A	N/A	N/A
Age 30-54 (32)	S=62.6% p=0.14	S=111% p=0.05	p=0.02	p=0.007
Age 55+ (22)	S=-24.0% p=0.56	S=-32.5% p=0.44	p=0.59	p=0.58
Oligodendroglioma				
>= 1 male case	S=39.7% p=0.07	S=68.1% p=0.03	p=0.008	p=0.003
>= 1 female case	S=57.8% p=0.03	S=69.2% p=0.04	p=0.02	p=0.01

NN: nearest neighbour; N/A: not available; S: strength.

[a] Cases are close in time if dates of diagnosis differ by less than 1 year. [b] Strength=((observed-expected)/expected)*100 counts of pairs which are close in time and space. [c] One-sided p-value derived from the Poisson distribution. [d] When using geographical distance cases are close in space if their locations are <5 km apart. [e] When using nearest neighbour thresholds cases are close in space if the locations of one (or both) is nearer than the other's 30th nearest neighbour in the total data set. [f] Cases are close in time if dates differ by <t where t is in the range 1-18 months. [g] p-value obtained by simulation (999 runs) with dates of diagnosis randomly re-allocated to the cases in the analysis. [h] When using geographical distance cases are close in space if distances between their locations differ by <s where s is in the range 0.5-7.5 km. [j] When using nearest neighbour thresholds cases are close in space if either is within the distance to the Nth nearest neighbour of the other (in the total data set) where N is in the range 23-37.

The only group to be analysed further was oligodendroglioma. Regarding age categories, statistically significant space-time clustering ($p<0.05$ using at least two

of the four methods and including a NN threshold version) was confined to cases aged 30-54 years (table 2). Although statistically significant in both groups, clustering was more pronounced for clustering pairs involving at least one male case than for pairs involving at least one female case (table 2).

Discussion

In a previous study in which we investigated space-time clustering in an all-glioma analysis, we showed that space-time clustering only exists for adult glioma.[268] Space-time clustering was evident in the eastern part of the south of the Netherlands, suggesting that an aetiological agent was acting in this area during the studied period. In the present study we focused on this southeastern area. We found statistically significant space-time clustering for oligodendroglioma. Clustering was present for adults aged 30-54 years and was more pronounced among males. There was no evidence for statistically significant space-time clustering in astrocytoma or ependymoma.

The Eindhoven Cancer Registry is characterised by high quality incidence data and almost complete ascertainment.[2,221] Pathological diagnoses were derived from different sources including the Dutch nationwide network and registry of histo- and cytopathology (PALGA) and the Dutch Medical Register (LMR), a hospital discharge registry. These registries are considered to be very reliable.[221] For all cases the address at diagnosis was recorded as well as the last known address which is regularly updated using municipal records. For less than 10 cases only, these addresses were different indicating a low possibility of bias due to migration. The analyses were performed using rigorous statistical methods although there could be a multiple testing problem. We however followed prior hypotheses and only the most conservative results were used. Further limitations in this study are the low numbers of cases in some of the analysed subgroups, possibly leading to false-negative results.

Several diagnostic groups were analysed. We chose to follow the major histological subgroups that are easily recognised at histopathological examination.[269] Most cases were examined by a neuropathologist at time of diagnosis. We only analysed histopathologically confirmed gliomas and excluded all tumours without pathological confirmation and gliomas of uncertain histology. High-grade gliomas can be found de novo but can also develop through dedifferentiation of lower-grade lesions.[5] These primary and secondary tumours may be due to different precipitating factors. We therefore analysed low-grade (WHO grade II) and high-grade (WHO grade III-IV) astrocytomas separately.

The interpretation of the results is not straightforward. The probability that our findings of space-time clustering in oligodendrogliomas are correct depends not only on the p-value, but also on the prior probability that the results are real and on the statistical power.[270] We have two main arguments that the observed clustering might be a false-positive result. First, the prior evidence for an infectious aetiology for oligodendroglioma is weak. In fact, many infectious agents have been considered a role in glioma aetiology,[268] but results from different epidemiological studies are conflicting and the importance of these findings to glioma aetiology are uncertain.[145,258-264,271] No other studies considering space-time clustering in subgroups of adult glioma have been published. However, space-time clustering has been examined for childhood brain tumours using data from the Manchester Children's Tumour Registry (MCTR). Evidence for space-time clustering was found, but mainly for astrocytoma and ependymoma.[249] Overall, the prior probability of involvement of an infectious agent in the aetiology of oligodendroglioma is low.

The second argument concerns the fact that space-time clustering was only found in middle-aged patients, particularly in males. This selectivity is difficult to explain and is therefore more likely to be a false-positive finding. We cannot exclude the possibility that an infectious exposure is more prevalent among adults aged 30-54 years and among males. However, clues about a specific aetiological agent that exhibits such a pattern are not obvious. Infectious agents specifically associated with occupation and lifestyle situations in males and in middle-aged adults might be involved, but also for these situations, no association with glioma is established.[44]

Despite the absence of space-time clustering in glioma subgroups, we previously showed that space-time clustering exists in adult glioma.[268] Is this all-glioma analysis, the observed clustering occurred within and between different diagnosis groups. Since clustering is lost in the analyses for glioma subgroups, there is probably no selectivity of space-time clustering for any of the adult glioma subgroups.

In conclusion, space-time clustering was only found for adult onset oligodendroglioma, and for males more than for females. Given the weak prior evidence for an infectious aetiology of oligodendroglioma, and given the positive findings in only a subgroup of patients, these results should probably be interpreted as false-positive. The effect of an infectious agent in the aetiology of glioma, as reported before,[268] can therefore not be attributed to a specific glioma subgroup. However, we cannot exclude the possibility of an infectious agent preferentially inducing adult oligodendroglioma. Further evidence for an infectious aetiology should now come from other studies, with emphasis on specific histological subgroups.

Chapter 3

Genetic risk factors

Chapter 3.1

Genetic epidemiological research designs for glioma

Difficulties in the genetic epidemiological research of glioma

The principal drawbacks of traditional epidemiological or molecular genetic study designs are fourfold: the rarity of glioma, the short survival time leading to reliance on proxies, the limited numbers of high-cancer-dense families and difficulty in distinguishing causative somatic mutations from chaotic neoplastic lesions within tumour tissue. One straightforward approach to clarify the pathogenesis of glioma is to start with the identification of germline mutations. Several strategies can be followed. Linkage analysis in families in which the disease is transmitted has been a powerful approach in the localisation of highly penetrant genes involved in a number of hereditary tumours.[272-277] However, the power of these studies is low in glioma. First, gliomas are difficult to study through classic linkage studies, as extended families in which multiple (living) affected subjects can be found in three generations or more are rare. Second, different genes may play a role in different families and pooling of families may result in false exclusion of linkage.[278] Finally, false-negative findings may occur because the disease may result from the interaction of a variety of genetic and environmental risk factors. An alternative approach to linkage studies is to examine affected sibling pairs. However, a large number of siblings (200-800 pairs) is required to study a genetically complex disease,[279] which limits the feasibility of affected sib-pair study of glioma, even with international collaborations.

In recent years, there has been growing interest in identifying disease genes through association studies using population-based patient series rather than families.[278-280] The basis of genetic association is that a disease gene and adjacent markers (haplotype) are transmitted together from a founding ancestor. Whenever a sizeable proportion of patients share a common ancestor, a genetic association should be detectable. A genomic screen with DNA-markers can then identify these haplotypes, thereby indicating the location of the disease gene.[281,282] The statistical power of association studies, however, is limited. Owing

to heterogeneity in complex genetic disorders, it is unlikely that a substantial number of patients descend from a common ancestor if patients are randomly drawn from the general population.

The situation is more favourable in isolated populations that are characterised by few founding ancestors (or a population bottleneck) and low migration. Due to several processes at the level of population genetics, genetic heterogeneity is markedly reduced.[278,283] The small number of founders cannot represent the total genetic diversity of the general population. Owing to random fluctuations in gene transmission and occasional disappearance of mutations from the population (genetic drift), genetic complexity is further reduced. Furthermore, limited migration prevents the introduction of new mutations from the general population. This benefit has been successfully used in the deCODE studies in the isolated population of Iceland, where 44 patients with Parkinson's disease were linked to a common ancestor in an extensive pedigree.[284] Patients with Parkinson's disease were significantly more related to each other than were subjects in matched groups of controls. Similar to glioma, familial aggregation in Parkinson's disease is weak. Genetic isolates have also been successfully used to study diverse genetic disorders,[285] including early-onset parkinsonism,[286] type 2 diabetes,[287] and dementia.[288]

Glioma in an isolated population

We evaluated the feasibility of such studies for glioma. From the Eindhoven Cancer Registry, twelve glioma patients diagnosed between 1988 and 1998 were identified in a genetically isolated population in the southwest of the province of North Brabant in the Netherlands. This population was founded around 1750 by approximately 150 ancestors, and was further characterised by limited migration and rapid expansion to more than twenty thousand inhabitants. By extensive genealogical research, a pedigree was constructed connecting nine of the twelve patients to one common ancestor (figure 1). Clinical details of the nine patients are given in table 1. Seven patients had an astrocytoma World Health Organisation (WHO) grade IV and two patients had an astrocytoma WHO grade II that reoccurred as grade III and IV tumours. The median age at diagnosis was 60 years (range 24-73) with a median survival time of 10 months. These clinical features do not appear to be markedly different from those of patients with sporadic tumours. However, the relatedness of the patients within seven to twelve generations suggests a common genetic origin of the tumours. Consanguinity

as early as four generations was also seen, which raises the possibility that an autosomal recessive mutation is segregating in this family.

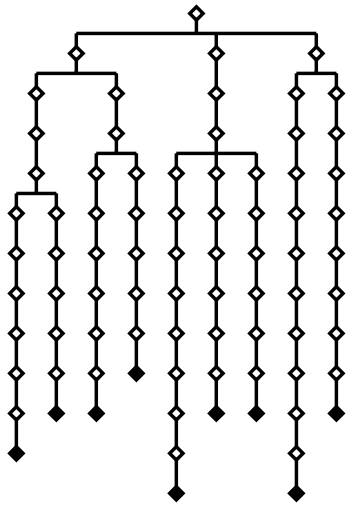

Figure 1. Pedigree of nine patients with glioma from a genetically isolated population in the Netherlands. Shaded diamonds represent patients with glioma recorded between 1988 and 1998. Open diamonds represent previous generations linked by a common ancestor. Marriages have occurred between arms of the family tree but have been omitted for simplicity.

Table 1. Clinical details of nine patients with glioma from a genetically isolated population in the Netherlands

Patient	Sex	Tumour type	WHO grade	Age at diagnosis, y	Survival, mo
1	M	Astrocytoma	II-III	24	72
2	F	Astrocytoma	IV	63	>18
3	M	Astrocytoma	IV	60	6
4	M	Astrocytoma	IV	30	15
5	F	Astrocytoma	IV	73	6
6	F	Astrocytoma	II-IV	33	59
7	M	Astrocytoma	IV	65	10
8	M	Astrocytoma	IV	73	0.5
9	F	Astrocytoma	IV	45	6

WHO: World Health Organisation; y: years; mo: months; F: female; M: male.

A common genetic origin cannot be proven based on genealogy alone. Pedigrees like these however provide exciting opportunities for genetic epidemiological research. One possibility is homozygosity mapping, a method to identify a recessive disease locus with only a very small number of consanguineous patients.[289] This method is based on the principle that patients can become homozygous for a recessive disease gene because it is inherited twice from the same ancestor, both via the mother and the father. Again, not only the disease

gene but also the surrounding haplotype is transmitted to the offspring. With a genomic screen, homozygous regions on the genome that are shared by all patients can be identified, and possible locations for the gene of interest can be mapped (figure 2).

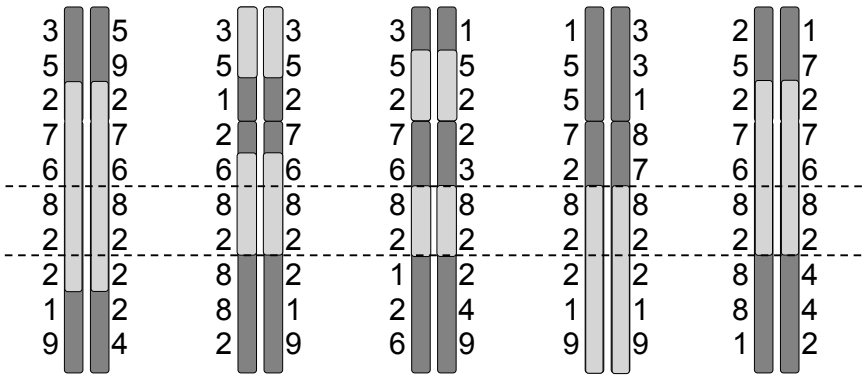

Figure 2. Imaginary experiment in which five distantly related and consanguineous glioma patients were genotyped with a genome-wide marker set. For the five patients, the markers on chromosome pair 1 are displayed. Homozygous regions are marked in light grey, the shared homozygous region indicates the possible location of a glioma gene (between dotted lines).

Chapter 3.2

Collection of glioma patients and genealogical research in genetically isolated populations

Summary

In chapter 3.1, we evaluated the feasibility of genetic epidemiological studies of glioma. The findings prompted us to study the genealogy of newly diagnosed glioma patients from genetically isolated populations, and to collect genetic material to be used in genetic studies. We also collected patients in a population-based approach. We selected three populations for the inclusion of patients, ranging in size from twenty thousand to two million inhabitants. Both prevalent and incident patients were selected from the Dr. Bernard Verbeeten Institute for radiotherapy and the neurosurgical centre of the St. Elisabeth Hospital in Tilburg. All patients were invited to provide genealogical information and to give a blood sample during a house visit. Genealogical research was performed by experienced genealogists. Two hundred-six patients were found eligible and 82 patients (40%) were willing to participate in the study. The distribution of histopathological subgroups of glioma was in favour of tumours with a better prognosis. Four pedigrees of distantly related glioma patients could be constructed. We show that by the systematic collection and genealogical research of newly diagnosed glioma patients, familial clusters of glioma can be identified. We also discuss potential problems that arise from the low response rate, and make recommendations for a more efficient procedure, that might be useful for diseases that are characterised by low incidence and high morbidity and mortality.

Introduction

The role of a genetically determined predisposition to glioma remains uncertain. Three segregation analyses yielded evidence for both a multifactorial mendelian model,[92,176] and for an autosomal recessive gene model, which could possibly

explain 2% of all glioma cases.[135] Low-penetrant or recessive diseases may appear to occur sporadically. Consanguineous relationships however greatly increase the possibility of acquiring such a disease, as discussed in chapter 3.1. If clustering of patients is recognised, particularly in a genetically isolated population, genealogical research is warranted.

We previously evaluated the feasibility of genetic epidemiological studies of glioma in genetically isolated populations (chapter 3.1).[220] We were able to connect nine sporadic glioma patients from such a population to one common ancestor within seven to twelve generations. The relatedness of the patients and consanguinity as early as four generations suggested a common genetic origin of the tumours, particularly an autosomal recessive mutation segregating in this family. We concluded that genetic research in isolated populations could be feasible for a genetically complex disease like glioma.[220] These findings prompted us to study the genealogy of newly diagnosed glioma patients and to collect genetic material to be used in genetic studies. We also collected patients in a population-based approach. Here we describe these patients and the results of the genealogical research. We end this chapter with recommendations for the collection of patients with rare diseases that are characterised by high morbidity and mortality.

Methods

Selected areas for patient collection

We selected three areas for patient collection that are summarised in table 1. The first area was the same as the population that we evaluated before.[220] This population was founded around 1750 by approximately 150 ancestors. The descendants lived in relative isolation until the middle of the 20th century. After decades of very slow growth, the population expanded rapidly to more than twenty thousand individuals. Because of the rarity of glioma and the expected small numbers of patients, patients from a wide region of rural areas and small villages surrounding this community were also included.

Another area for patient collection was located in a medium-sized city of approximately 200,000 inhabitants in the south of the Netherlands, and the small municipalities north of this city. This region was selected based on the experience of local genealogists, indicating that this area is characterised by limited migration and suitable for genealogical research with sufficient sources of genealogical data.

The third region was the complete province of North Brabant, which comprises approximately two million inhabitants. The estimated annual incidence in this area is less than 100 gliomas.[1]

Table 1. Characteristics of three selected areas for the collection of glioma patients

Area	Characteristic	Number of inhabitants	Source of patients	Genealogical research
1	Genetically isolated	20,000	Prevalent, Jan 2002 Incident, Jan 2002 - July 2003	Yes
2	Low migration	200,000	Prevalent, Jan 2002 Incident, Jan 2002 - July 2003	Yes
3	Total province	2,000,000	Incident, Jan - July 2003	No

Patient selection

All prevalent patients on January 1, 2002 were identified through the registers of the Dr. Bernard Verbeeten institute for radiotherapy in Tilburg. Prevalent patients were only collected for the two selected regions. Incident patients were derived from the St. Elisabeth Hospital Tilburg, which is the only neurosurgical centre in North Brabant. Specialised health care for glioma patients in this province is very much centralised in the St. Elisabeth Hospital, and most patients with glioma in North Brabant visit the hospital at least once during their illness. Incident patients were collected from January 2002 through July 2003 for the two selected regions, and from January through July 2003 for the total province (table 1). The diagnosis of each patient was confirmed by histopathological examination by a neuropathologist.

Data collection and analysis

All patients alive were sent a letter in which they were invited to fill in a questionnaire with genealogical data up to three generations, and to give a blood sample. After informed consent was signed, blood was drawn by a research physician during a one-hour house visit. We recruited volunteers with extensive experience in genealogical research in the selected areas to construct pedigrees. Genealogical research was performed for all patients from the two selected regions, but only if the genealogical questionnaire indicated that the patients' ancestors originated from the area. This study was approved by the medical ethics committees from the participating centres.

Results

Patients

Between January 2002 and July 2003, 206 patients were found eligible and were sent an invitation for participation in the study. Eighty-two patients (40%) gave their informed consent. Twenty-two patients (11%) explicitly refused participation and 11 patients were lost to follow-up, had their diagnosis revised to a non-glioma tumour or died before blood was drawn. Ninety-one patients (44%) did not respond to two invitations. Characteristics of the 82 participating patients and their diagnoses are given in table 2. As expected, the male/female ratio was 3:2 (62% males).[2] The most frequent diagnosis was astrocytoma (45), followed by oligodendroglioma (19) and ependymoma (10). The majority of the tumours was of low-grade malignancy (55%).

Table 2. Clinical characteristics of 82 patients

Characteristic	n	(%)
Area 1	14	
Area 2	17	
Area 3	51	
Males	51	(62)
Females	31	(37)
Astrocytoma	45	(55)
Oligodendroglioma	19	(23)
Ependymoma	10	(12)
Oligoastrocytoma	5	(6)
Other glioma	3	(4)
WHO grade II	45	(55)
WHO grade III-IV	35	(43)
Grade unknown	2	(2)

WHO: World Health Organisation.

Genealogy

Four patients with astrocytoma from area 1 were related within six to twelve generations (figure 1). Only one patient appeared to be born from a consanguineous marriage; the inbreeding coefficient for this patient was $9.77*10^{-4}$.

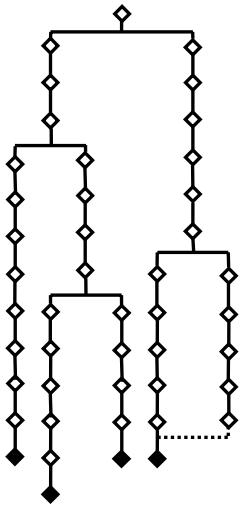

Figure 1. Pedigree of four patients with astrocytic glioma from a genetically isolated population in the Netherlands. Shaded diamonds represent patients, open diamonds represent previous generations. The patient on the right was born from a consanguineous marriage.

In area 2, three pedigrees were constructed with astrocytoma (figure 2), oligodendroglioma and ependymoma patients (figure 3). The pedigree with six astrocytoma patients showed the most interesting features. Clinical details of these patients are shown in table 3. Patients were related within four to fourteen generations and consanguinity was observed in two patients with pilocytic astrocytoma (patients 2 and 4). The family history of patient 4 was unremarkable.

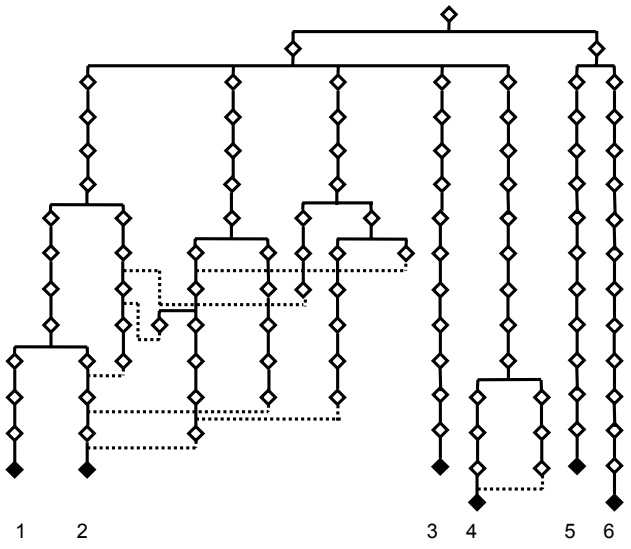

Figure 2. Pedigree of six patients with astrocytic glioma from a medium sized population in the Netherlands characterised by low migration. Shaded diamonds represent patients, open diamonds represent previous generations. Patient 4 was born from a consanguineous marriage, the family of patient 2 shows multiple consanguinity loops.

The father of patient 2 was diagnosed with liposarcoma (myxoid type) at age 61 years, her sister with breast cancer at age 42 years. Her brother developed epilepsy at age 3 years and is mentally retardated without definite diagnosis for this condition. Three more brain tumours were diagnosed in fourth and fifth degree family members of the mother, although the pathology of these tumours could not be verified. Patient 2 also has one healthy brother of 41 years old with one healthy daughter, and her sister who suffered from breast cancer had four healthy children. Patient 2 gave birth to two healthy daughters, aged 2 and 16 months. Screening for mutations in the TP53 gene in this patient revealed no pathogenic mutations.

Table 3. Clinical details of six patients with astrocytic glioma from a medium sized population in the Netherlands characterised by low migration

Patient	Sex	Tumour type	WHO grade	Age at diagnosis, y	Family history [a]
1	M	Astrocytoma	II	32	
2	F	Pilocytic astrocytoma	I	24	F: liposarcoma, age 61 S: breast carcinoma, age 42 S: mental retardation with epilepsy
3	M	Astrocytoma	IV	38	
4	F	Pilocytic astrocytoma	I	14	
5	M	Astrocytoma	II	31	
6	F	Angioglioma	II	30	F: lung carcinoma, age 59 M: melanoma, age 64

WHO: World Health Organisation; y: years; F: father; M: mother; S: sib.
[a] First degree relatives only.

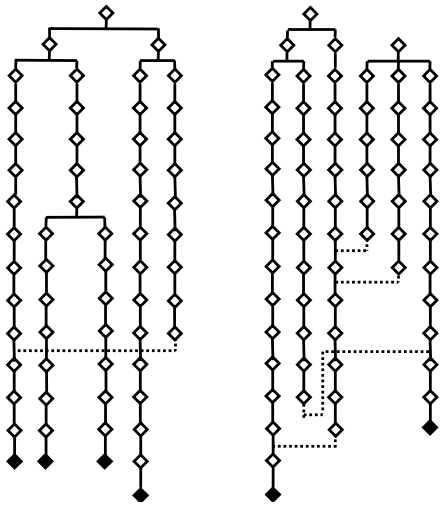

Figure 3. Pedigrees of patients with oligodendroglioma (left) and ependymoma (right) from a medium sized population in the Netherlands characterised by low migration. Shaded diamonds represent patients, open diamonds represent previous generations.

Discussion

We showed that by systematic collection and genealogical research in isolated populations, familial clusters of glioma can be identified. There are three main conclusions that can be drawn from our experiences. The interpretation of the findings is complicated by the fact that less than half of the patients (40%) were willing to participate. When asked for an explanation, the main reason to refuse participation was the emotional shock after a devastating diagnosis. Many patients refused because of their clinical condition, others had already died between diagnosis and invitation. The high mortality and morbidity of glioma therefore has great influence on the response rate. A second point of consideration is the presence of strong but inevitable selection of tumours with a better prognosis and of long survivors, which is reflected in the distribution of histological subtypes of glioma. This patient series is therefore unsuitable for epidemiological studies. However, selection of patients does not affect segregation of genes within families. Selection bias is therefore less of a problem in gene finding studies. Finally, for research purposes, it is of concern that during the genealogical exploration of the patients there appeared to be few relationships and, if related, they were not closely related. This might suggest that genealogy is not always complete. The main reason is that small communities like the genetically isolated population (area 1) are too small to contain sufficient numbers of patients with glioma (the world-standardised incidence rate is approximately 5 per 100,000 person-years).[2] We therefore invited patients from a wider area with less genetic isolation, which also complicated genealogical research to a great extent.

Recommendations

In contrast to more common diseases with lower mortality rates,[287,288] the described method of patient collection is less suitable for glioma. A number of recommendations can be made that might increase the efficiency of patient inclusion, and also limit selection due to morbidity and mortality. To increase response and to decrease selection, the collection and storage of blood samples should be standard protocol of hospitals. This could be done during routine blood examinations. The treating physician could then obtain written informed consent. When people are asked for participation by their own physician, response rates should go up. Furthermore, mortality and morbidity have less impact since blood is collected soon after diagnosis and no extra effort is asked from patients. The implementation of such a protocol should be relatively easy.

To include sufficient numbers of patients from localised geographical areas, e.g. genetically isolated populations, data collection has to span a long time period. If

genetic material of patients is stored in biobanks, future studies can readily make use of DNA from patients who originated from a certain geographical region. The Academic Medical Center in Amsterdam (AMC) has built such a biobank over the past 15-20 years. In chapter 3.3 we make use of this biobank in a study of seven glioma patients from a genetically isolated population near Amsterdam.

In summary, by the systematic collection and genealogical research of newly diagnosed glioma patients, familial clusters of glioma can be identified. The disease characteristics of glioma necessitate a modified protocol of data collection for genetic studies, compared with more prevalent and less malignant diseases. This protocol should dictate a long lasting and standardised infrastructure for the inclusion of patients soon after diagnosis, preferably involving the treating physician. A protocol for 'rapid case ascertainment' has already been proven to be successful in other studies.[67,68,93,146,290,291] A heightened awareness of the specific difficulties in glioma research, and the possible solutions, is needed to introduce such a protocol in all hospitals that participate in glioma treatment.

Acknowledgements

We are very grateful to genealogists Wim de Bakker, Hilda Kornman and Petra Veraart. The members of the neuro-oncology committee in the St. Elisabeth Hospital Tilburg are acknowledged for their cooperation in the selection of patients.

Chapter 3.3

Homozygosity mapping suggests a novel glioma susceptibility locus at 11p13

Summary

The genes involved in familial glioma are largely unknown, except for those involved in some mendelian tumour syndromes in which glioma is part of the clinical expression. A genetic origin for a subset of non-syndromic gliomas can be expected based on previous epidemiological studies. We identified a family of seven distantly related glioma patients from a genetically isolated population in the Netherlands. The pedigree contained multiple consanguinity loops. Six patients had an astrocytoma and one patient had an oligodendroglioma. There was no clinical evidence of involvement of any of the known tumour syndromes. We conducted a genome-wide search using homozygosity mapping with 9,409 single nucleotide polymorphisms (SNPs) from the Affymetrix GeneChip Human Mapping 10k array 2.0. The data were analysed with Merlin version 1.0-alpha. To prevent false-positive findings, we adjusted the analyses for the complex family structure with multiple consanguinity loops, and omitted SNPs that were in linkage disequilibrium. Four loci on chromosomes 1, 8 and 11 reached LOD-scores >3 in the initial analyses. After adjusting for linkage disequilibrium, only one locus at 11p13 remained statistically significant. This region spans 9.3 cM and reached a maximum LOD-score of 3.75. The susceptibility locus at chromosome 15 that was suggested in another study, could not be confirmed here. We conclude that we have identified a novel susceptibility locus at 11p13, involved in autosomal recessive familial glioma.

Introduction

Gliomas are malignant primary brain tumours of neuroepithelial origin. The aetiology of glioma remains largely unclear, and except for ionising radiation,[64,65] no major environmental factors have been established.[44] Less than 5% of glioma cases can be

explained by a number of monogenetic cancer syndromes like neurofibromatosis and the Li-Fraumeni syndrome, in which glioma is one of the occurring tumours.[90,91] Germline mutations in a variety of other genes such as *p16(INK4A)* and *PTEN* account for no more than a small subset of familial glioma cases.[126,127]

Although estimates are inconsistent, first-degree relatives of glioma patients appear to have a two- to ninefold increased risk of glioma.[93,130,132,134,136,139,143,175] This suggests that yet unknown genetic factors may be involved in the susceptibility to glioma, although exposure to shared environmental risk factors might explain part of the observed familial aggregation.[164] A study based on first-degree relatives of adult glioma patients suggested that familial glioma occurs in about 5% of all glioma cases, and that 1% may have a dominant mode of inheritance with reduced penetrance.[135] In a segregation analysis,[135] an autosomal recessive gene model provided the best fit, which could possibly explain 2% of all glioma cases. However, a multifactorial model was not rejected and others also found evidence for this mode of inheritance.[92,135,176] Most likely there are different genetic forms of the disease that are expressed in different glioma families. Paunu et al. performed a genome-wide linkage analysis in four families from a limited geographical area in Finland, followed by association analyses (haplotype pattern mining) and the transmission disequilibrium test (TDT) in fifteen families.[205] A low-penetrance locus for familial glioma at 15q23-q26.3 was suggested. The area contains several potential candidate genes, but none of them has been previously associated with hereditary brain tumours. Malmer et al. performed homozygosity mapping in five affected persons from three distantly related glioma families in Sweden, but found no alleles that were homozygous for all three families.[206] Non-parametric linkage analysis revealed a maximum allele-sharing LOD-score of 1.05 at chromosome 1q21-q25, consistent with a low-penetrant dominant gene.

Here, we studied seven glioma patients from a genetically isolated population in the Netherlands. All patients share a common ancestor within three to nine generations and a high degree of consanguinity suggests a recessive mode of inheritance. We hypothesised the presence of a founder mutation segregating in this family, and performed a genome-wide search using homozygosity mapping to localise the gene involved.

Methods

Patient selection

Over the past 15-20 years, the Academic Medical Center in Amsterdam has collected and stored information about all patients with glioma who received surgical

treatment. From this database we selected all patients from a community located in the central part of the Netherlands. This village was founded around 1500 and comprised approximately one hundred inhabitants in 1550. The population history of the village was characterised by very low migration owing to its geographically and socio-culturally isolated position. After centuries of very slow growth, the population expanded rapidly and the current population comprises over twenty thousand inhabitants. Most inhabitants are descendants from individuals who lived in the area around 1700. We identified seven patients that we could connect to one common ancestor from the seventeenth century within nine generations. By extensive genealogical research, multiple consanguinity loops (53-152) were found for each patient (table 1).

Clinical details

We derived clinical details from hospital records and medical correspondence (table 1). All patients were diagnosed between 1990 and 1996. One patient had a low-grade oligodendroglioma (World Health Organisation (WHO) grade II),[269] the others were diagnosed with low-grade and high-grade astrocytomas. Median age at diagnosis was 54 years (range 35-74), with a median survival of 8 months (range 1-28.5). The patient with oligodendroglioma suffered from a subarachnoid haemorrhage nine years earlier, the history of the other patients was unremarkable. There was no clinical evidence for involvement of any of the known tumour syndromes.[90,91] None of the patients had a positive family history of glioma or a remarkable pattern of other cancers in the family. These clinical features do not appear to be different from patients with sporadic tumours. The pedigree structure with a high level of inbreeding and the family history of these patients is consistent with an autosomal recessive mode of inheritance, and we therefore chose to perform a genome search using homozygosity mapping.[289]

Genotyping

Blood samples were stored for six patients and DNA was isolated from peripheral blood lymphocytes using standard protocols.[292] For one patient no blood was stored and DNA was therefore isolated from healthy choroid plexus tissue.[292]

We conducted a genome-wide search with the Affymetrix GeneChip Human Mapping 10K Array Xba 142 2.0. This array contains 10,204 single nucleotide polymorphisms (SNPs) distributed across the genome. The GeneChips were processed by an authorised Affymetrix Service Provider using standard Affymetrix protocols (ServiceXS, Leiden, the Netherlands). The SNP genotype call rate ranged from 96.01-98.85% with a mean of 97.92%. SNP locations, genetic maps

Table 1. Clinical and genealogical details of seven patients with glioma from a genetically isolated population in the Netherlands

Patient	Sex	Tumour type	WHO grade	Localisation	Age at diagnosis, y	Survival, mo	No. Loops [a]	Inbreeding coefficient [b]	Number of meioses	
									Shortest loop	Adjusted loop [c]
1	F	Astrocytoma	IV	Parietal-temporal	54	2.5	53	$3.887*10^{-3}$	11	10
2	F	Astrocytoma	IV	Parietal	74	6	55	$2.838*10^{-3}$	12	10
3	M	Astrocytoma	IV	Occipital	64	1	55	$1.001*10^{-2}$	10	8
4	F	Astrocytoma	II	Frontal	35	20	87	$1.062*10^{-3}$	15	11
5	F	Astrocytoma	IV	Parietal	52	9.5	70	$4.471*10^{-3}$	12	9
6	F	Oligodendroglioma	II	Frontal	44	28.5	152	$3.713*10^{-3}$	12	10
7	F	Astrocytoma	IV	Parietal-occipital	71	8	68	$4.089*10^{-3}$	12	9

WHO: World Health Organisation; y: years; mo: months; F: female; M: male.

[a] Total number of consanguinity loops in the family pedigree. [b] Inbreeding coefficient based on the complete family structure and all its consanguinity loops. The inbreeding coefficient represents the chance for each allele to be identical-by-descent. [c] Number of meioses in the adjusted consanguinity loop to match the inbreeding coefficient.

(Marshfield sex-averaged) and allele frequencies based on a sample of unrelated Caucasian individuals were obtained from Affymetrix (http://www.affymetrix.com/analysis/index.affx; June 2005). All but 172 SNPs have a known location on the genome. These 172 were therefore omitted from the analyses. We further omitted SNPs without heterozygosity in the Caucasian population (326) and SNPs that gave more than 1 'no call' in the seven patients (297). This resulted in 9,409 SNPs for analysis. Missing allele frequencies (137) were assumed to be equal. None of the SNPs were likely erroneous, as determined by the error-detection option of Merlin.[293]

Statistical analyses

The mode of inheritance was modelled as fully penetrant recessive with the population frequency of the disease allele set to 0.01. We considered the affection status of the parents and other ancestors to be unknown. To allow for locus heterogeneity among the patients, heterogeneity log odds-scores (HLOD) were computed using Merlin version 1.0-alpha.[293,294] The data were converted from Affymetrix to Merlin format using the affy2mega program (http://mga.bionet.nsc.ru/nlru/).

When analysing the shortest consanguinity loop of each patient (figure 1), distant genealogical loops are ignored and the degree of consanguinity is underestimated, resulting in inflated LOD-scores in homozygosity mapping.[295,296] As suggested by Liu et al.,[295] we therefore calculated the inbreeding coefficient based on the complete family structure, and constructed a new consanguinity loop to (conservatively) approximate this inbreeding coefficient (table 1). All analyses were performed with Caucasian allele frequencies specified by Affymetrix, and

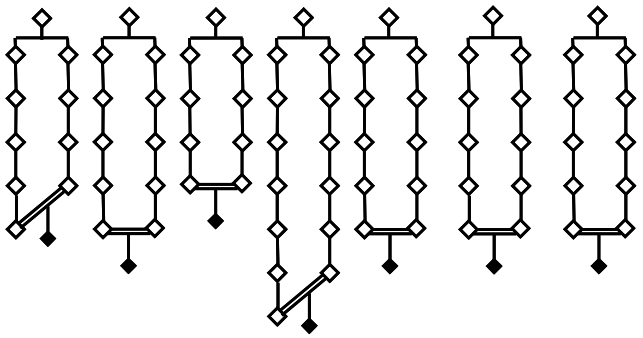

Figure 1. Shortest consanguinity loops of seven patients with glioma from a genetically isolated population in the Netherlands. All patients are descendants of one common ancestor from the seventeenth century (not shown). Shaded diamonds represent patients, open diamonds represent previous generations. Patients are in consecutive order, numbered 1-7 from left to right.

with allele frequencies that were calculated from the seven patients, which is a conservative approach.

The median intermarker distance on the GeneChip is 113 kb. Densely spaced markers in linkage disequilibrium (LD) may result in false-positive findings if parental genotypes are unknown.[297] The genomic regions with HLOD-scores of >3 were therefore additionally examined for the effect of LD. We obtained information about LD from the International HapMap Project (http://www.hapmap.org),[298] which utilises the data for 90 Utah residents with ancestry from northern and western Europe (Centre d'Etude du Polymorphisme Humain, CEPH). Haploview version 3.2 was used to visualise LD between the SNPs,[299] and SNPs in LD were manually removed if $|D'|>0.7$ or $r^2>0.4$, thereby excluding the SNPs that were less informative (lowest heterozygosity). SNPs that were not found in HapMap (20%) were also excluded. We then repeated the analyses with the reduced set of markers.

Written informed consent was obtained from all patients, and a medical ethics committee approved the protocol.

Results

Homozygosity mapping revealed four regions at chromosomes 1p, 8p and 11p, that reached statistically significant HLOD-scores of >3 using the conservative consanguinity loops based on the inbreeding coefficients. The positions of these regions are shown in table 2 and figure 2. After removing the SNPs that were in LD, only one region at 11p13 remained statistically significant (table 2, figure 3). This region spans 9.3 cM (4.15 Mb) between SNPs rs1002229 and rs1358054, with a maximum HLOD-score of 3.75 at SNP rs873469. For this region, patients 6 and 7 contributed most to the HLOD-score (not shown).

When we replaced Caucasian allele frequencies by estimated allele frequencies, HLOD-scores were slightly lower, but the regions of significance did not change (not shown). We found no evidence for linkage on chromosome 15, which was identified before as containing a susceptibility locus for familial glioma.[205] The maximum HLOD-score on this chromosome was 0.62. At the locus 1q21-q25, which was reported by Malmer et al.,[206] we found a maximum HLOD-score of 1.90 as the most conservative result.

A risk haplotype consisting of eleven consecutive SNPs could be constructed for the region at 11p13 (figure 4). For the other regions, no clear haplotype was seen at the position of the maximum HLOD-score, thereby making homozygosity-by-descent unlikely (not shown).

Table 2. Results of homozygosity mapping for four candidate regions for familial glioma

Location	No. SNPs	Maximum HLOD-score [a]	LD removed		SNP at max HLOD
			No. SNPs	Maximum HLOD-score [a]	
1p13.2	20	3.15	11	2.68	rs3860202
8p23.1	81	3.93	51	2.83	rs1073913
11p13	51	4.94	28	3.75	rs873469
11p11.2	44	3.17	22	2.21	rs1401417

SNP: single nucleotide polymorphism; HLOD: heterogeneity log odds; LD: linkage disequilibrium.
[a] Conservative analysis based on adjusted consanguinity loops to match the inbreeding coefficient.

SNP name	Marshfield map (cM)	Genotypes, patient							Haplotypes, patient						
		7	6	5	3	2	4	1	7	6	5	3	4	2	1
rs3898926	38.77	TT	CC	CT	CC	CT	CT	CT							
rs2761210 *	38.77	CC	AA	AC	AA	AA	AA	AC							
rs762044	39.17	AA	AA	AA	AA	GG	AG	AG	A	A	A	A	A	G	A
rs1321015	39.65	GG	GG	GG	GG	GG	GG	GG	G	G	G	G	G	G	G
rs953871	40.01	AA	AA	AG	AA	AG	AA	AA	A	A	A	A	A	A	A
rs939038	40.94	AA	AA	AA	AA	GG	AG	AG	A	A	A	A	A	G	A
rs873467 *	41.78	CC	CC	CC	CC	CC	CT	TT	C	C	C	C	C	C	T
rs873469	**41.78**	GG	GG	GG	GG	GG	GT	TT	G	G	G	G	G	G	G
rs910092 *	41.78	GG	GG	GG	GG	GG	AG	AA	G	G	G	G	G	G	A
rs1571163	42.12	GG	GG	GG	GG	GG	GG	GG	G	G	G	G	G	G	G
rs1553762 *	42.58	CC	CC	CC	CG	CC	CG	GG	C	C	C	C	C	C	G
rs952489	43.06	TT	TT	TT	CT	TT	CT	CC	T	T	T	T	T	T	C
rs496623	43.09	AA	AA	AA	AA	AA	AA	AG	A	A	A	A	A	A	A
rs353599 *	43.28	GG	AA	AA	AG	AA	AG	AG							
rs2421826	43.62	CC	CC	CC	CT	TT	CT	TT							

Figure 4. Genotypes (left) and most likely haplotypes (right) in the candidate region for familial glioma located at 11p13. Since patients 6 and 7 contributed most to the HLOD-score, their genotypes were used to define the haplotype. Note that the construction of haplotypes is subject to interpretation. Shared genotypes and haplotypes are shown in grey. The position of maximum heterogeneity log odds-score is shown in bold characters. The asterisk denotes SNPs that were omitted from the final analyses because of linkage disequilibrium. SNP: single nucleotide polymorphism; cM: centiMorgan.

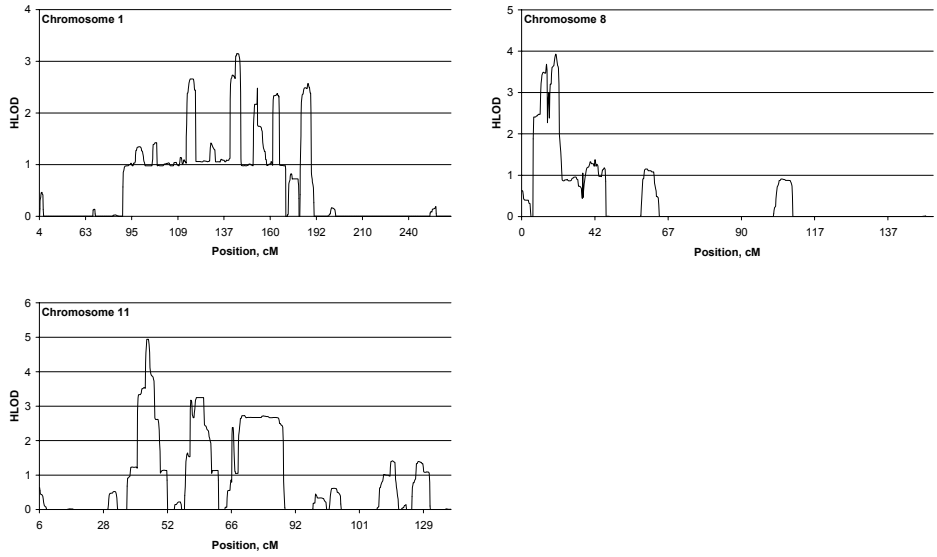

Figure 2. Whole-chromosome HLOD-scores for chromosomes 1, 8 and 11 found in homozygosity mapping of familial glioma. cM: centiMorgan; HLOD: heterogeneity log odds-score.

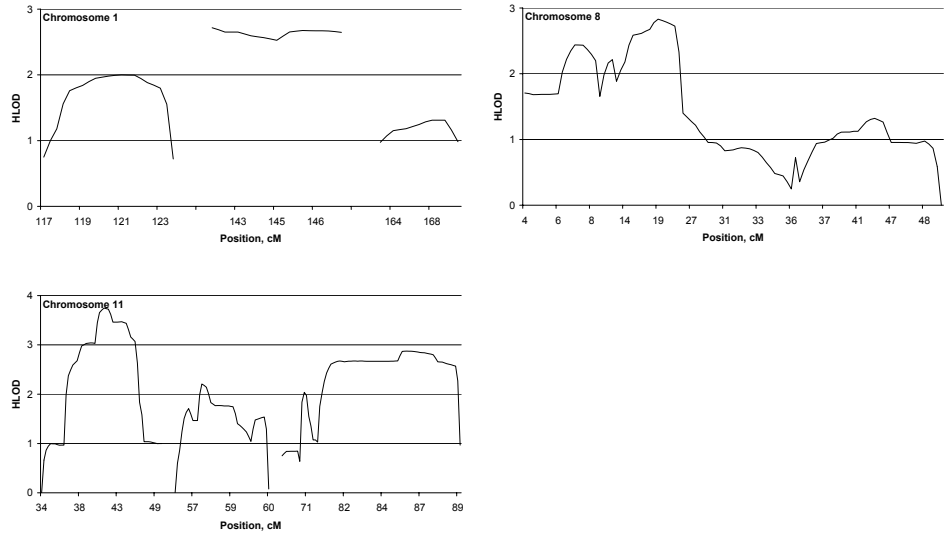

Figure 3. HLOD-scores for three peaks on chromosomes 1 and 11, and one peak on chromosome 8, found in homozygosity mapping of familial glioma after omitting SNPs in linkage disequilibrium. One region on chromosome 11 reaches HLOD-scores of >3. cM: centiMorgan; HLOD: heterogeneity log odds-score.

Discussion

This is the third genome-wide linkage analysis in familial glioma, and the second time that homozygosity mapping was used. Our findings suggest the presence of a susceptibility locus for familial glioma located at 11p13. Three other regions on chromosomes 1, 8 and 11 also showed statistically significant HLOD-scores. When we removed SNPs in LD, the HLOD-scores dropped below 3. Since we have chosen a very conservative approach, we cannot fully exclude these regions. Paunu et al. were the first to perform a genome-wide linkage analysis in familial glioma.[205] We cannot confirm most of the reported loci (6q27, 8p21.3 and 15q26.2). A possible locus at 1q21-q25, however, was identified by non-parametric analysis in the two previous studies,[205,206] and also showed a non-significant signal in our homozygosity mapping, although the presumed mode of inheritance is different.

Consanguineous families like the one described in this study are suitable for homozygosity mapping, a method to identify a recessive disease locus with only a very small number of patients.[289] This powerful genetic epidemiological method is based on the identification of homozygous chromosomal regions that are identical-by-descent. For the present study there are two potential problems. Miano et al. have shown that patients may become homozygous for large regions at multiple loci by chance, particularly if there are multiple (hidden) loops in the pedigree so that the degree of consanguinity is underestimated.[296] Following the reasoning of Liu et al.,[295] we solved this problem by using adjusted consanguinity loops, thereby taking the complex pedigree structure with all its inbreeding loops into account. Furthermore, the probability that the patients in this study share the same haplotype by chance is very unlikely. The second problem arises when markers are not in linkage equilibrium which results in inflated LOD-scores if parental genotypes are unknown. We therefore removed all markers in LD and repeated the analyses. It is unclear how accurately the samples from the CEPH families reflect the patterns of genetic variation in people with western European ancestry. Therefore, there is still a possibility of residual LD in our sample.

We used allele frequencies that are based on a sample of unrelated Caucasian individuals, as provided by Affymetrix. The allele frequencies in the genetically isolated population are unknown. However, analysis using the frequencies estimated from the seven patients, which is considered to be very conservative, did not lead to different conclusions. We also included SNPs with rare allele frequencies (<5%) since this is unlikely to influence LOD-scores.[299]

All except one of the patients suffered from astrocytoma, both low-grade and high-grade tumours. Although histologically different, we also included a

patient with oligodendroglioma in the analyses. The histological appearance of a glioma is not necessarily an indication of a different genetic aetiology, and the genetic predisposition may be the same in different types of tumours.[300] This is demonstrated by tumour syndromes that predispose to various malignancies of the central nervous system.[90,91]

The region at 11p13 contains 33 genes among which several interesting candidate genes. The hyaluronan receptor CD44 plays a role in the adhesion of glioma cells to the extracellular matrix and is involved in the infiltrating character of glioma cells and their migratory potential.[301] The glutamate transporter-1 (*EAAT2/GLT1*) actively removes the excitatory neurotransmitter glutamate from the extracellular space, and has been implicated in several neurological diseases, but not in neoplasma.[302,303] *CD59*, also called *Protectin*, is strongly expressed in H2 glioblastoma cells and is involved in the escape from complement-mediated cytolysis.[304] At the end of the region at 11p13 lies the Wilms tumor 1-gene (*WT1*). It has been suggested that *WT1* may play a role in the tumourigenesis of primary astrocytic tumours.[305,306] However, others examined *WT1* in gliomas and found no relevant mutations, making an aetiological role for *WT1* in glioma unlikely.[307] Two members of the *Ets* family of transcription factors (*ESE2* and *ESE3*) have been implicated in epithelial differentiation and carcinogenesis, but have never been associated with glioma.[308] There are also several predicted or hypothetical genes at 11p13. None of the 33 genes have been implicated in familial glioma before.

We conclude that we have identified a susceptibility locus involved in autosomal recessive familial glioma, by using homozygosity mapping in a consanguineous family. This region at 11p13 has not been reported before and therefore has to be confirmed in subsequent studies.

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Chapter 3.4

Secondary meningioma in a patient with a genetic predisposition for ependymoma

Summary

The occurrence of a meningioma in one of the patients from an ependymoma family with a genetic predisposition in chromosome region 22pter-q11.2 raised the question whether the meningioma is the result of this genetic predisposition or of radiation therapy. We performed a comparative loss of heterozygosity (LOH) analysis of the primary ependymoma and the secondary meningioma. The ependymoma showed LOH *22q* and no LOH *1p*, whereas the meningioma showed LOH *1p* and no LOH *22q*, the latter genetic changes being characteristic for radiation-induced meningiomas. This suggests that the two tumours are genetically different and that the meningioma, despite the presence of a genetic tumour predisposition in the patient, was probably induced by radiation therapy.

Introduction

Previously we presented a family in which two brothers each had two sons diagnosed with an anaplastic ependymoma before age 5 years. Molecular and cytogenetic analyses of the ependymomas of two of the affected boys and segregation analysis with chromosome 22 markers in this family suggested the presence of an ependymoma tumour suppressor gene in chromosome region 22pter-q11.2.[171,309] Recent additional molecular analysis of the ependymoma of a third affected boy gave very strong evidence for the existence of the ependymoma-predisposing locus at a slightly more distal position in 22q11.23-q12.1, but not including the neurofibromatosis type 2 (NF2) gene. More than twenty years after the ependymoma, the oldest of the four cousins is now diagnosed with a meningioma. This second tumour might be another appearance of the genetic predisposition locus in the proximal part of chromosome 22. Meningiomas can

however also be induced by radiation therapy which our patient received after removal of the primary tumour.

Here, we present a comparative loss of heterozygosity (LOH) analysis of the ependymoma and meningioma to investigate whether the meningioma is the result of a genetic predisposition locus on chromosome 22 or of the radiation therapy.

Methods

Details of patient history and radiation therapy were obtained from medical records and medical correspondence. DNA from the ependymoma was isolated from formalin-fixed and paraffin-embedded tumour material.[171] We extracted DNA from the meningioma from a fresh frozen sample as described previously.[309]

DNA markers and methods

A non-synonymous A to G mutation was found at position 167 in transcript variant 2 of the BID gene (GenBank accession number NM_001196), which predicts replacement of serine by glycine in the protein. The G-allele of this single nucleotide polymorphism (SNP) in coding exon 2 has a population frequency of 3.3% (Hulsebos and Redeker, unpublished results). To determine the status of this SNP, we amplified blood and tumour DNA of the patient by PCR using primers 5'-TTCCTGACTCCCCTTTCCC-3' and 5'-CTCTCTGCGGAAGCTGTTG-3'. The PCR-product has a length of 120 bp. Presence of the G-allele results in creation of a recognition site for restriction enzyme MspI, giving rise to fragments of 82 and 38 bp. Separation of the fragments was performed on an 8% polyacrylamide gel.

Other markers

Primer sequences for amplification of the other markers and conditions for PCR were taken from the Genome Database at http://www.gdb.org/. Loss of heterozygosity (LOH) analysis for these markers was performed as described previously.[309,310] Because of rather extensive degradation of DNA extracted from the ependymoma, only markers generating small PCR fragments (<150 bp) were applied.

Results

History

Our patient was diagnosed with a tumour in the fourth ventricle and right cerebellar hemisphere at age 4.5 years. Pathological examination after incomplete resection showed an anaplastic ependymoma World Health Organisation (WHO) grade III.[171] He received adjuvant chemotherapy (methotrexate, vincristine and prednisolone) followed by craniospinal radiotherapy: 3300 cGy in 22 doses with a boost of 2100 cGy on the location of the tumour. Fourteen years later a brain CT without contrast showed no signs of recurrent disease nor of other new pathology. Another six years later, 20.5 years after initial diagnosis, he presented again with bifrontal headaches, increasing ataxic gait disturbances and fatigue. CT examination showed a large isodense space-occupying lesion in the left temporal region with oedema, midline shift and contrast enhancement (figure 1). This tumour was resected. Histopathological examination revealed an atypical meningothelial meningioma WHO grade II with many vessels, a MIB-1 labelling index of 24% (measure of proliferative activity) and 1 mitosis per 10 HPF objective 40x.

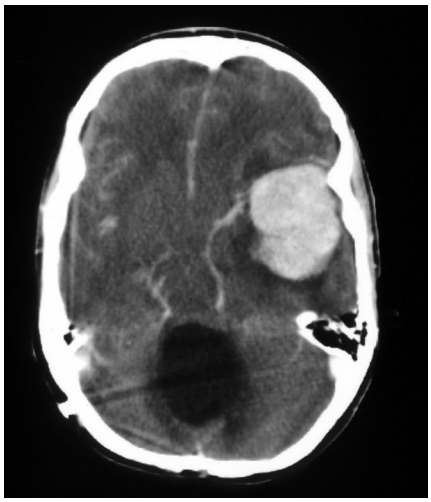

Figure 1. Brain CT with contrast enhancement showing a large space-occupying lesion in the left temporal region with oedema and midline shift. There is a defect in the cerebellar region due to surgical removal of an ependymoma of the fourth ventricle.

LOH analysis

Microsatellite analysis with markers BID, D22S156, D22S1176, D22S418 and D22S1165 on the long arm of chromosome 22 (region 22q11.21-q13.2) showed LOH for all markers in the ependymoma but not in the meningioma. Analysis for markers D1S214 and D1S228 on the short arm of chromosome 1 (region 1p36.21-p36.31) revealed LOH in the meningioma but not in the ependymoma.

Representative autoradiographs displaying the LOH analysis for selected markers on both chromosomes are shown in figure 2.

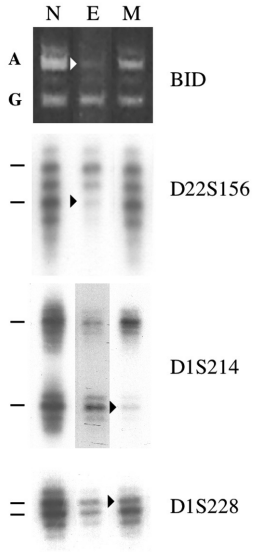

Figure 2. Loss of heterozygosity analysis using markers from chromosome arm 22q (BID and D22S156) and chromosome region 1p36 (D1S214 and D1S228), of primary ependymoma (E) and secondary meningioma (M) of the patient. N denotes normal DNA derived from the patients blood leucocytes. From top to bottom: loss of the A allele in the BID gene in the ependymoma and retention of both A and G alleles in the meningioma; loss of the bottom allele of marker D22S156 in the ependymoma and retention of top and bottom allele in the meningioma; loss of the bottom allele of marker D1S214 and the top allele of marker D1S228 in the meningioma and retention of two alleles for these markers in the ependymoma. Arrowhead indicates position of lost allele.

Discussion

To our knowledge, this is the first comparative LOH analysis of a primary brain tumour and a secondary brain tumour after radiation therapy from the same patient. We showed that the secondary meningioma is genetically different from the primary ependymoma. The ependymoma displayed LOH of *22q* and no LOH of *1p*, while the meningioma had LOH *1p* and no LOH *22q*. This discrepancy in LOH status for *1p* and *22q* suggests that the two tumours are genetically not related and that the meningioma is probably not the result of the genetic predisposition locus on chromosome 22.

Many reports have been published on meningiomas that were thought to be induced by radiation therapy for gliomas.[311] Radiation-induced meningiomas can occur after a latency period of 2-63 years. A radiation dose less than 1 Gy can already be tumourigenic, in particular at younger age,[64,65] with a clear dose-latency relationship.[312] In our patient, the meningioma fulfils the clinical prerequisites for a radiation-induced tumour: it was not present before the radiation therapy and it occurred in the irradiated pathway after a sufficient latency period (average 19-24 years for meningiomas).[312,313] The meningioma must have developed more than fourteen years after the diagnosis of the ependymoma as a brain CT at that time

showed no evidence of a meningioma. The rapid growth and atypical histology of the tumour is also in correspondence with a radiation-induced meningioma.

It is now increasingly recognised that the presence of LOH *1p* (and LOH *7p*, which was not studied here) and absence of LOH *22q* are characteristic genetic lesions in radiation-induced meningiomas.[314,315] Together with the clinical characteristics of the meningioma this strongly suggests that this tumour, despite the presence of a genetic predisposition locus on chromosome 22, was induced by the radiation therapy.

Chapter 4

Summary and general discussion

Chapter 4.1
Summary

Gliomas are primary brain tumours of neuroepithelial origin and comprise a heterogeneous group of glial malignancies. Less than one thousand patients are diagnosed with glioma each year in the Netherlands. Only about half of the patients is still alive after one year and no major improvements in survival have been noted over the past decades. Many environmental risk factors for glioma have been studied, but only for ionising radiation has an aetiological role been established. An additional 1-5% of glioma cases can be attributed to monogenetic cancer syndromes in which glioma is part of the clinical expression, but aggregation of gliomas in families without established cancer syndromes also occurs. The occurrence of gliomas in families can best be explained by a multifactorial model: environmental risk factors with a genetically determined susceptibility to these risk factors.

The objective of this thesis was to explore aetiological factors for glioma. In the studies for environmental and genetic risk factors, we used several different approaches in both population-based and family-based designs.

Studies of environmental risk factors

In the first part of this thesis, we presented studies exploring environmental risk factors for glioma. In **chapter 2.1** we described the trends in the incidence of glioma with data from the Netherlands Cancer Registry (NCR). The incidence of glioma was fairly stable in the Netherlands during the period 1989-2003, for both children and adults. Age-adjusted incidence rates were stable for all glioma combined and for oligodendroglioma/mixed glioma. In adult astrocytic glioma, a significantly increasing incidence of high-grade astrocytoma was balanced by simultaneous decreases in low-grade astrocytoma, astrocytoma with unknown malignancy grade and glioma of uncertain histology. The incidence of astrocytoma and ependymoma in children showed a decreasing trend, which was accompanied by an increasing incidence of glioma of uncertain histology. The overall incidence of glioma in children was stable. These incidence trends are comparable to those in other countries in Europe and the United States. Most variation since 1989 can be explained by better detection, improving histopathological diagnosis and the use of more diagnostics for the elderly. The stable incidence rates suggest that

no major changes in environmental risk factors have occurred which influenced the incidence of glioma.

In **chapter 2.2** we studied concomitant diseases in patients with a glioma. We therefore compared the prevalence of concomitant diseases in glioma patients with reference cancer populations from the Eindhoven Cancer Registry in a case-control study design. Patients with a glioma had strokes more often than other cancer patients. This can be explained by the high risk of bleedings in gliomas. Furthermore, in early stages it may be difficult to distinguish low-grade gliomas from ischemic strokes on CT scans. We also found an excess of hypertension in glioma patients, particularly for elderly men. We hypothesised that this association might be mediated through potentially neurocarcinogenic effects of antihypertensive medication. In **chapter 2.3** we therefore evaluated the effect of antihypertensive medication use on the risk of glioma. We used data from the PHARMO record linkage system, a database that links dispensing records of prescription drugs to patients' medical histories. The medical history of the study subjects was obtained from the Dutch Medical Register (LMR) and the Dutch nationwide network and registry of histo- and cytopathology (PALGA). Patients with a glioma used antihypertensive drugs more often than control subjects, particularly beta-blockers, but only for a duration of use of <2 years. When taking the possible delay between tumour induction and diagnosis into account, we found no associations between the cumulative use of antihypertensive medication and the risk of glioma. Both overall exposure and exposure to subgroups of antihypertensive drugs could not be related to a higher risk of glioma. It is therefore unlikely that the use of antihypertensive medication is a risk factor for developing a glioma. However, antihypertensive drugs are probably prescribed during the prodromal phase of the disease. The precise factors underlying a higher prevalence of hypertension in glioma patients, as reported in chapter 2.2, remain to be clarified.

One of the aetiological hypotheses in glioma research concerns a role for infectious agents. To obtain epidemiological evidence for an infectious aetiology, we investigated space-time clustering and seasonal variation in the incidence of glioma in the province of North Brabant. These analyses were performed with data from the cancer registries of the Comprehensive Cancer Centre South (IKZ) and the Comprehensive Cancer Centre of Rotterdam (IKR). Space-time clustering is said to occur when excess numbers of cases are observed within small geographical locations, but only at limited periods of time. In **chapter 2.4** we found this pattern of 'mini-epidemics' for glioma patients over 15 years of age, in an all-glioma analysis. Space-time clustering was only evident in the eastern part of the province, suggesting that an aetiological agent was acting in

this area during the studied period (1983-2001). No seasonal variation in glioma incidence could be demonstrated. In **chapter 2.5** we extended the analyses in the eastern part of the province to identify glioma subgroups for which an infectious aetiology might be involved. We could attribute space-time clustering to adult onset oligodendroglioma, particularly to middle-aged patients, and to males more than to females. However, given the low prior probability of an infectious aetiology for this specific subgroup, these results should be interpreted as false-positive. We concluded that space-time clustering of glioma cannot be attributed to a specific glioma subgroup. The observed clustering that was described in chapter 2.4 is therefore probably an overall effect within and between glioma subgroups.

Studies of genetic risk factors

The second part of this thesis aimed at the genetic basis of glioma. In **chapter 3.1** we discussed genetic epidemiological methods to study the genetic causes of glioma. We also presented a research design, based on genetic association, that might be very effective in genetically isolated populations. A pilot study in such a population in the southwest of North Brabant indicated that this approach might indeed be useful to study the genetic aetiology of glioma. In **chapter 3.2** we showed that by the systematic collection of patients and genealogical research in genetically isolated populations, familial clusters of glioma can be identified. The response rate of the patients was low due to the high morbidity and mortality of glioma. This leads to selection of tumours with a better prognosis, which might influence epidemiological studies and, to a lesser extent, genetic studies. The small numbers of patients also complicated the success of the genealogical research. We discussed these problems and made recommendations for a more efficient data collection procedure, that might be useful for diseases that are characterised by low incidence and high morbidity and mortality.

In **chapter 3.3** we identified a family of seven distantly related glioma patients from a genetically isolated community located in the central part of the Netherlands. These patients were prospectively collected since 1990 by the Academic Medical Center of Amsterdam (AMC). During the genealogical research we observed multiple consanguinity loops in the pedigree. We hypothesised the presence of a recessive founder mutation segregating in this family and leading to an increased risk of glioma. We therefore conducted a genome-wide search using homozygosity mapping with 9,409 single nucleotide polymorphisms (SNPs) from the Affymetrix GeneChip Human Mapping 10k array 2.0. To prevent false-positive findings, we adjusted the analyses for the complex family structure with multiple consanguinity loops, and dropped SNPs that were in linkage disequilibrium. Four

loci on chromosomes 1, 8 and 11 reached LOD-scores >3 in the initial analyses. After adjusting for consanguinity and linkage disequilibrium, only one locus at 11p13 remained statistically significant. This region spans 9.3 cM and reached a maximum HLOD-score of 3.75. The locus contains 33 genes among which several interesting candidate genes. We concluded that we identified a novel susceptibility locus, involved in autosomal recessive familial glioma. This region at 11p13 has not been reported before and therefore has to be confirmed in subsequent studies.

In **chapter 3.4** we presented a comparative loss of heterozygosity (LOH) analysis. Two studies were published previously about a family in which two brothers each had two sons diagnosed with an anaplastic ependymoma, with evidence for an ependymoma tumour suppressor gene in chromosome region 22pter-q11.2.[171,309] Twenty years after surgery, radiation and chemotherapy, one of these patients developed a meningioma. We showed that the primary ependymoma and the secondary meningioma were genetically different and that the meningioma, despite the presence of a genetic tumour predisposition in the patient, was probably induced by radiation therapy.

Chapter 4.2
General discussion

There are still many controversies about the genetic and environmental factors that are important in the aetiology of glioma. Despite the efforts of many investigators in diverse fields of glioma research, our knowledge of the disease is fragmentary, and no major factors in the aetiology of glioma that apply to a substantial number of patients have been identified. In this chapter, we focus on some methodological considerations in the light of general problems that often complicate glioma research. For specific methodological issues, we refer to the respective chapters. This discussion ends with suggestions for future research.

Methodological considerations

Amongst the problems that are frequently encountered in many studies are the pathological diversity and genetic heterogeneity of glioma, the relative rarity of the disease and its high mortality.

Pathological diversity

Classification of glioma is primarily based on a small number of histopathological criteria such as the resemblance of tumour cells to a specific glial cell type, mitosis and microvascular proliferation.[4] Diagnosis is not straightforward, as some criteria are not unambiguous and subject to interpretation of the pathologist. Secondly, gliomas also show regional heterogeneity in morphology and malignancy grade.[31-36] The accuracy of glioma classification and grading is therefore highly depending on the extent of sampling, and information from neuroimaging, clinical behaviour and histopathology must be combined to formulate a definite diagnosis. Thirdly, the lumping of biologically unrelated neoplasms under the designation of low-grade glioma, such as diffuse astrocytomas and pilocytic astrocytomas, also leads to classification errors.[6] Finally, classification can be difficult as some tumours show characteristics that fit more than one tumour type. An example is the distinction between astrocytic, oligodendrocytic, and mixed oligoastrocytic glioma, which is based on the observed amount of oligodendrocytic parts at histopathological examination.[4] Although there are no universally accepted criteria for mixed

tumours, correct classification is important as these tumours have different biological behaviour, treatment options and prognosis, and possibly different aetiology.[42,316,317] Because of increased awareness of these oligodendroglial features, more astrocytomas are now diagnosed as oligodendrogliomas or mixed oligoastrocytomas than before.[318,319] The classification, however, remains partly subjective and interobserver variability can be high.[320-324]

Over the past decades, glioma classification has changed repeatedly, and different classifications are often used simultaneously.[57,216,325-328] Today, the standard in classification is that of the World Health Organisation (WHO), based on the current knowledge of histopathology and genetics.[4] In general, a better understanding of glial malignancies and their natural history has led to the recognition of new glioma entities, and to the notion that other formerly distinguished subtypes are in fact different expressions of the same tumour. This better recognition is partly influenced by the increasing practice of genetic characterisation of tumours.

Genetic heterogeneity

As well as pathological heterogeneity, genetic heterogeneity of glioma forms a potential problem in genetic epidemiological studies. In genetic heterogeneity, mutations in different genes can result in the same disease or phenotype. When pathologically similar but genetically different tumours are combined in, for example, a linkage analysis, ambiguous 'linkage' assignments and slowing down of the gene finding process can result. The extent of this problem is not well known for glioma but is likely to play a role. Glioblastomas for example are heterogeneous as they may arise de novo (primary glioblastoma) or by an ongoing dedifferentiation of lower-grade lesions (secondary glioblastoma).[5,220] Within glioblastoma, further genetic subgroups can be recognised by array comparative genomic hybridisation experiments and expression profiles.[329,330] On the other hand, despite observed differences at the molecular genetic level, the same constitutional mutations may be responsible for the initial development of malignancy, which from there follows a different molecular genetic pathway.

It appears from segregation analyses that the occurrence of glioma in families can best be explained by a complex multifactorial model.[92,135,176] In a multifactorial model, the genetically determined susceptibility is unlikely to be the result of one or a few common alleles. A scenario in which hundreds of genes are acting together seems far more realistic. Because of the low relative risk of each allele, extremely large groups of patients are needed to identify these alleles.[279] On the other hand, a small number of rare high-risk alleles is responsible for a minority of patients. These alleles can be found in glioma families and are easier to identify

than the more common low-risk alleles. The well-known tumour syndromes like the Li-Fraumeni syndrome, neurofibromatosis and Turcot syndrome are examples of such high-risk alleles. Identification of high-risk alleles in glioma families is only feasible if patients are prospectively collected because of the high mortality of the disease. Genetic heterogeneity among different families however remains problematic.

One way to reduce genetic heterogeneity is to study patients from genetically isolated populations. The community that we studied in chapter 3.3 was founded around 1500 and comprised approximately one hundred inhabitants in 1550. The population history of the village was characterised by very low migration owing to its geographically and socio-culturally isolated position. After decades of very slow growth, the population expanded rapidly after 1800 and the current population comprises over twenty thousand inhabitants. Most inhabitants are descendants from individuals who lived around 1700. In populations such as these, genetic heterogeneity is markedly reduced as the small number of founders cannot represent the total genetic diversity of the general population. Owing to random fluctuations in gene transmission and occasional disappearance of mutations from the population (genetic drift), genetic complexity is further reduced. Finally, limited migration prevents the introduction of new mutations from the general population. However, a reduced genetic complexity also implies that results from studies in genetic isolates may be of limited importance for the general population. This has to be evaluated in additional studies for the prevalence of the discovered mutations in the general population.

Low incidence and high mortality

With less than one thousand patients per year in the Netherlands,[209] numbers of patients eligible for study are small, in particular for subgroups of glioma such as low-grade astrocytoma or oligodendroglioma. Additionally, glioma families are uncommon, thereby inhibiting genetic epidemiological studies. The high mortality of the disease makes the situation even less favourable: the median survival for the most common tumours is less than one year. Time to invite patients for participation in research, and to collect (genetic) material and informed consent is limited. A practical example of how low incidence and high mortality of glioma negatively affect glioma research is given in chapter 3.2.

To obtain a substantial group of patients, researchers have to extend the inclusion of patients over many years, or seek for nationwide or even international collaborations. Large-scale multicentre projects have to be designed to obtain sufficiently large patient groups. Examples are the population-based project in the San Francisco Bay area,[93,146,290] and the National Cancer Institute's hospital-

based study of brain tumours in three hospitals in Boston, MA, Phoenix, AZ and Pittsburgh, PA.[67,68] As an alternative, one can rely on existing data that was not intentionally collected for glioma research. Most studies in the first part of this thesis involved data from the Netherlands Cancer Registry (NCR) from the Association of Comprehensive Cancer Centres (ACCC). This population-based nationwide cancer registry records data of all malignant and in situ neoplasms in the Netherlands, including date of incidence, histology, topography, invasiveness, grade, stage and basis of diagnosis. These data are prospectively collected and of high-quality, and ascertainment is almost complete. Pathological diagnoses are derived from different sources including the Dutch nationwide computerised registry of histo- and cytopathology (PALGA) and the Dutch Medical Register, a hospital discharge registry (LMR). These diagnoses are confirmed by registration clerks on the basis of medical records. We also used other databases like the PHARMO record linkage system that links drug dispensing records of prescription drugs to patients' medical histories. Population-based databases have the advantage that there is no risk of selection but the information they contain is limited. As all data are routinely collected, there is often insufficient detail at the level of the individual, and potential risk factors (such as lifestyle factors) and family data are usually not recorded. Studies using pre-recorded data can therefore not always provide firm conclusions and are often hypothesis generating.

Suggestions for future research

Despite all efforts of glioma researchers worldwide, progress in the elucidation of glioma aetiology is slow. Some improvements in patient registration, data collection and methodology are thinkable that will increase research possibilities, and that may lead to the breakthroughs that everyone is looking forward to.

Glioma classification

As discussed before, the pathological diagnosis of glioma is subject to interobserver variability, changing classifications, changing concepts and sampling error. To avoid the problems posed by the pathologically and genetically heterogeneous nature of glioma, and to define homogeneous subgroups within this heterogeneous disease, a workable consensus on classification of glioma with increased use of molecular tumour markers is required. A start was made with a uniform histological cluster scheme for central nervous system tumours.[57] However, morphological criteria alone are probably insufficient for research purposes. For example, primary and secondary glioblastomas cannot be distinguished by histopathological features,

and glioblastoma is probably more heterogeneous than currently acknowledged. With the rapidly increasing insights in these matters, the challenge is to design a new classification that turns the current knowledge of pathology and genetics to practical use, but that can also be expanded with new insights that will definitely follow.

An improved classification will be helpful, not only in aetiological research but also for clinical practice. Prognostic information can already be derived from the genetic profile of a glioma.[79,331] Poor survival in oligodendrogliomas is associated with loss of *10q*, *p16/CDKN2A* deletion and *EGFR* amplification,[332-334] whilst activation of *PI3K*, mutation of *PTEN* and inactivation of the *RB1* pathway are associated with poor outcome in glioblastomas.[335,336] Furthermore, currently used therapeutic interventions could be tailored to the individual glioma patient. The chemosensitivity of oligodendrogliomas depends on allelic losses of *1p* and *19q*,[77,300,332,337,338] and on *MGMT* hypermethylation.[339-341] *MGMT* hypermethylation also seems to be of predictive value for the sensitivity of glioblastoma to Temozolomide.[342] In general, classification of gliomas based on gene profiling in addition to histology might provide a better prediction of survival.[343-345] It remains to be determined whether gene profiling studies can be used to determine aetiological subgroups. These genetic profiles might be useful in defining candidate genes after the identification of a susceptibility locus by linkage studies. New technologies will be very helpful in the practical application of these principles.[345-348]

Patient registration and prospective data collection

Efforts should also focus on the registration of patients and the collection of epidemiological data. The Netherlands Cancer Registry, as well as cancer registries in many other countries, already provides researchers with valuable information. However, epidemiological studies for risk factors will benefit from a more detailed registration of patient characteristics. The Comprehensive Cancer Centre South (Integraal Kankercentrum Zuid, IKZ) is one of the regional cancer registries in the Netherlands that also collects comorbidity data,[221] which was used in chapter 2.2 to study concomitant diseases in glioma patients.[239] Information on sociodemographic indicators and occupational history would be very useful in the study of glioma aetiology as well. Additional registration of this information by the cancer registries, if possible, or linking with existing databases (e.g. municipal records or the tax authorities) is therefore a logical step to take. When molecular diagnostic methods are more commonly used, registration should be adjusted accordingly. Obstacles such as the strict privacy legislation in the Netherlands have to be solved first to make all this possible.

Almost all of the studies in this thesis are based on prospectively collected epidemiological data, DNA and tumour material. Many studies worldwide relied on prospectively collected data and would otherwise not have been possible in the relatively short period of time that is usually available for research projects. In particular for rare and lethal diseases like glioma, more efforts should be taken to collect and store epidemiological data, (genetic) material and informed consent from patients and families for future research.

Increasing technical possibilities

The year 2001 was marked by the completion of the Human Genome Project in which the complete nucleotide sequence of the human DNA became available.[349,350] Also in the near future, genetic research will benefit further from the current explosion of knowledge and technical possibilities. A striking example is the recent introduction of DNA microarrays to fast and efficiently genotype thousands of SNPs for genomic screens.[351] Highly polymorphic microsatellite marker sets have been widely accepted for linkage analysis. SNP markers on the other hand are less informative than microsatellites as they are only di-allelic, but this can be compensated by new high-throughput methods. In general, a dense map of SNPs extracts far more information than a scarce map of 300-400 microsatellites,[352] and a screen with a dense map of SNPs without parental genotypes available equals or exceeds that of a microsatellite screen with parental genotypes. Additionally, SNPs are less sensitive to genotyping errors and are amenable to automation.[353] The use of high-density SNP mapping in linkage studies has been shown to substantially increase the information extracted from families, and achieves higher rates of genotyping success and accuracy as compared to conventional microsatellite marker sets.[297,352,354] Previous studies using microsatellite markers could therefore benefit substantially from reanalysis using a dense SNP marker set.[352,354] Examples can be found for bipolar disorder, prostate cancer, neonatal diabetes and rheumatoid arthritis.[297,355-357] After its recent introduction, already many studies were published in which these arrays were successfully adopted for genome-wide searches, and high-throughput arrays will probably become the new standard in genomic screens. The new generation of arrays already contains over 500,000 SNPs. Data management and analysis of the enormous output now need our attention to prevent future restrictions in this field.

The increasing amount of information generated by techniques like genomic screens and expression profiling should be combined into bioinformatics resources, together with the current knowledge on molecular interaction networks, metabolic and regulatory pathways for diseases and cellular processes.[298,358-363] This will maximise the extraction of information and optimise the comparison of

results from different research groups. The developing field of proteomics might prove to be useful in the future.[348,364]

New hypotheses and genetic concepts

In general, there is a need for explanations or aetiological hypotheses regarding some consistently observed characteristics of glioma epidemiology.[59] The sex differences with a male predominance and the geographical variations in incidence remain (partly) unexplained. Another puzzling observation is that glioma seems to be a disease of higher educated middle class men.[365] Yet unknown aetiological factors might therefore be associated with affluence and lifestyle. Any compelling hypothesis would be worth investigating. It seems possible that some of the crucial aetiological questions have not yet been posed.[59]

The role of many putative risk factors remains unresolved. Results are difficult to replicate, and more than once the observed effects point in different directions, as is the case for farm residence,[366,367] exposure to lead,[234,368] and consumption of fruits.[369] Differences in ethnic distribution, aetiologically heterogeneous patient groups and small sample sizes may all account for the difficulties in determining the precise risks. Perhaps the largest problem is the difficulty to precisely assess the level of exposures such as infections or dietary factors. Tools for risk factor assessment such as blood levels of substances or their metabolites, concentrations in brain and other tissues, detailed questionnaires,[370,371] and other techniques therefore have to be developed further. Also the effects of combined exposures to risk factors should be studied in more detail. Particularly the research for dietary factors would benefit from this, as the effects of potential carcinogens are often modulated by other dietary compounds like antioxidants. Ideally, a combined approach would also include relevant genetic polymorphisms to study gene-environment interactions.[372] Evaluation of exposure to specific carcinogenic chemicals with respect to an individuals susceptibility to those chemicals, as determined by polymorphisms in carcinogen metabolising enzymes and DNA repair capacity, might be the direction of the future and could lead to a breakthrough in both fields of research. It is however important to realise that these studies require large datasets, which is problematic in the light of what we discussed before.

New concepts in neurocarcinogenesis may be required to obtain a more comprehensive view of glioma aetiology.[44] Progress is being made in the field of epigenetic phenomena: epigenetic mechanisms are increasingly recognised as important tumourigenic events.[72] Gene silencing by aberrant methylation has been demonstrated to be a highly prevalent alteration in glial cells and glial neoplasms.[340,373-376] Researchers should therefore focus more on these (epi)genetic

alterations and mechanisms. This may also facilitate new therapeutic strategies targeted to these genetic defects or their incorrectly functioning gene products.

Conclusion

Although glioma aetiology remains largely not understood, we are now faced with rapidly increasing possibilities for further research. More attention to diagnosis and registration of patients, recognition of molecular genetic subgroups of glioma, the evolution of genetic (epidemiological) methods, new approaches in bioinformatics and new concepts in neurocarcinogenesis will all contribute to the exciting developments. It is now warranted to combine forces and to forge new collaborations between researchers, to gain as much from these developments as possible.

Hoofdstuk 5

Samenvatting

Hoofdstuk 5.1

Gliomen: de rol van omgevingsfactoren en genetische predispositie

Ieder jaar worden in Nederland ongeveer duizend primaire maligniteiten van de hersenen gediagnosticeerd. De meeste van deze tumoren zijn gliomen: maligniteiten van neuro-epitheliale oorsprong die behoren tot de kwaadaardigste neoplasmata. Ongeveer de helft van alle patiënten is na een jaar nog in leven en in de afgelopen decennia is de prognose slechts beperkt vooruitgegaan.

Gliomen worden ingedeeld naar de veronderstelde cel van origine: astrocyten, oligodendrocyten en ependymcellen. Het meest komt het astrocytoom voor (80%), gevolgd door het oligodendroglioom, het oligoastrocytaire menggloom en het ependymoom. Op basis van histologische kenmerken worden gliomen verder onderverdeeld naar maligniteitsgraad. Een praktische verdeling is die in laaggradige (graad II) en hooggradige tumoren (graad III en IV, inclusief glioblastoma multiforme). Gliomen tonen meestal in de loop van de tijd een voortschrijdende dedifferentiatie naar hogere maligniteitsgraden. Er zijn enkele bijzondere vormen van indolente gliomen waaronder het pilocytaire astrocytoom. Deze tumor wordt voornamelijk op de kinderleeftijd gezien, is gelokaliseerd in het cerebellum of de nervus opticus, en kenmerkt zich door een goedaardig beloop met een uitstekende prognose. Deze tumoren worden gegradeerd als graad I.

Epidemiologie

Ongeveer 85% van alle primaire maligne intracraniële neoplasmata zijn gliomen, waarvan 20% laaggradig is. Gliomen komen meer bij mannen voor dan bij vrouwen, met een verhouding van 3:2. De incidentie van laaggradige gliomen is in Nederland min of meer constant over de leeftijd en bedraagt 1 per 100.000 persoonsjaren. Hooggradige gliomen kennen een gemiddelde incidentie die drie tot vier maal zo hoog ligt en deze neemt toe met de leeftijd. De piekincidentie van ongeveer 9 per 100.000 persoonsjaren wordt bereikt tussen de 50 en 70 jaar, waarna weer een afname volgt. Waarschijnlijk is deze afname artificieel en wordt ze veroorzaakt door een sterke toename op hogere leeftijd van het aantal klinisch gediagnosticeerde tumoren zonder pathologische verificatie.

Er zijn in Europa en de Verenigde Staten geen duidelijke temporele trends in incidentie waarneembaar die niet kunnen worden verklaard door een betere detectie, zoals de toename ten tijde van de introductie van CT en MRI. Ook geografisch bezien is er in vergelijking met andere maligniteiten weinig variatie in incidentie. In het algemeen komen gliomen vaker voor in Scandinavië. In andere Europese landen en Noord-Amerika worden weer hogere incidenties gezien dan in Afrika, Azië en Zuid-Amerika. Tenminste een deel van de verklaring ligt in verschillen in beschikbaarheid van medische zorg of in verschillen in registratie en classificatie van deze tumoren. Gezien de beperkte variatie in incidentie valt te verwachten dat oorzakelijke omgevingsrisicofactoren zeldzaam zijn, een beperkte rol spelen, dan wel alom aanwezig zijn.

Omgevingsrisicofactoren

De rol van omgevingsrisicofactoren bij het ontstaan van gliomen blijft grotendeels onduidelijk. Deze tumoren vormen een histologisch zeer diverse groep; eenduidige en reproduceerbare classificatie geeft daardoor problemen. Hierdoor, en door hun relatieve zeldzaamheid, is men er in veel studies naar risicofactoren niet in geslaagd om oorzaken van gliomen aan te wijzen. Ook grote studies die voortkomen uit internationale samenwerkingsverbanden leveren dikwijls tegenstrijdige resultaten. Tot op heden is alleen van (lage) doses ioniserende straling aangetoond dat die het risico op gliomen verhogen. Het aantal patiënten waarbij deze risicofactor een rol speelt is echter zeer klein. Andere soorten elektromagnetische straling, bijvoorbeeld afkomstig van mobiele telefoons, lijken vooralsnog het risico op gliomen niet te verhogen. De verontrustende berichten die hierover toch herhaaldelijk in de media verschijnen berusten meestal op ondeugdelijk epidemiologisch onderzoek. Voor andere factoren, zoals blootstelling aan tabaksrook, bestanddelen van het dieet, beroepsgebonden factoren en hoofdletsel, zijn de resultaten van onderzoek op zijn minst tegenstrijdig of ontbreekt bewijs voor een causaal verband.

Erfelijke tumorsyndromen: monogenetische aandoeningen

Bij een aantal monogenetische syndromen maken gliomen deel uit van het klinisch beeld. Bij deze syndromen wordt één enkel gendefect overgedragen van generatie op generatie, waarbij het risico op het krijgen van maligniteiten, waaronder gliomen, verhoogd is bij dragers van deze mutaties. Deze erfelijke syndromen zijn verantwoordelijk voor naar schatting 1-5% van alle patiënten met een glioom. De meeste genen die verband houden met predispositie voor tumoren van het centraal zenuwstelsel zijn tumorsuppressorgenen en vertonen een autosomaal dominant patroon van overerving.

Syndromen waarbij gliomen frequent worden gezien zijn neurofibromatose type 1 en 2, het Li-Fraumeni-syndroom, tubereuze sclerose, het Turcot-syndroom, het Gorlin-syndroom, en het melanoom-astrocytoomsyndroom. Door de autosomaal dominante overerving met een duidelijk patroon van kanker in meerdere generaties van een familie zijn deze aandoeningen vaak te herkennen. Dit heeft in veel gevallen geleid tot het identificeren van het onderliggende gendefect en tot een goed omschreven fenotype. Hoewel deze tumorsyndromen zeldzaam zijn, kan kennis over de oorzakelijke genetische afwijkingen leiden tot inzicht in de pathogenese van sporadisch voorkomende gliomen. Zo worden mutaties in het *TP53*-gen, verantwoordelijk voor het Li-Fraumeni-syndroom, ook aangetroffen als een vroege verworven genetische afwijking in sporadische laaggradige astrocytomen.

Regelmatig worden in de literatuur ook families beschreven bij wie er aanwijzingen zijn voor een mendeliaans overervingspatroon van gliomen, maar zonder tekenen van een van de bekende tumorsyndromen. Er zijn verschillende studies verschenen waarin werd gezocht naar oorzakelijke mutaties, onder meer in genen als *TP53*, *PTEN, p14, p15*, *p16* en *CDK4*. Meestal worden in dit soort studies echter geen afwijkingen gevonden, hetgeen suggereert dat mutaties in een van deze genen slechts verantwoordelijk kunnen zijn voor een zeer beperkt aantal families met gliomen.

Aggregatie van gliomen in families zonder aanwijzingen voor een tumorsyndroom

Verreweg de meeste gliomen treden sporadisch op zonder dat andere leden van de familie zijn aangedaan. Aan de andere kant komt ook familiare clustering van twee of meer gliomen voor zonder duidelijke erfelijkheid. Het patroon van overerving is dan meestal atypisch voor erfelijke tumoren: vaak zijn er slechts patiënten in een of twee generaties en gaat het niet duidelijk om kanker op jonge leeftijd, en in het geval van ouder-kindparen met een glioom wordt de ziekte bij het kind vaak op jongere leeftijd gediagnosticeerd dan bij de ouder. Families met meerdere patiënten komen niet vaak voor, en als er meerdere patiënten zijn gaat het meestal om een zeer beperkt aantal.

Hoewel de schattingen sterk uiteenlopen, is het risico op een glioom voor bloedverwanten van patiënten waarschijnlijk verhoogd met een factor twee tot negen, waarbij het risico lijkt toe te nemen naarmate de verwantschap met de patiënt nauwer is en de patiënt op jongere leeftijd is aangedaan. Ook lijkt dit met name het geval te zijn voor laaggradige astrocytomen. Overigens wordt eveneens voor andere vormen van kanker een dergelijk verhoogd risico beschreven voor familieleden van patiënten, ook voor veelvoorkomende tumoren als mamma-, colon-, prostaat- en longcarcinoom. Studies naar het risico op andere tumoren

dan gliomen bij patiënten of familieleden rapporteren tegenstrijdige resultaten. Het is niet duidelijk of er een verband met gliomen bestaat, en voor welke vormen van kanker dat geldt.

Dit alles suggereert dat nog onbekende erfelijke factoren een rol spelen bij het optreden van gliomen. Het is echter ook mogelijk dat een deel van de familieaggregatie veroorzaakt wordt door gedeelde omgevingsrisicofactoren, hoewel dit door sommigen wordt betwist. Zo lijken partners die de omgevingsfactoren van patiënten delen geen verhoogd risico op gliomen te hebben. Ten slotte geldt ook voor een zeldzamere ziekte als het glioom uiteraard, dat zij op basis van toeval meerdere personen binnen een familie kan treffen.

Genetische risicofactoren bij sporadische gliomen

In segregatieanalyses wordt het meest waarschijnlijke overervingspatroon van een ziekte bepaald, door berekening van de waarschijnlijkheid van het waargenomen overervingspatroon, onder de voorwaarde van verschillende vormen van erfelijkheid. Hieruit is gebleken dat het vóórkomen van gliomen in families waarschijnlijk het best verklaard kan worden met een multifactorieel model. Hierbij wordt het ontstaan van gliomen bepaald door omgevingsrisicofactoren tegen de achtergrond van een genetisch bepaalde gevoeligheid voor die risicofactoren. Deze gevoeligheid wordt bijvoorbeeld bepaald door genetische polymorfismen. Dit zijn genen met meer dan één variant (meerdere allelen) in de populatie, waarbij de varianten een (meestal klein) positief of negatief effect hebben op de werking van het genproduct. Deze genen spelen een rol bij processen als de detoxificatie van carcinogenen, DNA-herstelmechanismen en het oxidatief metabolisme. Het best bestudeerd zijn polymorfismen van de carcinogeenmetaboliserende enzymen glutathion *S*-transferase. De rol hiervan blijft echter onduidelijk, evenals die van vele andere onderzochte genvarianten. In veel studies zijn belangrijke methodologische tekortkomingen aan te wijzen, met als gevolg fout-positieve bevindingen die niet kunnen worden bevestigd in andere studies.

Uit de segregatieanalyses blijkt echter dat mogelijk ook autosomaal dominante en recessieve aandoeningen verantwoordelijk zijn bij een klein deel (minder dan 2%) van de patiënten zonder een bekend tumorsydroom. Tot op heden werden slechts twee studies gepubliceerd waarin men trachtte de desbetreffende genen te lokaliseren. In een onderzoek uit Finland werd koppelingsonderzoek (linkage analyse en genetische associatie analyses) verricht in vijftien families uit dezelfde geografische regio in Finland. Daarbij werd een mogelijk locus met lage penetrantie gevonden op chromosoom 15q23-q26.3. Dit grote gebied van 40 centiMorgan bevat echter honderden genen, waaronder enkele kandidaat-

genen die geen van alle ooit eerder in verband werden gebracht met gliomen. In een Zweedse studie werd 'homozygosity mapping' toegepast op drie families met gliomen met een verre gemeenschappelijk voorouder. Eerder werd op basis van segregatieanalyses al een autosomaal recessieve aandoening vermoed in deze families, maar dit kon nu niet worden bevestigd. Een non-parametrische linkage analyse gaf echter aanwijzingen voor een gen met lage penetrantie op chromosoom 1q21-q25.

Conclusie

Onze kennis van de etiologische achtergrond van gliomen blijft beperkt. Ioniserende straling is de enige bekende risicofactor, en <5% van de gliomen kan verklaard worden door een klein aantal tumorsyndromen. Hoewel men aanneemt dat de etiologie van gliomen multifactorieel bepaald is, zijn de factoren die hierbij een rol spelen nog altijd grotendeels onbekend.

Hoofdstuk 5.2
Samenvatting van dit proefschrift

Het doel van het in dit proefschrift beschreven onderzoek was om mogelijke oorzaken van gliomen nader te bestuderen. In de studies naar genetische en omgevingsrisicofactoren maakten we gebruik van verschillende methoden, zowel in populaties als in families.

Studies naar omgevingsrisicofactoren

In het eerste deel van dit proefschrift beschreven we studies waarin we zochten naar omgevingsrisicofactoren voor gliomen. In **hoofdstuk 2.1** gaven we een gedetailleerde beschrijving van trends in de incidentie van gliomen. Deze studie werd uitgevoerd met gegevens van de Nederlandse Kankerregistratie (NKR). De incidentie van gliomen bleef in Nederland stabiel tussen 1989 en 2003, zowel bij kinderen als bij volwassenen. De incidentie, gestandaardiseerd voor leeftijd, bleef stabiel voor alle gliomen samen en voor oligodendrogliomen/menggliomen. Wel zagen we een significant stijgende trend in de incidentie van hooggradige astrocytomen bij volwassenen, die echter werd gecompenseerd door een gelijktijdige afname van laaggradige astrocytomen, astrocytomen met onbekende maligniteitsgraad en gliomen met onduidelijke histologie. De incidentie van astrocytomen en ependymomen bij kinderen vertoonde een dalende trend die werd gecompenseerd door een toename van gliomen met onduidelijke histologie. De incidentie van alle gliomen op de kinderleeftijd bleef wel stabiel. Deze trends in incidentie zijn vergelijkbaar met die in andere landen van Europa en de Verenigde Staten. De meeste trends sinds 1989 konden we verklaren door een betere detectie, verbeteringen in de histopathologische diagnostiek en het toenemend gebruik van diagnostische middelen bij ouderen. De overwegend stabiele incidenties suggereren dat er geen belangrijke veranderingen in de blootstelling aan omgevingsfactoren hebben plaatsgevonden tijdens de duur van de studie.

De Eindhoven Kanker Registratie van het Integraal Kankercentrum Zuid (IKZ) is een van de regionale kankerregistraties in Nederland die niet alleen persoonsgegevens en tumorpathologie registreert, maar ook comorbiditeit. In **hoofdstuk 2.2** beschreven we een patiënt-controle onderzoek waarin we de prevalentie van verschillende gelijktijdige ziekten vergeleken tussen patiënten

met een glioom en patiënten met een andere vorm van kanker, die geregistreerd werden door dezelfde kankerregistratie. Patiënten met een glioom bleken vaker dan andere kankerpatiënten een herseninfarct of -bloeding te hebben gehad. Dit komt waarschijnlijk doordat gliomen door neovascularisatie gemakkelijk bloeden. Ook zijn herseninfarcten en laaggradige gliomen in de praktijk niet altijd gemakkelijk van elkaar te onderscheiden op CT scans. We ontdekten ook dat patiënten met een glioom vaker lijden aan hypertensie, vooral oudere mannen. We veronderstelden dat dit verband mogelijk het gevolg is van neurocarcinogene effecten van antihypertensiva. Daarom onderzochten we ook het effect van het gebruik van antihypertensieve medicatie op het risico voor gliomen, hetgeen beschreven werd in **hoofdstuk 2.3**. We gebruikten daarvoor gegevens van het PHARMO Instituut voor farmaco-epidemiologisch onderzoek. In het 'PHARMO record linkage system' wordt receptmedicatie geregistreerd en gekoppeld aan de medische geschiedenis van patiënten. Deze medische gegevens worden verkregen via onder meer de Landelijke Medische Registratie (LMR) en het Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA). Patiënten met een glioom bleken vaker kortdurend antihypertensiva te hebben gebruikt, waaronder bètablokkers. Als we echter corrigeerden voor de tijd tussen het ontstaan van een glioom en het stellen van de diagnose, vonden we geen duidelijk verband tussen het gebruik van antihypertensiva en het risico op een glioom. Zowel het gebruik van antihypertensiva in het algemeen, als het gebruik van de verschillende soorten bloeddrukverlagers, bleek het risico op een glioom niet te verhogen, ongeacht de duur van gebruik. Het is daarom onwaarschijnlijk dat het gebruik van antihypertensieve medicatie een risicofactor is voor het krijgen van een glioom. Wel worden antihypertensiva waarschijnlijk vaker voorgeschreven in de prodromale fase van de ziekte. De precieze factoren die maken dat de prevalentie van hypertensie hoger is bij patiënten met een glioom, zoals we beschreven in hoofdstuk 2.2, moeten nog verder opgehelderd worden.

Eén van de etiologische hypothesen in het gliomenonderzoek betreft een mogelijke rol voor infecties. Om op populatieniveau epidemiologisch bewijs te vinden voor deze hypothese, onderzochten we in **hoofdstuk 2.4** ruimte-tijdclustering en seizoensfluctuaties in de incidentie van gliomen in Noord-Brabant. We maakten daarvoor gebruik van gegevens van het IKZ en het Integraal Kankercentrum Rotterdam (IKR). Ruimte-tijdclustering wil zeggen dat er op bepaalde geografische locaties meer patiënten zijn dan op grond van toeval mag worden verwacht, maar alleen in bepaalde tijdsperioden. Dit patroon van 'mini-epidemieën' werd gevonden voor patiënten ouder dan 15 jaar, wanneer we keken naar alle gliomen gezamenlijk. Ruimte-tijdclustering trad alleen op in het oostelijk deel van Noord-Brabant, hetgeen suggereert dat een etiologisch agens actief was

in dit gebied in de bestudeerde periode (1983-2001). Seizoensfluctuaties in de incidentie werden niet gevonden. In **hoofdstuk 2.5** breidden we onze analyses uit voor het oostelijk deel van Noord-Brabant, en concentreerden ons daarbij op de verschillende histologische subgroepen van gliomen. Ruimte-tijdclustering werd alleen gevonden bij oligodendrogliomen, met name op middelbare leeftijd, en was meer uitgesproken bij mannen dan bij vrouwen. Echter, gezien de lage waarschijnlijkheid dat alleen deze groep een infectieuze oorzaak heeft, moeten deze resultaten waarschijnlijk beschouwd worden als een fout-positieve bevinding. We concludeerden daarom dat ruimte-tijdclustering niet uitsluitend kan worden toegeschreven aan een van de histologische subtypen van gliomen. De clustering die we beschreven in hoofdstuk 2.4 is daarom waarschijnlijk het resultaat van clustering binnen en tussen histologische groepen.

Studies naar genetische risicofactoren

In het tweede deel van dit proefschrift richtten we ons op de genetische basis van gliomen. In **hoofdstuk 3.1** bespraken we methoden in de genetische epidemiologie die gebruikt kunnen worden om de genetische oorzaken van gliomen te bestuderen. We presenteerden daarbij ook een genetisch epidemiologische methode, gebaseerd op genetische associatie, die zeer effectief zou kunnen zijn in genetisch geïsoleerde gemeenschappen. Een voorstudie in een dergelijke gemeenschap in het zuidwesten van Noord-Brabant toonde al aan dat deze aanpak waarschijnlijk bruikbaar is om de genetische etiologie van gliomen te onderzoeken. In **hoofdstuk 3.2** lieten we zien dat het mogelijk is om familiaire clusters van gliomen te identificeren door systematisch patiënten te verzamelen en genealogisch onderzoek te doen in genetisch geïsoleerde gemeenschappen. Door de hoge morbiditeit en mortaliteit van gliomen was het aantal patiënten dat toestemming gaf voor onderzoek klein. Hierdoor ontstond selectie van tumoren met een betere prognose, hetgeen invloed heeft op epidemiologische studies en, in mindere mate, genetisch studies. Het kleine aantal patiënten in de genetisch geïsoleerde gemeenschappen beperkte ook het succes van het genealogisch onderzoek. We bespraken deze problemen en deden aanbevelingen voor een efficiëntere werkwijze, die bruikbaar kan zijn voor ziekten die worden gekenmerkt door een lage incidentie en hoge morbiditeit en mortaliteit.

In **hoofdstuk 3.3** beschreven we zeven patiënten met een glioom, allen afkomstig uit een genetisch geïsoleerde gemeenschap in het midden van Nederland en verre afstammelingen van dezelfde voorouder. Deze patiënten werden sinds 1990 prospectief verzameld door het Academisch Medisch Centrum Amsterdam (AMC). Tijdens het genealogisch onderzoek vonden we een hoge mate van consanguiniteit in de stamboom. Hierdoor rees het vermoeden op een

recessieve mutatie die in deze familie wordt doorgegeven, en waarbij dragers een verhoogd risico lopen op het krijgen van een glioom. Om deze mutatie te vinden verrichtten we een genoom scan met 9.409 'single nucleotide polymorphisms (SNPs)' van de Affymetrix GeneChip Human Mapping 10k array 2.0. We brachten daarna de homozygote gebieden van het genoom in kaart met 'homozygosity mapping'. Om fout-positieve bevindingen te voorkomen, corrigeerden we de analyses voor de complexe structuur van de stamboom en verwijderden we SNPs die te sterk aan elkaar gekoppeld waren (linkage-disequilibrium). De analyses suggereerden een interessant gebied op chromosoom 11 waar mogelijk de genetisch oorzaak van gliomen in deze familie zou kunnen liggen. Dit gebied op de korte arm van chromosoom 11 (11p13) is 9,3 centiMorgan (4,15 miljoen basenparen) groot en bereikte een maximale HLOD-score van 3,75. Het gebied bevat 33 genen waaronder een aantal interessante kandidaat-genen. We concludeerden dat we een nieuw locus voor autosomaal recessieve familiaire gliomen hadden gevonden. Dit gebied op chromosoom 11 is nog nooit eerder beschreven en moet daarom bevestigd worden in andere studies.

In **hoofdstuk 3.4** presenteerden we de resultaten van een vergelijkende 'loss of heterozygosity' (LOH) analyse. In het verleden werden reeds twee studies gepubliceerd over een familie waarin twee broers ieder twee zoons kregen met een anaplastisch ependymoom. Uit onderzoek bleek al dat er in deze familie aanwijzingen zijn voor een tumorsuppressorgen op chromosoom 22pter-q11.2. Twintig jaar nadat een van de vier kinderen werd behandeld met chirurgie, radio- en chemotherapie ontwikkelde hij een meningeoom. We lieten zien dat het primaire ependymoom en het secundaire meningeoom genetisch van elkaar verschillen, en dat het meningeoom waarschijnlijk veroorzaakt werd door de radiotherapie, ondanks de aanwezigheid van een erfelijke predispositie voor tumoren.

Ten slotte bespraken we in **hoofdstuk 4** de bevindingen, en gingen we dieper in op de bijzondere moeilijkheden die onderzoekers van gliomen vaak parten spelen. Ook bespraken we een aantal methodologische aspecten van de studies. We besloten met mogelijkheden en wenselijke verbeteringen voor toekomstig onderzoek.

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