A Novel Low-Penetrance Locus for Familial Glioma at 15q23-q26.3

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ABSTRACT

Epidemiological studies and case reports suggest that familial clustering of gliomas may occur in families that do not fit any known tumor syndromes. In the present study, 15 familial glioma pedigrees from a limited geographical area were hypothesized to carry the same low-penetrance susceptibility allele. We used a two-stage strategy for disease gene mapping. A genome scan in four glioma families revealed four interesting loci at chromosome arms 1q, 6q, 8p, and 15q. Additional markers in these regions provided evidence of significant linkage to 15q23-q26.3 with a maximum nonparametric linkage score of 3.35 with marker D15S130. Investigation of all 15 glioma families by association analysis (haplotype pattern mining) and through use of the transmission/disequilibrium test gave further evidence of significant association/transmission distortion at the same 15q locus (P = 0.02 and P = 0.03, respectively). No evidence of involvement of known tumor syndromes was obtained from the data provided by the linkage analysis or hospital records. Thus, the first genome-wide linkage analysis of familial glioma suggests a novel susceptibility locus at 15q23-q26.3.

INTRODUCTION

Gliomas are known to occur in association with several well-defined hereditary tumor syndromes such as neurofibromatosis 1 and 2, tuberous sclerosis, Li-Fraumeni syndrome, and Turcot’s disease (1). Familial clustering of gliomas, however, often occurs in the absence of these tumor syndromes. Although families with multiple glioma patients have been described in several case reports and epidemiological studies (2–7), the genetic basis behind such occurrences remains undiscovered. On the basis of segregation analyses, both an autosomal recessive mode of inheritance and a multifactorial Mendelian model have been suggested (8, 9).

In previous studies, a number of candidate genes for familial glioma have been investigated. Some of these genes are known to predispose individuals to established brain tumor syndromes, and others are involved in the pathogenesis of sporadic gliomas. The p53 tumor suppressor gene has been the most frequently investigated candidate gene, but mutations have only seldom been found in the germ line of glioma families without Li-Fraumeni syndrome (4, 10–15). Interestingly, large germ-line deletions at the INK4 tumor suppressor region involving the p16, p15 and p14 genes have been found in some families featuring both gliomas and skin melanomas (13, 16). Such a brain-tumor-melanoma syndrome, however, seems to affect only a small proportion of glioma families (13, 16). Other candidate genes studied, namely PTEN, NF1, and CDK4, have not been found to harbor mutations in the germ line of familial glioma patients (11–14).

The results of previous studies therefore indicate that there may be other genes responsible for hereditary predisposition to familial gliomas. In the present study, we undertook a genome scan by linkage analysis to identify novel chromosomal candidate loci for a glioma susceptibility gene. Linkage disequilibrium methods were also applied to test for the presence of a founder mutation in families from a limited geographical area inhabited by a relatively stable population (17).

MATERIALS AND METHODS

Identification of the Glioma Families

A total of 25 families with two or more verified gliomas were identified among glioma patients operated on at Tampere University Hospital, as described previously (18). The patients who undergo surgery at Tampere University Hospital reside in an area of 1 million inhabitants in western Finland.

A detailed family history of cancer was verified for the familial glioma pedigrees. The descendants of the shared ancestors of the glioma patients in each family were traced through church parish registers and the Central Population Register. For all those living in Finland in 1953–1996, cancer diagnoses were retrieved through the files of the Finnish Cancer Registry, as described elsewhere (18). According to this information and the data obtained from the hospital records of the glioma patients, evidence of involvement of neurofibromatosis 1 or 2, Li-Fraumeni syndrome, tuberous sclerosis, or Turcot’s disease was not found. Nor were germ-line mutations of the p53 tumor suppressor gene found among these families (15).

Genotyping

Description of the Families

Fifteen of the 25 families were subjected to genotyping. Four of these were multiplex pedigrees (with more than one affected glioma patient sampled) informative for the linkage analysis (Fig. 1A).

In these families, genotyping included 4–14 family members.

In addition to the 4 multiplex pedigrees, the remaining 11 families were included in association analysis and the TDT.3 In nine of these families, only one of the affected individuals could be sampled (simplex pedigrees; data not illustrated), and in two families, an affected parent-offspring pair was sampled (Fig. 1B). In each family, three members were analyzed such that the unaffected relatives were siblings or parents of the glioma patients.

The relationship between the nearest glioma patients in the 15 pedigrees was first degree in five families, second degree in three families, third degree in three families, and fifth degree in four families. The number of verified gliomas was two in 12 families, three in 2 families, and six in 1 family. In one family in which there were two verified gliomas, an additional brain tumor was reported in the questionnaire, but no verification could be obtained for this tumor (see Fig. 1A). The family with six gliomas had two family branches stemming from the 18th century, and in one of them, all of the glioma patients were deceased; therefore, information for linkage analysis could be obtained from only one family branch (see Fig. 1A). Overall, these families were consistent with the concept of a low-penetration gene with a possible founder effect, which we adopted as our working hypothesis.

Laboratory Experiments for Genotyping

Blood samples for extraction of constitutional DNA for genotyping were available from 65 members (21 affected members and 44 relatives) of the 15 families. Genomic DNA was isolated from peripheral blood lymphocytes using Puregene DNA Isolation

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3 The abbreviations used are: TDT, transmission/disequilibrium test; NPL, nonparametric linkage analysis; HPM, haplotype pattern mining.
Kits (Genta Systems, Minneapolis, MN). A total of 363 microsatellite markers from Applied Biosystems Linkage Mapping Set MD-10 were used for genome-wide genotyping. The average interval between the markers was 10 cM. DNA (20 ng) was dried on microtiter plates for each PCR assay. The PCRs were performed in 5-μl volumes using reagent concentrations and temperature profiles as recommended by the reagent manufacturer (Applied Biosystems). The fluorescence-labeled PCR products were pooled (10–20 markers/pool), and electrophoresis was run on a MegaBace 1000 capillary electrophoresis instrument (Molecular Dynamics, Sunnyvale, CA). The alleles were visualized using Genetic Profiler 1.1 software (Molecular Dynamics). The fine mapping markers for the interesting regions in chromosomes 1, 6, 8, and 15 were selected between the original genome-wide scan markers with average intervals of 2–3 cM based on the positions stated in the Marshfield genome map (19). The PCRs were performed as described above.

**Statistical Methods**

**Linkage Methods.** First, nonparametric and parametric multipoint linkage analyses were performed with GENEHUNTER 2.1 software (20) for the four multiplex pedigrees depicted in Fig. 1A.

The chromosomes showing a positive signal in the genome-wide analysis were then reanalyzed with a denser map, using NPL.

**Association Analysis.** Association was analyzed by using the HPM method. HPM is a data mining-based algorithmic approach to genetic association analysis, in which frequent patterns of haplotypes associated with a trait are sought (21).

HPM analyzes case-control data using nontransmitted parental chromosomes as controls (i.e., pseudo-controls). In total, 40 disease-associated chromosomes and 40 control chromosomes were obtained from the 15 glioma families. HPM was carried out for chromosomes showing positive signals in the nonparametric linkage analyses. The following parameters were used for HPM: (a) maximum length of the pattern, 10 markers; (b) maximum number of gaps, 2; and (c) maximum χ² for a pattern, 2 (a low threshold was set because of the limited amount of data). To compensate for differing marker densities and marker informativeness, a total of 10,000 permutations, fashioned as described by Toivonen et al. (21), were run to obtain empirical Ps.

**TDT.** The TDT was carried out separately for each allele of each marker in the data, lumping together the remaining alleles, as implemented in the GENEHUNTER 2.1 package.

Empirical Ps were obtained in three steps, each of which represented a different degree of correction for multiple testing. (a) For a single chromosome, the distribution of the highest χ² value obtained from any marker and any allele within the chromosome...
produces an empirical P that corrects for multiple testing within the chromosome.

(b) For densely mapped chromosomes, the distribution of the highest $\chi^2$ value was obtained from any of the four chromosomes. In each iteration, the highest of the four chromosome-specific values was selected, and the distribution was created for these highest values. This provides a correction of multiple testing in the four chromosomes analyzed with the denser map.

(c) For the whole genome, assuming that the four chromosomes correspond roughly to one-fifth of the human genome, multiplying the P obtained from step b by 5 gives the conservatively corrected P, assuming hypothetical, equally dense map and equal sample size everywhere in the genome.

Study Ethics

The study protocol was approved by the Ethics Committee of Tampere University Hospital and by the Ministry of Social Affairs and Health, Finland. Written informed consent was obtained from all those giving blood samples.

RESULTS

Genome-wide Linkage Analysis. Genome-wide NPL revealed several interesting chromosome regions. The highest NPL scores were 3.19 near marker D15S130 of chromosome 15 and 2.28 near marker D8S258 of chromosome 8. The next highest scores were 1.25 at 1q25 and 1.18 at 6q27.

In reanalysis of the four candidate regions with additional markers, the NPL scores for chromosomes 8 and 15 were clearly increased (2.96 and 3.35, respectively), whereas those for chromosomes 1 and 6 were decreased (1.23 and 0.77, respectively; Table 1 and Fig. 2).

In the genome scan, no interesting signals were obtained from chromosome regions known to involve hereditary tumor syndromes with gliomas, such as loci harboring the NF1, NF2, TSC1, TSC2, RB, p53, p16, and hMLH1 genes. Because parametric two-point analysis with dominant and recessive models yielded very modest LOD scores (all were between −0.5 and 0.5), exclusion of these chromosome loci was not successful either.

Association Analysis by HPM. HPM analysis was performed for the four chromosomes chosen for fine mapping on the basis of the genome-wide linkage analysis. Analysis of 20 trios (obtained from the 15 families) gave the highest empirical Ps for marker D6S446 at chromosome 6 ($P = 0.0119$) and for marker D15S642 at chromosome 15 ($P = 0.0154$).

TDT. TDT was performed among all 15 families for the four candidate locations. The strongest transmission disequilibrium was observed at 15q with marker D15S642, where allele 8 was transmitted every time from the 12 heterozygous parents. Based on 10,000 iterations, the empirical P for the observed degree of transmission distortion ($\chi^2 = 12.00$) was 0.0009. When correction was carried out for all four chromosomes reanalyzed, the P was 0.0062. Under the most stringent correction of multiple testing, the four chromosomes studied correspond roughly to one-fifth of the human genome and, thus, the conservatively corrected P for the whole genome is 0.03.

DISCUSSION

In the present study, we report the results of the first genome-wide linkage analysis in familial glioma. We present the mapping of a novel low-penetrance susceptibility locus at 15q23-q26.3 suggested by linkage and association analyses. Although several potential candidate genes for familial glioma reside in this region, none of them has been previously associated with hereditary brain tumors.

The clinical and epidemiological data on gliomas clustered in families suggest the presence of a low-penetration gene possibly present in the local population as a founder mutation. A two-stage strategy was used for disease gene mapping to test this hypothesis. Although a total of 25 pedigrees with familial gliomas were identified for this study, only 15 of them presented one or more living glioma patients to yield tissue samples for genotyping. In four of these families, the relationship between the two living glioma patients was informative for the linkage analysis. Our strategy was to search for chromosomal candidate areas by carrying out a genome scan of the four informative pedigrees. Thereafter, association analysis and transmission/disequilibrium testing were applied to the larger number of families to investigate the presence of a founder effect. Thus, the choice of strategy was dependent on our working hypothesis of a local founder effect.

NPL of the four multiplex pedigrees revealed a candidate locus for familial glioma at 15q23-q26.3. Interestingly, significant association and transmission disequilibrium were revealed in the same genetic area. Consistent results using these three methods are remarkable for two reasons: (a) the linkage analysis and HPM/TDT methods mostly involved different families; and (b) these methods use different kinds of information present in the data. With regard to a, all family trios with an affected individual were analyzed by HPM and TDT, whereas NPL only involved the four families with adequate affected relative pairs. With regard to b, the inherent natures of the three methods largely differ from each other. Linkage measures cosegregation of alleles or haplotypes with the disease status in extended pedigrees, whereas HPM, as an association method, measures population association of haplotypes to a trait irrespective of linkage. Finally, the TDT measures linkage in the presence of association; the transmission of a possibly associating allele from parent to affected offspring is measured trio by trio, and if population association exists, the test statistic indicates bias from the null hypothesis transmission probability of 0.5. Thus, the fact that three independent approaches all point to the same genetic area gives evidence of true gene localization near the telomere at 15q.

The area exceeding NPL score 3 at 15q23-q26.3 covers 40 cM, which is a relatively wide area even for linkage. The reason for this is the limited number of recombinations due to the limited amount of data. The HPM and TDT analyses suggest the gene location to be at the telomeric end of the candidate region at 15q. However, the NPL scores decrease toward these most telomeric markers (see Fig. 2). Because closer examination of markers in the linkage pedigrees revealed this to be due to only one of four informative families (see Fig. 1A), the overall decrease in the NPL score through the four most telomeric markers is probably explained by the multipoint nature of the NPL score method itself: the discrepancy in the last marker expresses itself in the wider area. Thus, it seems likely that the actual disease locus is situated in the telomeric end, possibly between the two most distal markers used.

The tumor histologies in the 15 families analyzed in this study included various types of gliomas. Glial cell tumors, i.e., astrocytomas, oligodendrogliomas, and ependymomas, present different histological patterns and genetic changes. However, embryonic origin from a common stem cell implicates that the same regulatory mechanisms may be involved (22). This hypothesis is supported by already known tumor syndromes (e.g., neurofibromatosis 1 and 2 and Li-Fraumeni syndrome), each of which predispose individuals to more than one glioma subtype. Similarly, in the present study, the linkage analysis or

Table 1 Results of nonparametric linkage analysis for four candidate locations for familial glioma

<table>
<thead>
<tr>
<th>Cyto genetic location</th>
<th>Closest marker</th>
<th>NPL score (initial scan)</th>
<th>NPL score (dense map)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q25.1</td>
<td>D1S218</td>
<td>1.25</td>
<td>1.23</td>
</tr>
<tr>
<td>6q27</td>
<td>D6S446</td>
<td>1.18</td>
<td>0.77</td>
</tr>
<tr>
<td>8q21.3</td>
<td>D8S258</td>
<td>2.28</td>
<td>2.96</td>
</tr>
<tr>
<td>15q26.2</td>
<td>D15S130</td>
<td>3.19</td>
<td>3.35</td>
</tr>
</tbody>
</table>
disequilibrium methods did not distinguish between individuals with different glioma histologies.

The candidate area at the distal end of 15q is not among those areas most frequently altered in gliomas. This area, however, harbors several interesting candidate genes for familial glioma. For example, the cellular oncogene FES and the insulin-like growth factor 1 receptor gene (IGF1R) reside at 15q26. The NTRK3 gene at 15q25 encodes a tyrosine kinase receptor, which is responsible for signal transduction in neuroepithelial of the central nervous system (23). Mutations at the RECQL3 locus at 15q26.1 cause Bloom syndrome, a recessively inherited tumor predisposition syndrome characterized by pre- and postnatal growth deficiency, skin lesions, and a wide variety of cancers, which are caused by chromosomal instability (24). Other interesting candidate genes for familial glioma at 15q26 are the homologous PACE and PACE4 loci that code for paired amino acid-cleaving enzymes. Furin, the translational product of the PACE gene, converts pro-transforming growth factor β1 protein to biologically active transforming growth factor β, which is involved in the SMAD signaling pathway (25, 26).

The familial occurrence of brain tumors has been confirmed in several epidemiological studies (2, 3, 5–7). With the exception of therapeutic X-irradiation, consistent association between specific environmental exposure and brain tumors has not been identified (27–29). In the present study, we give the first molecular genetic evidence for a hereditary background in families with gliomas as the major manifestation. Our results suggest the presence of a founder mutation at 15q23-q26.3 in familial gliomas in western Finland. Whether the results are consistent in other populations remains to be clarified.

Fig. 2. NPL scores of four chromosomal candidate regions found in the genome-wide linkage analysis. The results are obtained from four pedigrees with familial gliomas in a reanalysis with additional markers.
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