Molecular and Clinical Evaluation of a Patient Hemizygous for TIGR/MYOC

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Objective: To determine if a patient with an interstitial deletion of chromosome 1 is hemizygous for the TIGR/MYOC gene and if that patient has glaucoma.

Methods: A patient with an interstitial deletion of chromosome 1 was clinically examined for evidence of glaucoma. DNA samples from the patient and her family were used for molecular studies to determine the boundaries of the chromosome 1 deletion using polymorphic markers located on chromosome 1q21 to 1q24. Additional markers located in the vicinity of the TIGR/MYOC gene, including 2 derived from the ends of the gene, were used to determine if it was included in the deletion.

Results: The patient and her family showed no evidence of glaucoma. Molecular analysis demonstrated that a complex deletion of the maternal copy of chromosome 1 included the entire TIGR/MYOC gene.

Conclusions: We have determined that the patient has only 1 functional copy of TIGR/MYOC. The lack of clinical evidence of glaucoma suggests that haploinsufficiency of the TIGR/MYOC protein is not the cause of early-onset glaucoma associated with mutations in TIGR/MYOC.

Clinical Relevance: Missense and nonsense mutations in the TIGR/MYOC gene have been associated with juvenile- and adult-onset primary open-angle glaucoma. Although many different mutations have been correlated with the disease, the underlying genetic mechanism (haploinsufficiency, gain of function, or a dominant negative effect) remains unknown. Information regarding the genetic mechanism responsible for TIGR/MYOC-associated glaucoma is necessary for further studies designed to develop transgenic animal models and gene-related therapy.

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The chromosomal region GLC1A was first identified segregating in families with an autosomal dominant form of juvenile-onset primary open-angle glaucoma. The responsible gene was originally cloned from cultured human trabecular meshwork cells as a steroid response protein named trabecular meshwork-induced glucocorticoid response (TIGR) protein. The gene was independently isolated from a retinal complementary DNA library, and the protein was shown to localize to the cilium connecting the inner and outer segments of photoreceptor cells (myocilin). Mutations in the TIGR/MYOC gene have been detected in juvenile- and adult-onset glaucoma pedigrees and in sporadic populations of patients with both forms of this disease. Mutations are more commonly associated with early-onset glaucoma (8%-20% of patients with this disease) than with the adult-onset form (3%-5% of patients with adult-onset glaucoma). Most mutations are DNA sequence variants that result in missense substitutions in the third exon of the gene that encodes the olfactomedin-like domain. Different missense mutations are associated with a range of ages of onset. Several missense mutations correlate with an early onset before the age of 10 years, whereas others are associated with an onset in the second decade. The same missense mutation can produce a range of ages of disease onset in different individuals; however, the mutations associated with an early onset of disease affect most individuals before the age of 30 years. Several truncating mutations have also been described; one of these, Gln368STOP, is primarily associated with adult-onset disease.

Although many different TIGR/MYOC mutations have been identified...
in patients with primary open-angle glaucoma, the underlying genetic mechanism remains unknown. Autosomal dominant disorders can be caused by 3 general mechanisms: haploinsufficiency, gain of function, or a dominant negative effect.\(^13\) Haploinsufficiency results from a loss of function of the protein encoded by 1 of 2 gene copies. A gain-of-function mechanism is caused by the mutant protein acquiring a novel function that is detrimental to the cell. A dominant negative effect is caused by the mutant protein losing its function and interfering with that of the normal protein. Studies have shown that missense mutations and truncating mutations are responsible for each of these mechanisms in humans.\(^14\-\)\(^21\) Knowledge of the underlying genetic mechanism responsible for a disease is a critical component of studies designed to develop transgenic animal models and gene-related therapy. If the formation of the mutant protein causes a dominant negative or gain-of-function mechanism, the removal of one or both normal copies of the gene (a “knockout” mouse) may not produce an appropriate animal model. Also, if a dominant negative or gain-of-function mutation is responsible for the disease, gene therapies designed to add a normal gene without inactivating the abnormal gene may not be useful. Because missense mutations and truncating mutations contribute to each of these genetic mechanisms, the variety of glaucoma-associated mutations found in \(TIGR/MYOC\) does not conclusively identify the genetic mechanism responsible for \(TIGR/MYOC\)-associated glaucoma.

Gene dosage can provide useful insights into the biochemical mechanisms of genetic disease. This concept has been applied to human disorders such as Charcot-Marie-Tooth disease\(^22\) and is routinely applied in \(Drosophila\) genetics, where the dosage of the chromosomal region under study can be varied experimentally.\(^23\) Occasionally, individuals with segmental aneuploidy allow these concepts to be applied to human genetic disease.

To address the question of haploinsufficiency as an underlying mechanism of \(TIGR/MYOC\) glaucoma, we evaluated a patient with a known interstitial deletion of 1q23 to 1q25.\(^24\) Although most individuals with constitutional, cytogenetically detectable deletions of the proximal long arm of chromosome 1 do not survive beyond infancy,\(^25\) a woman with such a deletion who has survived to age 29 years was available for ocular examination and DNA studies to determine if she was hemizygous for \(TIGR/MYOC\) and if she showed evidence of glaucomatous disease.

Our patient had been clinically evaluated previously and demonstrated developmental delay, and the dysmorphic features typically associated with an interstitial deletion of the long arm of chromosome 1 (1q23-1q25).\(^24\) The purpose of this evaluation was to perform a detailed ocular examination to identify any evidence of glaucoma. Our results suggest that at age 29 years, the patient showed no signs of elevated intraocular pressure or damage to the optic nerve.

DNA samples were obtained from the patient, her parents, and her sister and tested with polymorphic microsatellite markers located near the \(GLC1A\) locus. Comparison of marker alleles between the patient and her parents identified a large deletion of approximately 17 cM on the maternal chromosome 1. The proximal

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**RESULTS**

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breakpoint occurred between D1S2844 and D1S426, and the distal breakpoint occurred between D1S1589 and D1S2769. We detected a smaller deletion of approximately 10 cm centromeric to the proximal breakpoint, also involving the maternal chromosome 1 (Figure 1A). Amplification of polymorphic markers using a cell line containing only the deleted chromosome 1 confirmed these boundaries. Haplotypes were constructed using markers located throughout the chromosomal region containing the TIGR/MYOC gene. Our data demonstrate that the patient and her sister inherited different copies of this region of chromosome 1 from their mother and that the patient’s maternal chromosome carries the deletion that includes the GLC1A locus and the TIGR/MYOC gene (Figure 1B). To confirm this result, we tested DNA from a previously described somatic cell hybrid line, 88H5, that contains a copy of the patient’s shortened maternal chromosome 1 but not her intact paternal homologue.24 Analysis of TIGR/MYOC (GenBank [National Institutes of Health, Bethesda, Md] accession number U85257), PCR assays developed from the 5’ and 3’ coding sequence of TIGR/MYOC (GenBank [National Institutes of Health, Bethesda, Md] accession number U85257), demonstrated that the gene was missing from the patient’s maternal chromosome 1 (Figure 2). Therefore, the patient harbors a true null allele of TIGR/MYOC.

<table>
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<tr>
<th>Band 1q21</th>
<th>D1S15 152 cm +</th>
<th>D1S44 155 cm +</th>
<th>D1S49 156 cm +</th>
<th>D1S234 156 cm +</th>
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<td>D1S256 165 cm -</td>
<td>D1S484 169 cm -</td>
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<td>TC16263 177 cm +</td>
<td>D1S2844 175 cm +</td>
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<th>D1S42 151 cm +</th>
<th>D1S196 178 cm +</th>
<th>D1S335 181 cm +</th>
<th>D1S432 184 cm +</th>
<th>D1S191 185 cm +</th>
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<td>D1S232 184 cm -</td>
<td>D1S265 185 cm -</td>
<td>D1S484 186 cm -</td>
<td>D1S242 188 cm -</td>
<td>D1S484 190 cm -</td>
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**Figure 1.** A, Microsatellite repeat analysis demonstrating the deleted portions of chromosome 1. Deleted alleles identified from the comparison of genomic DNA from pedigreed members are shown in regular type. Deleted alleles identified from polymerase chain reaction amplification using DNA from a somatic cell hybrid containing the patient’s deleted chromosome 1 (88H5) are shown in boldface. Plus signs indicate the presence of a marker; minus signs, the absence of the marker. B, Haplotype analysis of the patient and her family. Alleles are identified as base pair lengths. P indicates paternal; M, maternal; and the term del, deleted allele. Squares indicate males; circles, females; and the solid circle, affected individual. Genetic distances were obtained from the Marshfield genetic map (research.marshfieldclinic.org/genetics/). CM indicates centimorgan.

We have determined that the patient has only 1 functional copy of the TIGR/MYOC gene. The lack of clinical evidence of glaucoma suggests that haploinsufficiency of TIGR/MYOC is not the cause of early-onset glaucoma associated with GLC1A. These results indicate that the TIGR/MYOC missense mutations associated with severe early-onset glaucoma do not cause a simple loss of function of the protein. Instead, it is more likely that these mutations result in a gain of function or cause a dominant negative effect. Wild-type TIGR/MYOC protein is secreted from human trabecular cells2 and associates into dimers and possibly oligomers.9,27 Recent studies have shown that mutant forms of TIGR/MYOC protein expressed in cell culture are not secreted28 and may form precipitates in vivo.27 Mutant protein may form a complex with wild-type protein and prevent its normal action, creating a dominant negative effect. Alternatively, the precipitation of abnormal TIGR/MYOC protein could have a more general effect on trabecular function by interfering with the secretion and/or processing of 1 or more other proteins. Dominant negative or gain-of-function mechanisms are also suggested by a recent study indicating that the Glu323Lys missense mutation may lead to a pause in the processing and abnormal folding of the nascent protein.29

Some missense mutations in TIGR/MYOC are associated with an older average age of onset than that of the patient we have examined.5,10 Because of the variable age of onset connected with these mutations, we cannot conclude that adult-onset disease associated with defects in TIGR/MYOC is not caused by haploinsufficiency.

The effect of the truncating mutations remains an intriguing question. It is not clear if these mutations lead to null alleles (complete loss of function) or result
in gain-of-function or dominant negative effects. Protein truncations that cause dominant negative effects have been described in a variety of organisms, including humans.14,30 Previous studies have indicated that the Gln368STOP mutation, which results in a loss of about 25% of the TIGR/MYOC open reading frame, is associated with a mild form of open-angle glaucoma that generally has a later onset.8,10,12 Gln368STOP may be a null allele due to nonsense-mediated decay of the messenger RNA or to instability or lack of function of the hypothetically truncated protein. Alternatively, the mutation could cause a gain of function or a dominant negative effect on the cell. Recently, a 77-year-old Chinese woman has been identified who is homozygous for a truncating mutation in both copies of her TIGR/MYOC gene.31 This mutation occurs at codon 46 and is predicted to result in a severely truncated protein that is missing more than 90% of the amino acid residues found in the wild-type protein. It is likely that the messenger RNA carrying this mutation is degraded via nonsense-mediated decay and that little or no mutant protein is produced. Because the patient is homozygous carrier of this mutation, she probably does not have any functional TIGR/MYOC protein. Interestingly, this woman does not have any evidence of glaucoma. However, these results must be tempered in light of the report of a Korean patient who is homozygous for the same codon 46 stop mutation and has juvenile-onset glaucoma.32 Other family members who carry 1 copy of the 46 stop mutation are not affected by the disease.

The results of our study indicate that the loss of 1 copy of the TIGR/MYOC gene does not cause severe early-onset glaucoma. It remains to be determined if the mutant forms of TIGR/MYOC interfere with the function of the remaining normal copy of the protein, causing a dominant negative effect, and/or if the mutant forms of the protein gain a function that interferes with the action of other proteins necessary for aqueous outflow.

References


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