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ORIGINAL ARTICLE

Homozygosity mapping of familial glioma in Northern Sweden

BEATRICE MALMER[1], SUSANN HARALDSSON[2], ELISABET EINARSDOTTIR[2], PETTER LINDGREN[2] & DAN HOLMBERG[2]

[1]*Department of Radiation Sciences, unit of Oncology, and* [2]*Department of Medical Biosciences, Unit of Medical Genetics, Umeå University Hospital, 901 87 Umeå, Sweden*

Abstract
About 5% of glioma cases are familial. Most glioma families are not ascribed to the well-known glioma predisposing syndromes. One segregation analysis has supported an autosomal recessive gene in glioma families, which could be studied by homozygosity mapping. The ancestors of seven glioma families from the northern region of Sweden were traced through genealogical databases. A common ancestor and inbreeding were traced to give support to an autosomal recessive gene. Homozygosity mapping was performed with a genome-wide scan of 811 markers with linkage calculations. The families were geographically mapped to see if familial glioma was more common in northern compared with southern Sweden. Three of the seven families were remotely related. Homozygosity mapping did not reveal any allele homozygous for all three families. However, there was a geographical clustering of glioma families in the northern region of Sweden. A non-parametric analysis showed an allele-sharing LOD score of 1.05 for marker D1S196 on chromosome 1q23. Genealogical studies linking glioma families might be a tool for linkage in a small set of families. This study did not support an autosomal recessive gene, implicating a low penetrant dominant gene as a possible explanation to the glioma family clustering.

Introduction

The aetiology of primary brain tumours is to a large extent unknown. The only well established exogenous factor is ionising radiation [1]. Familial aggregation of glioma occurs in about 5% of all glioma cases [2,3] which is lower compared with breast and colon cancer where a positive family history for same-site tumour is observed in 15–20% of all cases. In one case/control study [2] and two cohort studies an increased risk of glioma for first-degree relatives of glioma patients has been observed [3,4]. Some studies have observed familial cases, especially in elderly patients with glioma, and have advocated an environmental cause for the familial aggregation [5]. However, two segregation analyses have been performed, one of all cancer cases in the family favouring a multifactorial cause for the familial aggregation [6]. The other segregation analysis including glioma patients only coded as affected where an autosomal recessive gene provided the best fit [7].

Genetic analyses of candidate genes in familial glioma have not been very successful [8,9]. In one family with both glioma and melanoma in the family a p16/INK4 locus mutation has been observed [10]. Mutations in the tumour suppressor p53 have also been found in Li-Fraumeni like families with almost exclusively brain tumours [11]. Linkage of Finnish glioma families recently gave support for a novel locus at 15q23-q26.3 [12]. In this study we wanted to investigate whether the glioma families in Northern Sweden had a common ancestor, which could indicate a common founder mutation. A homozygosity-mapping project spanning 810 markers over the whole genome was also performed in order to find areas of interest harbouring an autosomal recessive gene.

Material and methods

Family data

The glioma families were identified from incident cases of glioma in the northern region of Sweden,

Correspondence: Beatrice Malmer, Department of Radiation Sciences, Unit of Oncology, Umeå University Hospital, 901 87 Umeå, Sweden. E-mail: beatrice.malmer@onkologi.umu.se

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1985–1993. This study has been reported previously [3]. There were altogether 25 glioma families and many family members were deceased as a result of the glioma. It was therefore only possible to collect blood samples in 5 of the families from the cohort study and from two additional families recruited at the Oncology Department in Umeå. The simplified pedigrees of the families are presented in Figure 1. There were two families with three affected persons with glioma in the family and five families with two persons affected with glioma. In these seven families the ancestors were traced through investigations in the Regional Archives and also through an Internet database ("Indiko") including all persons living in a small specific catchment area of northern Sweden. All ancestors in all branches of the families were traced back as far as possible, often to the seventeenth century. We searched for ancestors in common and indications of inbreeding. The population in Sweden comprises Caucasians, and people from southern Sweden populated the northern part of Sweden during the sixteenth century. Before this area was sparsely inhabited by a Sami population. The medical records of the affected persons in the families have been analysed to find signs of neurofibromatosis type 1 and 2 and no signs of these or other cancer syndromes could be found.

The Multigeneration Register for all of Sweden was also used to study the geographic distribution of familial glioma in Sweden. This register comprises almost all citizens in Sweden born between 1932 and 1997. It has been used in previous studies and is presented in more detail elsewhere [13]. The identified glioma families were mapped according to county of birth, to investigate whether familial glioma is more common in northern Sweden compared with southern Sweden. The number of families was correlated with a χ^2 distribution to the number of inhabitants in southern Sweden (8 million) versus the inhabitants in the northern region (0.9 million) and in the most northern county, Norrbotten (0.26 million).

Methods

The samples have previously been screened for p53 mutations in all exons to exclude Li-Fraumeni syndrome and for microsatellite instability to rule out Turcot's syndrome [8].

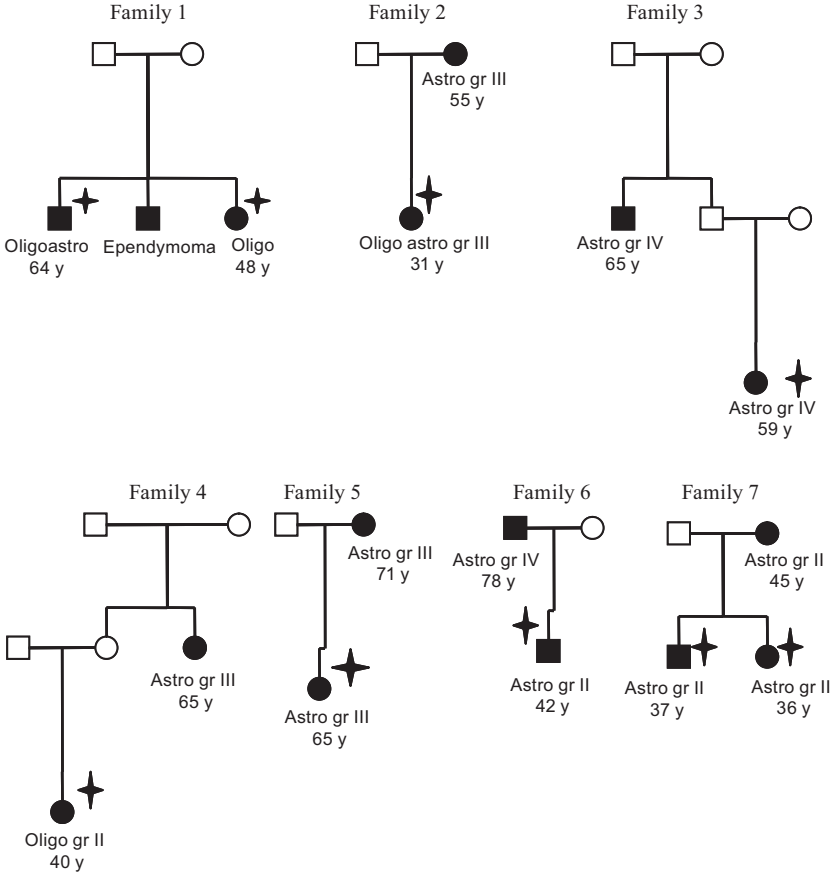

Figure 1. Simplified pedigree of the seven glioma families from Northern Sweden included in the genealogical study. Family nos 1, 5, and 7 were linked as shown in Figure 2. Markers indicate affected patients with DNA available.

Blood samples were collected from nine affected cases in the family and from five unaffected persons. Genomic DNA was isolated from blood using the Qi-amp tissue kit.

Genomic DNA (30 ng) was mixed with 250 μM dNTP (Pharmacia), Gene Amp PCR buffer II, 0.4 μM primer, 2.5 m M MgCl2, 0.3U AmpliTaq-Gold (Applied Biosystems) at a total volume of 7.5 μl. Multiplex PCR was performed by using a 9600 GeneAmp thermal cycler (Applied Biosystems), according to the manufacturer's conditions. The PCR product was pooled and diluted 1:10 and 1:20 times. Electrophoresis was run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and analysis of alleles was performed with GeneMapper 3.0 (Applied Biosystems).

A total of 811 markers with an average distance of 5 cm from the Linkage mapping set v2.5 MD-5 (Applied Biosystems) were used for genome-wide screening. The fine mapping markers were ordered from DNA Technology with a median distance of 0.5–3.0 Mb. The largest gap between markers due to technical difficulties or difficulties in getting valid markers was 10.9 cm. Whole-chromosome haplotypes were constructed and, owing to the small number of families available, regions of homozygosity were analysed visually. The exact markers can be obtained from the first author upon request.

Statistical analysis

Analysis of the power of the pedigree to detect linkage was carried out by means of MSIM of the SLINK software [14,15]. In the simulation, genotype data for one disease marker with five equally distributed alleles was generated a thousand times under two recessive and two dominant models. For the two recessive models, a penetrance of 80% and disease allele frequencies of 10% and 0.1%, respectively, were assumed. In the dominant case, the two models differed in penetrance, 40% compared with 60%, while the same population disease allele frequency of 0.1% was used.

Both non-parametric and parametric multipoint linkage analysis was performed using the software Allegro [16] and the Genethon marker map [17]. Markers with distances not available in the map were excluded from the analysis. Due to the small number of individuals genotyped, equally distributed marker allele frequencies were assumed. For the allele-sharing analysis, we used the s_{pairs} scoring function that assesses identity by descent (IBD) sharing among all pairs of affected individuals within families. Also, the NPL Z scores were converted into allele-sharing LOD scores using the exponential model described elsewhere [18]. For the parametric analysis, the same model parameters as in the simulations were used.

Results

The genealogical research found three of seven families that were remotely related to each other in several different branches of the pedigree (Figure 2). Each family was related to each other both on the paternal as well as the maternal side of the pedigree, giving support for an autosomal recessive gene with one disease allele from each side of the family. Family number 1 with the most severe phenotype, including a mother and two children with glioma before the age of 45, was especially closely related (see Figure 2). The patterns in the families were not clearly autosomal recessive but since there was inbreeding in the family this could have a pseudo-dominant appearance. There was no over-representation of any kind of cancer in the seven families as described in previous studies and in the three families that were remotely related there was no other cancer in the family [7]. Four families were not linked to the others; one of those families came from southern Sweden and two families from Finland. Only one family, which also originated from the northern region of Sweden, was not linked in the compound pedigree. The simplified geographic analysis detected that 10/53 families came from families in northern Sweden, which, compared with the number of citizens, is significantly higher number than in southern Sweden ($p < 0.05$). The aggregation was especially pronounced in Norrbotten county ($p < 0.001$).

Homozygosity mapping of the three pedigrees that were linked did not show any homozygotic chromosomal region in common for all five affected persons. Simulation using the recessive model with a disease allele frequency of 10% resulted in an average LOD score of 0.84, with 41% of the simulations giving a LOD score of 1 or more. The other three models resulted in lower values, especially the dominant models. Although the power to detect linkage is quite low, we decided to do linkage analysis of the genome-wide scan to obtain indications of regions linked to glioma.

The parametric calculation under this recessive model did not yield any significant LOD scores.

Non-parametric analysis of the material did reveal an allele-sharing LOD score of 1.05 for marker D1S196 on chromosome 1q23. There were several markers in this region 1q21-q25 showing an increased LOD score close to 1 (Table I). We did not find any support for the Finnish autosomal dominant locus at 15q23-q26.3 in our families.

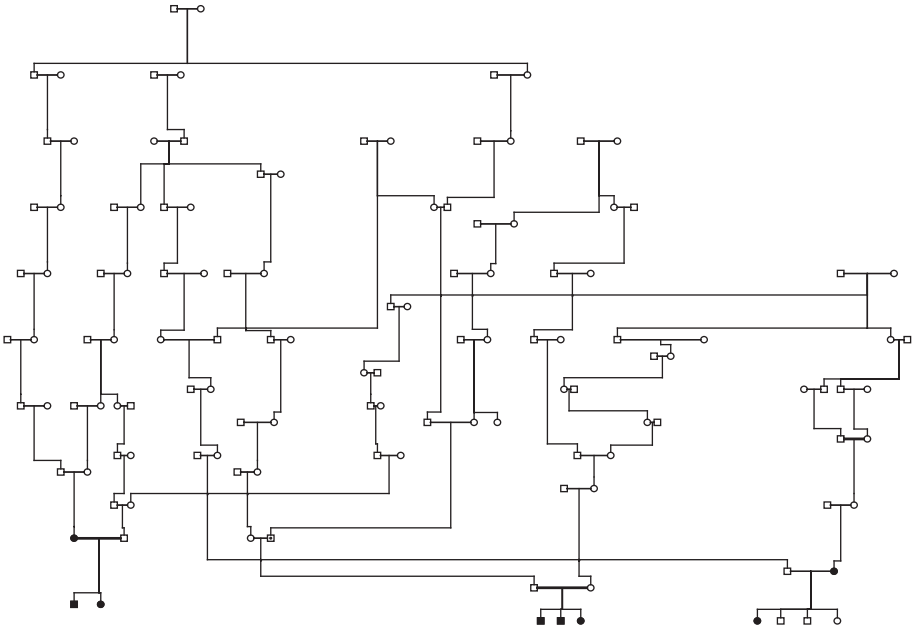

Figure 2. Three glioma families from northern Sweden and their interrelationships back to the seventeenth century.

Discussion

Genetic susceptibility of brain tumours has been described in several uncommon inherited disorders as for example in Li-Fraumeni syndrome, neurofibromatosis, and Turcot's syndrome [19]. The question has been raised as to whether familial gliomas exist beside these well-characterized syndromes. First-degree relatives of glioma patients seem to have an increased risk of glioma according to some epidemiological studies [2–4] but not according to others in an Icelandic population [20]. The familial aggregation in one study was partly explained by p53 mutations in 6/44 glioma families' probands [21] but that has not been confirmed in other studies [8]. Therefore few families have previously been linked to candidate genes or well-known tumour syndromes so other as yet unidentified genes might be involved.

Table I. Markers on chromosome one in the area 1q21–1q25 showing increased LOD scores.

Non-parametric LOD score	Marker on chromosome 1
0.9738	D1S2878
0.9971	D1S2681
1.0052	D1S2762
1.0517	D1S196**
1.0395	D1S2799
1.0099	D1S2851
0.9806	D1S452
0.0668	D1S218*
0.9660	D1S2818
1.0262	D1S238
1.0492	D1S2877
0.9563	D1S412

Adjacent markers showed lower LOD scores of 0.8 or lower. *D1S218 was the marker with increased LOD score of 1.24 in the Finnish linkage study. **D1S196 was the marker with highest LOD scores although several markers yielded similar LOD scores.

Few attempts have previously been made to perform a genome-wide scan on familial glioma. The only linkage analysis previously reported is a Finnish study where seven families identified in the Tampere region gave support for a novel low-penetrance locus at 15q23-q26.3 with a LOD score of 3.35 [12]. No glioma gene responsible in this region has yet been identified. In the present study the main hypothesis was to identify a locus for an autosomal recessive gene, and therefore homozygosity mapping was performed. There were no specific regions with homozygotic markers for all affected persons in the family (see Figure 2). There are several possible explanations as to why we could not identify a chromosomal region in common. First of all, the proposed inheritance pattern could be false and an autosomal low-penetrance gene or a polygene inheritance could be the explanation for the glioma aggregation in these families.

Although the usage of equal allele frequencies in the linkage analysis is not likely to be an accurate reflection of the truth, and despite the very small number of individuals in the families, the LOD score of 1.05 at 1q23 on chromosome 1 is a potentially interesting finding. This marker is the only one in the entire genome-wide scan that shows elevated LOD scores, and this region has elevated LOD scores in several consecutive markers. Therefore this region is an interesting candidate for further studies on glioma. The fact that this region has previously

been implicated in the Finnish study that found a non-parametric LOD score of 1.25 in the region of 1q25 at marker D1S218, close to our marker, only strengthens this argument. A confirmation in a larger data set is needed.

The aggregation of glioma families is rare and the affected persons often succumb to the disease rather rapidly, so larger sample sizes are difficult to achieve. The segregation analysis favouring an autosomal recessive gene could be misinterpreted when only allowing exclusively for first-degree relatives. However, the interrelationships on both the maternal and the paternal side of the pedigrees indicate the possibility of an autosomal recessive gene. Another explanation could of course also be a detection failure with the mapping of 811 markers, which might not be dense enough [22]. Nevertheless, this method has previously and successfully been used with few affected family members who are remotely related [23]. Since the families are very remotely linked to each other this might be due to chance and equal to the inbreeding factor in the population of northern Sweden. Environmental causes for the familial aggregation of glioma can of course not be excluded, though very few environmental causes have been identified to date. Different studies of familial aggregation of gliomas have given conflicting results. However, several studies have identified families of at least two persons affected with glioma in the family in about 5% of all glioma cases, which according to some more recently performed studies is more than expected in the population [3]. Also, the geographic cluster analysis indicated higher frequency in northern Sweden, which gives an indication that if there is a major gene involved this catchment area is important to study. There are also other hereditary disorders that specifically cluster in this region as for example familial amyloidosis and familial ALS. To advance in knowledge of familial glioma, larger sets of families ought to be collected prospectively in a collaborative setting to allow for general linkage calculations. Faced with the knowledge of glioma susceptibility where high penetrant families including many affected persons are extremely rare and most families have two affected members only, genes with a low penetrance are more likely be involved than major autosomal genes.

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