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Brief Communication

BRIEF COMMUNICATION

Germline Mutations in Shelterin Complex Genes Are Associated With Familial Glioma

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Abstract

Gliomas are the most common brain tumor, with several histological subtypes of various malignancy grade. The genetic contribution to familial glioma is not well understood. Using whole exome sequencing of 90 individuals from 55 families, we identified two families with mutations in POT1 (p.G95C, p.E450X), a member of the telomere shelterin complex, shared by both affected individuals in each family and predicted to impact DNA binding and TPP1 binding, respectively. Validation in a separate cohort of 264 individuals from 246 families identified an additional mutation in POT1 (p.D617Efs), also predicted to disrupt TPP1 binding. All families with POT1 mutations had affected members with oligodendroglioma, a specific subtype of glioma more sensitive to irradiation. These findings are important for understanding the origin of glioma and could have importance for the future diagnostics and treatment of glioma.

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Genetic factors in glioma etiology are poorly understood; less than 5% of glioma cases are familial in origin (1), with only a few described by rare genetic syndromes (2). Both long and short telomeres are associated with cancer risk (3). With regards to sporadic glioma, case-control studies examining telomere length have shown inconsistent associations with risk (4,5), possibly confounded by age-dependent interactions with the variant (6). Here, we describe three familial glioma kindreds characterized by mutations in *protection of telomeres protein 1* (*POT1*), whose product is a member of a protein complex that binds to the TTAGGG repeats of telomeres to regulate length and to protect chromosome ends from abnormal events.

The Gliogene Consortium recruited 435 glioma families from 14 centers in the United States, Sweden, Denmark, the United Kingdom, and Israel between 2007 and 2011 (7). Eligible families were consented, interviewed, and blood or saliva samples were obtained from case patients and unaffected family members. All glioma case patients with medical record or pathology report were adjudicated (Supplementary Methods and Supplementary Table 1, available online).

Whole exome sequencing was conducted on genomic DNA from blood or saliva of 90 affected individuals from 55 glioma families, as previously described (8). Only variants shared among affected individuals within a family were brought forward. We mapped reads and called and annotated variants using previously described methods and prioritized variants that were rare, predicted to disrupt gene function, and occur in genes with reported cancer association or predisposition (Supplementary Methods, available online) (9).

With this approach, we identified two previously undescribed protein-changing variants in *POT1* (NM_015450:p.G95C, HG19:chr7:g.124503667C>A; NM_015450:p.E450X, HG19:chr7:g.124481048C>A) in families A and B, respectively (Figure 1; Supplementary Table 2, available online). Subsequently, polymerase chain reaction (PCR) amplification followed by high-throughput sequencing to interrogate *POT1* in a cohort of 264 glioma patients from 246 families resulted in the identification of a third, novel protein-changing mutation (NM_015450:p.D617Efs*8, HG19:chr7:g.124464068TTA>T) in family C (Figure 1).

Using Sanger sequencing, we verified and determined the genotypes of all available family members in each pedigree (Figure 1). In family A, six members and one obligate carrier were found to harbor the mutation, whereas three of those individuals developed glioma. Similarly, in family B, six individuals

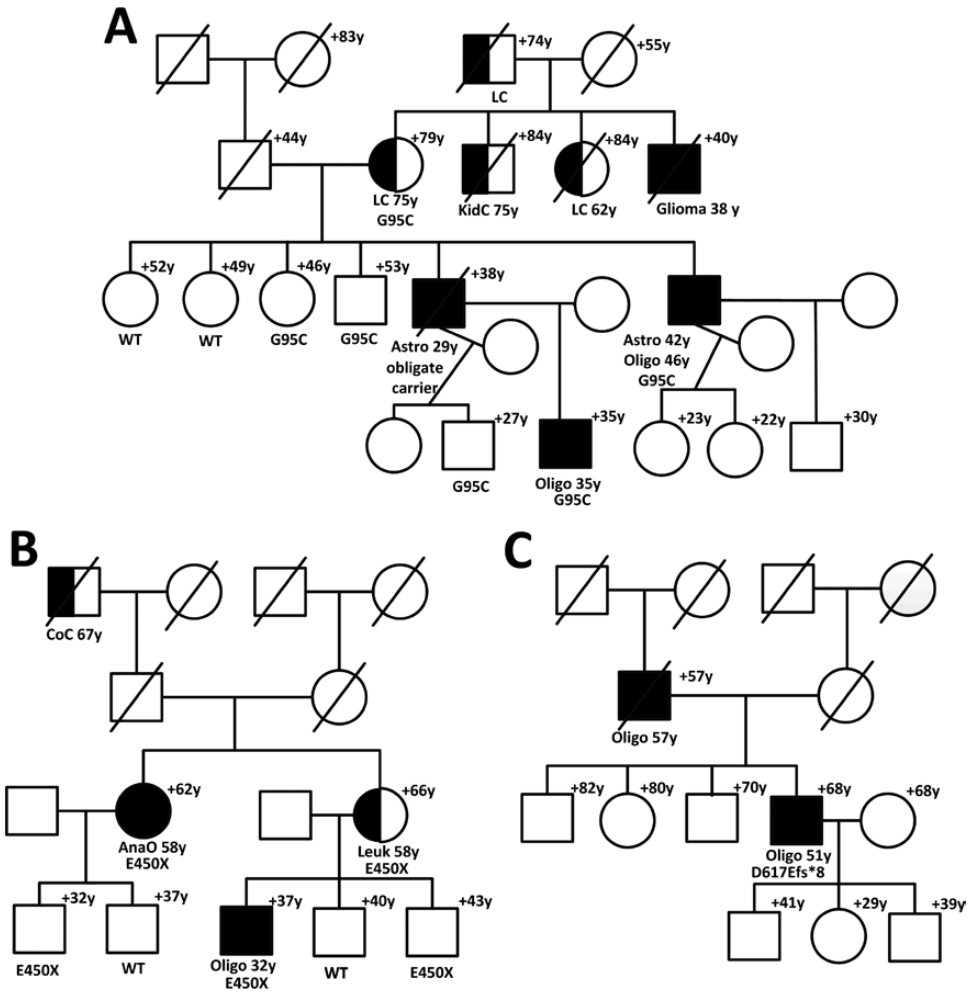

Figure 1. *POT1* mutations in familial glioma pedigrees. Individuals with glioma are shown as filled. Individuals with other cancers are shown as half filled. Disease and age in years at first diagnosis are given underneath the symbol, with current age or age at death (+) above it. Deceased individuals are designated with a slash through the symbol. Glioma type is shown (AnaO = anaplastic oligodendroglioma; Astro = astrocytoma; GBM = glioblastoma multiforme; Glioma = glioma unknown; Oligo = oligodendroglioma). Other cancers in the pedigree are shown (CoC = colon cancer; LC = lung cancer; Leuk = leukemia; Mel = melanoma). Mutations for all sequenced individuals are shown or listed as WT (wild-type).

carried the mutation and two developed glioma, which clearly shows incomplete penetrance of the alleles.

Both truncating mutations in families B and C delete highly conserved residues from the C-terminus of POT1 (although in family C, this occurs at the extreme end of the gene) and are predicted to affect binding to TPP1 and, subsequently, impair association with the shelterin complex and telomeres. In addition, p.E450X is predicted to undergo nonsense-mediated decay, and these individuals may be haploinsufficient for POT1. The p.G95C mutation occurs in the evolutionarily conserved DNA binding domain, OB1, and is computationally predicted to be damaging by multiple algorithms (Supplementary Methods, available online). Interestingly, it is identical to a high-quality *somatic* mutation observed in a lung tumor (10). We further examined an internal database of 6200 unselected, ethnically matched exome-sequenced individuals for mutations in *POT1* to understand the frequency of mutation types in this gene (Supplementary Methods, available online). We found one truncating mutation (p.R363X) and 64 nonsynonymous mutations in *POT1*. Of the latter, only four (6.25%) of the variants occurred in the OB1 region of POT1, despite that region representing about 25% of the protein length. Thus, not only are the specific mutations identified here very rare (~0% MAF), the mutation types themselves are uncommon across a normal population and are likely deleterious.

POT1 localizes to 7q31 and encodes a component of the shelterin complex, which is additionally comprised of TPP1, TRF1, TRF2, TIN2, and RAP1. POT1 binds single stranded telomeric DNA (11) and accumulates at telomeres via TPP1's interaction with TIN2. At telomeres, POT1/TPP1 modulate telomerase recruitment and processivity (12,13) and inhibit ATR DNA damage signaling (14). The POT1 N-terminus contains two DNA binding domains (OB1 and OB2) and the C-terminus is responsible for binding to TPP1 and anchoring to the shelterin complex (Figure 2) (15). In human cell lines, loss of *POT1* expression induces telomere lengthening. To date, germline mutations in *POT1* have only been associated with susceptibility to melanoma (Figure 2) (16,17). Interestingly, an excess of melanoma has been observed in familial glioma kindreds (18); however, there was no history of melanoma in any of the *POT1* families studied here. This may relate to the specific mutation identified in the families, epistatic effects or potentially other genetic modifiers that impact the specific cancer seen in POT1 mutation carriers. Further, somatic mutations in *POT1* were reported in 3.5% of chronic lymphocytic leukemia (CLL) case patients, and mutations were associated with telomere elongation with unprotected ends as well as biologically significant chromosomal aberrations (15). This is consistent with studies of POT1 knock-down in human cell lines and knock-out in animal cell-lines, which show telomere elongation, chromosome reduplication, and deprotection (19).

Given evidence for an association between *POT1* mutations and alterations in telomere length, we assessed telomere content (TC) in DNA isolated from peripheral blood or saliva from affected and unaffected carriers as well as noncarrier, healthy control patients. We analyzed 26 DNA samples by qPCR, including 13 samples from subjects with the reference allele (three of which were familial controls), and 13 samples from subjects with *POT1* mutation. The latter includes five affected carriers (TC distribution shown in Supplementary Figure 1, available online). Most likely because of sample size limitations, there was no statistically significant difference in mean TC by unpaired *t* test, nor did a multivariable analysis using TC as a continuous explanatory variable reveal point estimates that were statistically significant for either glioma case patients or mutation carriers (see Supplementary Methods, available online). However, given the possibility of utilizing TC as a clinical test to screen potential carriers, we generated receiver operating characteristic (ROC) curves to determine the area under the curve (AUC) statistic. These results were statistically significant for mutational status (wild-type vs POT1), with an AUC of 0.72 and a 95% confidence interval (CI) of 0.5 to 0.94 (Supplementary Figure 2, available online) but not for affected status (data not shown), likely because of small sample size and incomplete penetrance of the allele. As another means for assessing TC, we used an orthogonal method that utilizes telomere aligned "off-target" reads from capture data (Supplementary Methods, available online). Using an additional regional capture data set from 204 glioma case patients and family members, *POT1* mutant carriers exhibited statistically significantly higher TC when compared with the remainder of the cohort, even after excluding those with glioma ($P < .002$) (Supplementary Figure 3, available online).

In summary, germline mutations in *POT1* represent a previously undescribed glioma predisposition syndrome characterized by telomere dysfunction and extend the spectrum of cancer types associated with POT1 mutation. In all *POT1* glioma families, one or more affected family member had oligodendroglioma; a histological association that suggests a glioma type–specific susceptibility in these families.

This study was limited by the small sample size of families with *POT1* mutations. This study does not account for potential modifying variants in the genome that may have an effect on allele penetrance. Additional research needs to be conducted before we can determine the clinical relevance of *POT1* mutations in individuals carrying the mutation. Finally, although the evidence presented here strongly supports a causal role for *POT1* mutations in glioma susceptibility, such a role cannot be fully assumed in the absence of direct experimental evidence.

Our results indicate that families with multiple oligodendrogliomas or multiple primary cancers (eg, melanoma and glioma) may benefit from sequenced-based screening for *POT1* mutations to assess their risk and for guiding screening and surveillance plans individualized to carriers. Further studies are called for to examine the mutational status of *POT1* in individuals who

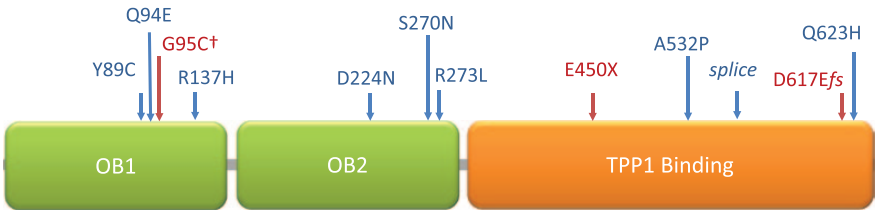

Figure 2. Germline variants identified in this study (**red arrow**) and associated with melanoma (**blue arrow**) in OB1 and OB2 regions (**green**) and TPP1 binding region (**orange**) in *POT1*. † indicates variant was also seen in a tumor sample. OB1/2 = Oligonucleotide binding; TPP1 = tripeptidyl peptidase I.

carry germline mutations in the gene. To our knowledge, this is the first article that identifies telomeric genes associated with glioma susceptibility.

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Notes

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