Biochimie Génétique, Institut de Biologie, Montpellier

Acknowledgments

We thank the European Community Concerted Action for Cystic Fibrosis for providing amplification primers for the DGGE analysis. The technical assistance of Mrs. Britta Hitschfeld and Mr. Jürgen Wansch is gratefully acknowledged. This work was supported in part by Deutsche Forschungsgemeinschaft grant Ni-130/15-1.

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Address for correspondence and reprints: Dr. Dieter Meschede, Institute of Human Genetics of the University, Vesaliusweg 12-14, D-48149 Münster, Germany. E-mail: dmesche@uni-muenster.de

0002-9297/97/6105-0027$02.00

Am. J. Hum. Genet. 61:1202–1204, 1997

Mutations in the *TIGR* Gene in Familial Primary Open-Angle Glaucoma in Japan

To the Editor:

As described in an editorial by Raymond (1997), glaucoma is characterized by progressive excavation of the optic disk, with both loss of retinal nerve fiber and visual field defects. This disease is one of the most common causes of bilateral blindness, and it is estimated that by the year 2000 ~66.8 million people worldwide will be affected by it (Quigley 1996). Recently, the glaucoma gene*GLC1A* was shown to be identical to the trabecular meshwork–inducible glucocorticoid response (TIGR) gene (*TIGR*) (Stone et al. 1997). The *TIGR* gene was cloned by Polansky and colleagues (Nguyen et al. 1993; Polansky et al. 1997) and, also, was called "myocilin" (gene *MYOC*) when it was cloned by Kubota et al. (1997). Three different mutations in the gene were shown to be responsible for the development of primary open-angle glaucoma (POAG), the most common form of glaucoma (Stone et al. 1997). The prevalence of these mutations was reported to be 4.4% in familial POAG patients and 2.9% in unselected POAG patients (Stone et al. 1997). We investigated whether Japanese patients with familial POAG carry identical or other mutants on the same gene. As a result, two new mutations in the *TIGR* gene were found. The prevalence of mutations in the *TIGR* gene in Japanese patients with familial POAG was also investigated.

Peripheral blood samples were collected, with in-

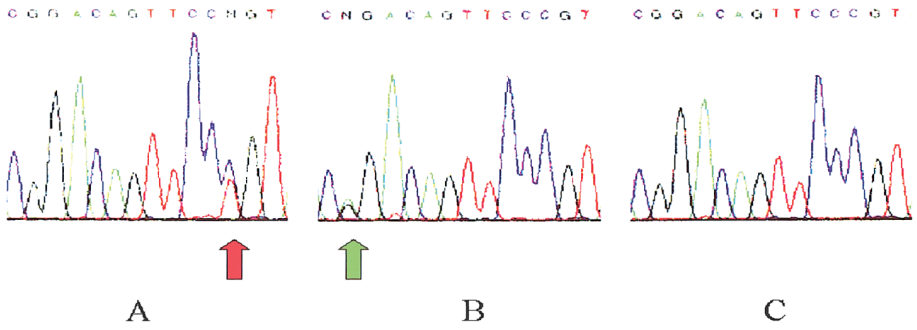

Figure 1 Chromatograms of nucleotide sequences from patients with mutations (*A* and *B*) and from a normal control (*C*). The double peak of cytosine (*blue line*) and thymine (*red line*) (*A, red arrow*) represents a heterozygous mutation in the codon corresponding to the 370th amino acid residue of the TIGR protein (Pro370Leu). The double peak of guanine (*black line*) and adenine (*green line*) (*B, green arrow*) represents a heterozygous mutation at the codon of 367th amino acid residue (Gly367Arg).

formed consent, from 52 POAG patients of 50 pedigrees with a family history. The patients were diagnosed with POAG, by ocular and systemic examinations. The family history was obtained by direct interview with the patients. Subjects having at least one relative with POAG within the third degree of relationship were defined as belonging to a pedigree having familial POAG. They all had an elevated intraocular pressure ($\geqslant$22 mmHg), open-angle (Shaffer grade III or IV), visual-field loss characteristic of glaucoma, and glaucomatous optic-disk damage. Blood samples from five normal healthy volunteer were also obtained, as controls, with informed consent. Genomic DNA was purified from these blood samples by use of a QIAGEN QIAamp Blood Kit. A DNA fragment encoding a portion of the TIGR protein (amino acid residues 317–476, exon 3; GenBank accession number U85257-AF001620) was amplified, with samples of the purified genomic DNA used as templates, by PCR. The nucleotide sequences of primers used are 5′-ATACTGCCTAGGCCACTGGA (sense strand) and 5′-CATGCTGCTGTACTTATAGCGG (antisense strand). A 150-ng template was mixed with 10 μl of 10 × buffer, 8 μl of the deoxynucleotides mixture, 10 pmol of each primer, and 0.5 μl of *Taq* polymerase (AmpliTaq Gold; Perkin Elmer), to produce a 100-μl PCR mixture. The nucleotide sequences of both strands of the PCR products were directly determined with the terminator cycle–sequencing method, by use of fluorescent dideoxynucleotides and an automatic DNA sequencer (Applied Biosystems). Mutation was recognized by the approximately equal peak intensity of two fluorescent dyes at the mutation site. When a mutation was detected, the whole procedure of PCR and sequencing was repeated, and the existence of the mutation was confirmed.

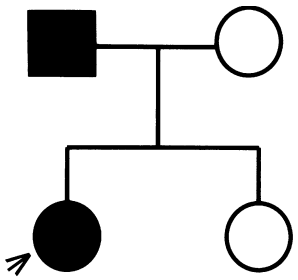

Figure 2 Pedigree of patients with the Pro370Leu mutation. The proband is indicated by an arrow.

Of the 52 patients from the 50 pedigrees, 2 patients of one family (a father and a daughter [who was the proband]) carried a heterozygous C→T mutation at the second nucleotide position in the codon corresponding to the 370th amino acid residue of the TIGR protein, resulting in an amino acid change from proline to leucine (Pro370Leu) (figs. 1*A* and 2). The father was diagnosed with POAG at age 26 years old, the daughter at age 16 years. One other patient, from a different pedigree, had a heterozygous G→A mutation at the first nucleotide position in the codon corresponding to the 367th amino acid residue, resulting in an amino acid change from glycine to arginine (Gly367Arg) (fig. 1*B*). She was diagnosed with POAG at age 45 years. Both mutations were different from those reported elsewhere (Stone et al. 1997). In the pedigree with the Pro370Leu mutation, the mother and a sister of the proband were examined and proved to have neither symptoms of glaucoma nor mutations in the *TIGR* gene portion examined (fig. 2). Therefore, the mutation was inherited in an autosomal dominant manner. The patient with the Gly367Arg mutation had a family history, in that at least her two aunts and five cousins had POAG; but we could not obtain

blood samples from her relatives. The prevalence of the mutations in the *TIGR* gene was 4.0% (2/50 families), which was comparable to that reported in a previous study (Stone et al. 1997).

The present study has revealed that mutations in the *TIGR* gene are also responsible for familial POAG in Japan. The mutations in the third exon of the *TIGR* gene were found to be associated with ~4% of Japanese familial POAG patients. The prevalence of the mutations in the gene was comparable in Japanese patients and in the population previously studied. The mutated sites, however, were different from those reported in the previous study, and no common mutations were found. Therefore, the distribution of the mutated sites in the *TIGR* gene in Japanese POAG may be different from those in other races. Known mutated sites in the *TIGR*-gene disease alleles are at the 364th, 367th, 368th, 370th, and 437th amino acid residues. The apparent focus of the reported mutations—around the 367th amino acid residue—indicates that the amino acid change around this position may play a critical role in the pathogenesis of POAG. The TIGR-protein product is overexpressed with glucocorticoid stimulation and is thought to contribute to steroid-responsive intraocular-pressure increase, by the obstruction of aqueous outflow (Nguyen et al. 1993; Polansky et al. 1997). Further investigations of the *TIGR* gene will reveal more information about the pathogenesis of POAG.

YASUYUKI SUZUKI,[1] SHIROAKI SHIRATO,[2]
FUMIKO TANIGUCHI,[2] KUNITOSHI OHARA,[1]
KIYOMI NISHIMAKI,[3] AND SHIGEO OHTA[3]
[1]*Department of Ophthalmology, Nippon Medical School, and* [2]*Department of Ophthalmology, University of Tokyo School of Medicine, Tokyo; and* [3]*Department of Biochemistry and Cell Biology, Institute of Gerontology, Nippon Medical School, Kanagawa, Japan*

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Address for correspondence and reprints: Dr. Yasuyuki Suzuki, Department of Ophthalmology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo, 113, Japan. E-mail:ysuzuki@nms.ac.jp

0002-9297/97/6105-0028$02.00

Am. J. Hum. Genet. 61:1204–1205, 1997

Childhood Cancer and Neural Tube Defects

To the Editor:

In the March 1997 issue of the *Journal,* Narod et al. (1997) concluded that cases of spina bifida and of abnormalities of the eye, ribs, and spine were more common in children with cancer than among population-based controls. In an invited editorial in the same issue, Friedman (1997) called for additional work to define more clearly the associations between congenital anomalies and cancer and to differentiate those associations that are spurious from those that are biologically important.

I wish to comment on the reported association between spina bifida and cancer, which was based on comparison of the relative frequency of specific types of anomalies in children with cancer who were diagnosed in Great Britain during the period 1971–86 with the corresponding relative frequency in liveborn children born in British Columbia during the period 1969–88. In addition to the differences in methods of ascertainment discussed by Narod et al. (1997), this comparison appears to be inappropriate, since the prevalence of neural tube defects at birth was markedly higher in the British Isles than in Canada, from the 1950s to the early 1980s (Little and Elwood 1992). This difference was apparent despite the decline in the prevalence of these defects at birth in the British Isles since the early 1970s. In Canada, there has been a persistent east-west gradient, with the prevalence at birth declining from the east toward the west.

In the study by Narod et al. (1997), when the 275 cases with an established genetic cause were excluded, the prevalence of neural tube defects at birth among children diagnosed with childhood cancer was 1.2 per 1,000 births. As noted by Narod et al., anencephalus is associated with a high rate of infant mortality, and most children with neural tube defects are likely to have had