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## Germline rearrangements in families with strong family history of glioma and malignant melanoma, colon, and breast cancer

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**Background.** Although familial susceptibility to glioma is known, the genetic basis for this susceptibility remains unidentified in the majority of glioma-specific families. An alternative approach to identifying such genes is to examine cancer pedigrees, which include glioma as one of several cancer phenotypes, to determine whether common chromosomal modifications might account for the familial aggregation of glioma and other cancers.

**Methods.** Germline rearrangements in 146 glioma families (from the Gliogene Consortium; <http://www.gliogene.org/>) were examined using multiplex ligation-dependent probe amplification. These families all had at least 2 verified glioma cases and a third reported or verified glioma case in the same family or 2 glioma cases in the family with at least one family member affected with melanoma, colon, or breast cancer. The genomic areas covering *TP53*, *CDKN2A*, *MLH1*, and *MSH2* were selected because these genes have been previously reported to be associated with cancer pedigrees known to include glioma.

**Results.** We detected a single structural rearrangement, a deletion of exons 1-6 in *MSH2*, in the proband of one family with 3 cases with glioma and one relative with colon cancer.

**Conclusions.** Large deletions and duplications are rare events in familial glioma cases, even in families with a strong family history of cancers that may be involved in known cancer syndromes.

**Keywords:** CDKN2A/B, family history, glioma, MLH1, MSH2, TP53.

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Diffuse gliomas are the most common group of primary malignant brain tumors.<sup>1</sup> Family history is an important risk factor for glioma, with first-degree relatives of glioma patients having an increased risk of developing the disease.<sup>2–4</sup> Although a small percentage of these families with glioma are attributed to hereditary genetic disorders such as neurofibromatosis types I and II, Li-Fraumeni syndrome, and Turcot's syndrome,<sup>5,6</sup> the genes underlying the appearance of multiple gliomas in most families remain ill defined. In addition to the familial aggregation of glioma-specific risk, the risk of other cancers in first-degree relatives of glioma patients has been noted, and significantly more melanoma cases than expected have been identified.<sup>7</sup> High-penetrance genes such as the tumor suppressor gene *TP53* have been described in families with Li-Fraumeni syndrome; these families include persons diagnosed with glioma as well as other malignancies such as breast cancer, sarcoma, and leukemia. Moreover, these genes have also been associated with glioma and low-penetrant genetic variants in the *CDKN2A* (*p16INK4A/p14ARF*) and *TP53* genomic area.<sup>8–11</sup>

Gliomas have been observed in families with mutations in the *CDKN2A* and *TP53* genes, but most of the studies published to date are based on small sample sizes with limited power to assess the contribution of mutations in these genes with familial glioma.<sup>12–17</sup> In an earlier study, we used standard sequencing, which was ineffective in detecting large rearrangements of *TP53* and *CDKN2A* in 96 unselected glioma families. Only one proband had a *TP53* mutation, and no functional mutations were found in *CDKN2A*.<sup>18</sup>

The association between glioma and melanoma has been previously reported in aggregation studies<sup>3,19–21</sup> and is supported by linkage of melanoma to regions of chromosome 9,<sup>22,23</sup> which has been reported to be deleted or mutated in glioma.<sup>24–26</sup> Furthermore, recent genome-wide association studies of both glioma<sup>9,10</sup> and melanoma<sup>27</sup> have identified variants in chromosome 9p21 near the cyclin-dependent kinase inhibitor genes, *CDKN2A*, *CDKN2B*, and other genes. Although the variants identified for glioma and melanoma are not in the same linkage block, the results indicate the plausibility that deletions or other chromosomal modifications in the region might account for some familial aggregation of glioma and melanoma. The melanoma-neural system tumor syndrome, in which affected families have increased risk of melanoma and astrocytoma, was recently linked to loss of the *CDKN2A/B* genes located on chromosome 9.

The mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, play a basic role in maintaining genome integrity by correcting single-base pair mismatches after DNA replication.<sup>28</sup> It is well established that the etiological basis for Lynch syndrome is heterozygous germline mutations within one of the mismatch genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, with *MLH1* and *MSH2* mutations playing a major role.<sup>29</sup> Lynch syndrome patients are susceptible to colorectal, endometrial, and other cancers recognized by microsatellite instability (MSI), which is a hallmark of MMR defects.<sup>30–32</sup> Lynch syndrome is associated with an increased risk of brain tumors.<sup>33</sup> In carriers of pathogenic *MLH1* or *MSH2* mutations or their first-degree relatives, the cumulative risk of brain tumors to the age of 70 years was 1.7% for carriers of *MLH1* mutations and 2.5% for carriers of *MSH2* mutations.<sup>36</sup> Mean age (38 years) at the time of brain tumor diagnosis is lower in those with Lynch syndrome than in the general population, and the most common tumor types are glioblastoma and

astrocytoma.<sup>37</sup> Biallelic mutations in *MSH2* have been shown to be associated with childhood brain tumors.<sup>38</sup> A heterozygous germline mutation in *MSH2* is also known to be involved in patients with a syndrome diagnosis (eg, Turcot's syndrome), in which some patients have an inherited predisposition for brain tumors and colorectal cancer.<sup>39</sup> The results listed above suggest the possibility that deletions or other chromosomal modifications in common chromosomal regions might account for some familial aggregation of glioma and other cancers, notably melanoma, colon, and breast cancer.

## Materials and Methods

### Ascertainment and Collection of Families

All families were identified through the Gliogene Consortium, and the exclusions were based on reported information obtained from the questionnaire in which we asked about the clinical criteria used for these hereditary conditions. We excluded all families with a reported or confirmed diagnosis of neurofibromatosis I, neurofibromatosis II, Turcot's syndrome, or tuberous sclerosis. The recruitment protocol and data collection procedures for this study have been previously described.<sup>40</sup> We identified 146 (34%) families meeting the criteria of having both familial glioma and associated cancers out of 428 probands recruited from 14 569 screened cases of incident glioma cases. The cases were initially screened for family history of glioma and had been diagnosed between 2007 and 2011 at one of our 14 recruitment centers. DNA was extracted from EDTA-venous blood samples and/or saliva samples. Biospecimen and clinicopathological information from probands and the above description of selected family members were collected after obtaining informed consent according to protocols approved by each center's institutional review board in accordance with the Declaration of Helsinki. The genomic areas covering *TP53*, *CDKN2A*, *MLH1*, and *MSH2* were selected because these genes have previously been reported to be associated with cancer pedigrees known to include glioma. Families with 2 or more verified gliomas were recruited between 2007 and 2011. Distributions of demographic characteristics of the probands, pathological characteristics of the glial tumors, and clinical variables of glioma in the families were described based on information derived from personal questionnaires<sup>40,41</sup> (Table 1). Glioma families were included from Sweden ( $n = 14$ ), Denmark ( $n = 36$ ), Israel ( $n = 10$ ), and the United States ( $n = 86$ ) (Table 2). The first category was families with at least 2 glioma cases verified and a third reported or verified in the same family ( $n = 67$ : Sweden  $n = 7$ , Denmark  $n = 12$ , Israel  $n = 5$ , United States  $n = 43$ ). (International Classification of Diseases codes for oncology: low grade glioma [WHO grades I and II]: juvenile pilocytic astrocytoma [9421/3], fibrillary astrocytoma [9420/3], protoplasmic astrocytoma [9410/3], gemistocytic astrocytoma [9411/3], diffuse astrocytoma [9400/3], oligodendroglioma [9450/3], oligoastrocytoma [9382/3], ependymoma [9391/3]; high-grade glioma [WHO grades III and IV]: anaplastic astrocytoma [9401/3], anaplastic oligodendroglioma [9451/3], anaplastic oligoastrocytoma [9382/3], anaplastic ependymoma [9392/3], gliosarcoma [9442/3], gliomatosis cerebri [9381/3], and glioblastoma [9440/3]). The second category was families with  $\geq 2$  glioma cases plus a report of at least one family member affected with colon cancer, breast cancer, or malignant melanoma ( $n = 128$ : Sweden  $n = 12$ ,

**Table 1.** Demographic characteristics of the probands and pathological characteristics of the glial tumors from Sweden, Denmark, Israel, and United States ascertained for multiplex ligation-dependent probe amplification analyses of *TP53*, *CDKN2A/B*, *MLH1* and *MSH2*

Glial Tumor (pathological characteristics)	Number of Affected Individuals	Median Age at Diagnosis (y) <sup>a</sup>	Sex Male/Female	Race White/Black/Hispanic/Arabic
<i>Astrocytic tumors</i>				
Astrocytoma, unclassified	3	43.0	2/1	2/0/1/0
Astrocytoma, fibrillary	1	43.0	0/1	1/0/0/0
Astrocytoma, gemistocytic	1	31.0	0/1	1/0/0/0
Astrocytoma, juvenile pilocytic	1	2.0	0/1	1/0/0/0
Astrocytoma, diffuse	9	29.0	3/6	8/0/1/0
Astrocytoma, anaplastic	18	47.0	11/7	18/0/0/0
Ganglioglioma	2	29.0	0/2	2/0/0/0
Glioma, unclassified	5	39.0	2/3	5/0/0/0
Glioblastoma	64	56.0	35/29	61/2/0/1
<i>Oligodendroglial tumors</i>				
Oligodendroglioma	17	42.0	9/8	16/0/1/0
Oligodendroglioma, anaplastic	10	51.5	2/8	10/0/0/0
Oligoastrocytoma	3	34.0	1/2	3/0/0/0
Oligoastrocytoma, anaplastic	3	45.0	1/2	3/0/0/0
<i>Ependymal tumors</i>				
Ependymoma, myxopapillary	2	24.5	0/2	2/0/0/0
Ependymoma	3	28.0	0/3	3/0/0/0
Ependymoma, anaplastic	1	60.0	0/1	1/0/0/0
<i>Neuronal and mixed neuronal-glia tumors</i>				
Dysembryoplastic neuroepithelial tumor	1	28.0	0/1	1/0/0/0
Paraganglioma of spinal cord	1	51.0	0/1	1/0/0/0

<sup>a</sup>Median age at diagnosis of probands.

Denmark  $n = 38$ , Israel  $n = 8$ , and United States  $n = 70$ ). Some families belonged to both categories, having  $\geq 3$  cases of glioma, and another cancer in the family ( $n = 37$ : Sweden  $n = 5$ , Denmark  $n = 9$ , Israel  $n = 3$ , and United States  $n = 20$ ) (Table 2).

### Multiplex Ligation-dependent Probe Amplification

#### *MLH1* and *MSH2*

The samples were screened for large deletions/duplications by multiplex ligation-dependent probe amplification (MLPA). MLPA is a method for copy number detection by the multiplex PCR method. Small (50–70 nt) sequences are targeted, enabling MLPA to identify single exon aberrations. The samples were ligated and amplified using the SALSA MLPA P003 *MLH1/MSH2* probe mix version B2 according to the protocol manufacturer's recommendation (MRC-Holland). The P003 *MLH1/MSH2* probe mix version 2 contains probes for each of the 19 exons of the *MLH1* gene and for each of the 16 exons of the *MSH2* gene. Also, 2 probes are included for the most 3' exon of *EPCAM*, a gene located just upstream of the *MSH2* gene. Deletions of the most 3' exon of the *EPCAM* gene can result in silencing of the *MSH2* gene. In addition, the P003 *MLH1/MSH2* probe mix also covers 7 genes in the *CDKN2A-9p21* region + *PAX5* (9p13) *DOCK8* (9p24.3), and *GLDC* (9p21.1). The samples were analyzed on a CEQTM 8000 GeneticAnalysis System (Beckman Coulter Inc.). Data normalization and analysis were performed with GeneMarker Software version 1.75 (SoftGenetics) using standard parameters.

#### *TP53* and *CDKN2A/B*

Standard MLPA analysis was performed following the manufacturer's instructions (MRC-Holland), version 31; 17-06-211. One hundred nanograms of genomic DNA were denatured and then hybridized with SALSA MLPA probe mixes that covers 6 genes in the *TP53-17p13.1* region + *NF2* and *CHEK2* (included *CHEK2\*1100-delC*). Probe mixes used were P056-A2 for *TP53* and ME024-B1 9p21 for *CDKN2A/2B*. Following ligation, PCR was performed in a Bio-Rad 1000series Thermal Cycler (Bio-Rad Laboratories). Fragment separation was carried out as suggested by MRC-Holland on an ABI 3100 sequencer using POP7 polymer and GeneScan-500 ROX sizing standard (Applied Biosystems). 8.75  $\mu\text{L}$  of Hi-Di Formamide and 0.25  $\mu\text{L}$  of GeneScan-500 ROX sizing standard were mixed with 1  $\mu\text{L}$  of the MLPA PCR product per sample for a total volume of 10  $\mu\text{L}$ . Data were analyzed with the SoftGenetics GeneMarker software version 1.6 from SoftGenetics LLC.

#### Next-generation Sequencing

Since some of the variants found in this study were not standardized and clinically validated mutations, we used massively parallel sequencing of hybrid-captured DNA to further evaluate preliminary findings from MLPA screening of genes in the 9p21 region. Agilent SureSelect probes were designed to capture the genomic regions of *CDKN2A* and *CDKN2B*, including introns and 20 kb adjacent 5' and 3' regions, which covered the regions implicated by MLPA. Paired-end sequencing 2  $\times$  100 bp was performed on

**Table 2.** Descriptive characteristics of glioma families from Sweden, Denmark, Israel, and United States ascertained for multiplex ligation-dependent probe amplification analyses of *TP53*, *CDKN2A/B*, *MLH1* and *MSH2*

Categories	Number of Affected Individuals <sup>b</sup>	Median Age at Diagnosis <sup>a</sup>	Non-GBM n (%)	GBM n (%)
<i>Pedigrees available for MLPA analysis</i>				
United States	85	45.0	49 (57.0)	36 (43.0)
Sweden	14	57.0	8 (57.1)	6 (42.9)
Denmark	36	51.0	18 (50.0)	18 (50.0)
Israel	10	49.5	5 (50.0)	5 (50.0)
<i>Pedigrees with ≥3 glioma</i>				
United States	43	48.0	20 (46.5)	23 (53.5)
Sweden	7	60.0	6 (85.7)	1 (14.3)
Denmark	11	56.0	5 (45.5)	6 (54.5)
Israel	5	56.0	1 (20.0)	4 (80.0)
<i>Pedigrees with ≥2 glioma + colon cancer</i>				
United States	53	45.0	19 (35.2)	34 (64.8)
Sweden	10	52.0	4 (40.0)	6 (60.0)
Denmark	25	50.0	10 (40.0)	15 (60.0)
Israel	1	35.0	1 (100.0)	NA
<i>Pedigrees with ≥2 glioma + breast cancer</i>				
US	35	48.0	15 (42.9)	20 (57.1)
Sweden	5	60.0	3 (60.0)	2 (40.0)
Denmark	24	45.0	12 (50.0)	12 (50.0)
Israel	8	41.0	5 (62.5)	3 (37.5)
<i>Pedigrees with ≥2 glioma + malignant melanoma</i>				
United States	16	51.5	10 (62.5)	6 (37.5)
Sweden	0	NA	NA	NA
Denmark	9	61.0	5 (55.6)	4 (44.4)
Israel	2	41.0	2 (100.0)	NA

<sup>a</sup>Median age at diagnosis of probands.

<sup>b</sup>Overlap because some of the probands were included in several categories.

Abbreviations: MLPA, multiplex ligation-dependent probe amplification; N, number of affected individuals; NA, not applicable.

the Illumina HiSeq2000 instrument to an average depth of >100 reads, followed by alignment to the reference genome. Coverage over the suspected deleted/duplicated regions was not found to be different from coverage in control samples.

## Results

We were able to successfully analyze 127 out of 146 glioma cases for *TP53* and *CDKN2A/B*. One hundred thirty-seven out of 146 glioma cases were also successfully analyzed for *MLH1* and *MSH2*. One mutation found was a deletion of exon 1-6 in *MSH2*; this mutation was present in the proband of a single family. The family included 3 glioma cases and 1 relative with colon cancer (Table 3). The proband in this family was diagnosed with anaplastic oligodendroglioma at age 63 years. The other affected relatives in this family were a maternal first cousin diagnosed with anaplastic astrocytoma at age 32 years, a maternal first cousin's child diagnosed with oligodendroglioma at age 51 years, and a maternal aunt diagnosed with colon cancer at age 84 years (Table 3). Another aberration found was the variant *CHEK2* 1100delC, and this aberration was present in one family that included 3 cases with glioma and one relative with breast cancer

(Table 3). The proband in this family was diagnosed with an oligodendroglioma at age 70 years. The other affected relatives were the proband's mother, who was diagnosed with a glioblastoma at age 72 years, the child of the mother's first cousin diagnosed with a glioblastoma at age 41 years, and a maternal aunt diagnosed with breast cancer at age 38 years.

In addition, we found, a duplication at the promoter of *CDKN2A*prom dupl1022before ex1 (in 3 of the families), a deletion at exon 2 of *EFNB3* delex2 (in 2 of the families) and a duplication of *GLDC* dupl9p24.1 (in one family) but these aberrations could not be verified by next-generation sequencing (Table 2).

## Discussion

In this large family study of gliomas, we found one large deletion in exons 1-6 of *MSH2* in one of the Swedish families with a family history of colon cancer. This mutation was originally detected in 9 apparently unrelated multigenerational kindred with Lynch syndrome. The sequence of the breakpoints of the exon 1-6 deletions and the haplotypes surrounding the mutation were identical in all 9 kindred, suggesting a common origin of the mutation.<sup>42</sup> A similar mutation was reported as an American Founder Mutation in

**Table 3.** Description of aberrations detected in glioma families from Sweden, Denmark, Israel, and United States by multiplex ligation-dependent probe amplification

Family ID	Maternal/paternal <sup>a</sup>	Gliomas	Colon Cancer	Breast Cancer	Melanoma	Gene	MLPA Status
1	Bilineal	3	1	–	–	<i>MSH2</i>	Del exon 1-6
2	Maternal	3	–	1	–	<i>CHEK2</i>	1100 delC
3	Paternal	4	1	1	–	<i>CDKN2A</i>	Prom dupl1022 before exon 1
4	Paternal	2	1	1	–	<i>CDKN2A</i>	Prom dupl1022 before exon 1
5	Paternal	3	1	2	–	<i>CDKN2A</i>	Prom dupl1022 before exon 1
6	Maternal	3	–	1	–	<i>EFNB3</i>	Del exon 2
7	Paternal	2	1	2	–	<i>EFNB3</i>	Del exon 2
8	Bilineal <sup>b</sup>	3	–	1	–	<i>GLDC</i>	dupl 9p24.1

<sup>a</sup>The maternal (mother's side)/paternal (father's side) refer only to the glioma in the family.

<sup>b</sup>Unconfirmed glioma on the maternal side.

Abbreviation: MLPA, multiplex ligation-dependent probe amplification.

families with Lynch syndrome, an autosomal-dominant cancer syndrome traced back to a single couple who migrated from Germany, and settled in Pennsylvania in the early 1700s. Lynch syndrome is known to be associated with hereditary colorectal cancer<sup>43,44</sup> and several extracolonic cancers including endometrial, gastric, small-bowel, renal, ovarian, and brain.<sup>33,36</sup> Despite a low incidence, brain tumors were the third highest cancer-related cause of death in a large Dutch cohort of patients with Lynch syndrome.<sup>45</sup> Germline mutations in *MSH2* have also been described in families with a syndrome diagnosis such as Turcot's syndrome, which is clinically characterized by occurrence of primary brain tumors and colorectal cancer.<sup>39</sup> Mutations in *MSH2* result in production of a faulty, truncated, or absent protein, which impairs the ability of the MMR system to recognize and repair DNA mismatches.<sup>46</sup> We also identified rearrangements in the promoter of *CHEK2*, the variant *CHEK2* 1100delC, in one American family having a family history of breast cancer. *CHEK2* acts as a checkpoint gene, activated in response to DNA damage, and encodes a serine/threonine-protein kinase that phosphorylates P53. The germline 1100delC variant of *CHEK2* is a frameshift mutation, resulting in a truncated and nonfunctional protein.<sup>47</sup> Nevertheless, *CHEK2* is a well-known median penetrant gene that is quite common in the population. Published data suggest that *CHEK2* is not involved in familial glioma.<sup>48,49</sup>

In addition, a novel duplication was identified in the promoter region of *CDKN2A*. To our knowledge, this specific aberration in the promoter has not been previously described in the literature. The aberration in *CDKN2A* was present in 3 families, all of which have a family history of both breast and colon cancer. Unfortunately, we were unable to confirm this aberration by additional deep-sequencing methods. Because of the unusual structure of *CDKN2A*, mutations in this locus may affect both p16<sup>INK4a</sup> and p14<sup>ARF</sup> depending on the localization and type of sequence alteration. The p16<sup>INK4a</sup> has been found to be inactivated in the vast majority of melanomas through mutation, deletion, or promoter hypermethylation of *CDKN2A*.<sup>50</sup> The *CDKN2A* has, as a low penetrant risk loci, been associated with risk of glioma and melanoma in genome-wide association studies. The aberration discovered in *CDKN2A* supports the finding that germline mutations in *CDKN2A/CDKN2B* could cause the co-occurrence of the melanoma-astrocytoma syndrome reported previously.<sup>51–53</sup>

However, we did not observe the *CDKN2A* aberration in our families with a family history of melanoma, so it might be possible that other low-penetrance genes contributed to the melanoma-astrocytoma syndrome in this study.

In conclusion, candidate genes in known syndromes do not explain these glioma-prone families. Large rearrangements are uncommon events explaining cancer-prone glioma families, and novel strategies of exome and whole genome sequencing of glioma families with similar phenotypes are one likely strategy for the future.

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