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Report

Mutations in the Cone Photoreceptor G-Protein α-Subunit Gene *GNAT2* in Patients with Achromatopsia

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Achromatopsia is an autosomal recessively inherited visual disorder that is present from birth and that features the absence of color discrimination. We here report the identification of five independent families with achromatopsia that segregate protein-truncation mutations in the *GNAT2* gene, located on chromosome 1p13. *GNAT2* encodes the cone photoreceptor–specific α-subunit of transducin, a G-protein of the phototransduction cascade, which couples to the visual pigment(s). Our results demonstrate that *GNAT2* is the third gene implicated in achromatopsia.

Achromatopsia, also referred to as "rod monochromacy" (ACHM2 [MIM 216900] and ACHM3 [MIM 262300]), is a congenital ocular disorder characterized by total color blindness, low visual acuity, photophobia, and nystagmus. It is inherited as an autosomal recessive trait, with an estimated prevalence of 1/30,000 (François 1961).

Recently, we and others have shown that mutations in *CNGA3* and *CNGB3*, which encode the α- and β-subunits of the cone photoreceptor cGMP-gated channel, cause clinically indistinguishable forms of achromatopsia, including Pingelapese blindness (Kohl et al. 1998, 2000; Sundin et al. 2000).

However, screening for *CNGA3* and *CNGB3* mutations in a large cohort of patients with achromatopsia revealed further genetic heterogeneity in approximately one-third of the cases. The pursuit of additional genes involved in achromatopsia led us to consider *GNAT2* as a prime candidate gene, because of its cone photoreceptor specificity and functional importance in the visual transduction process (Lerea et al. 1986; Blatt et al. 1988; Morris and Fong 1993). *GNAT2* encodes the cone-specific α-subunit of transducin (MIM 139340), a heterotrimeric G-protein that couples to the cone visual pigments. Excited pigment molecules induce both the exchange of GDP to GTP at the guanosine-binding site of the transducin α-subunit and its subsequent release from the inhibitory β/γ-subunits. The activated GTP • transducin complex then binds and activates a phosphodiesterase that hydrolyzes cGMP and thereby effectively reduces its intracellular concentration. This results in closure of the cGMP-gated channels and, subsequently, in membrane hyperpolarization (Müller and Kaupp 1998). Transducin thus mediates one of the first steps of the phototransduction cascade, whereas the cGMP-gated channel represents the final component of the very same process.

It has been shown that a missense mutation in *GNAT1*, which encodes the rod photoreceptor–specific transducin α-subunit, is implicated in autosomal dominant congenital stationary night blindness of Nougaret (Dryja et al. 1996), and a homologous phenotype has been described for the corresponding knockout-mouse model (Calvert et al. 2000). In contrast, no association between the *GNAT2* locus and hereditary retinal disorders has been detected in previous studies (Gerber et al. 1995; Magovcevic et al. 1995). The human *GNAT2* gene comprises eight exons and covers ~10 kb of genomic sequence on human chromosome 1p13. *GNAT2* encodes a polypeptide of 354

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Table 1

Sequences of PCR Primers Used to Amplify *GNAT2* Coding Segments

Exon(s)	Primer		PCR Product Size (bp)
	Forward	Reverse	
1	GCTTTTAGGGGAGCCAAGTT	CCCCTAGCCAGACTGTGTAGA	452
2 and 3	CCCCCAAGCAAGCAG	CAGCAGGTGGGATTTTAGTT	691
4	TCTGCATCTGGGGAGCATAC	TTGGGTTGGGTGAGGTTT	434
5 and 6	TGGCTTCTAGTCCTGAGGTC	GATTGGTCTGCTGGAGG	716
7 and 8	TTTTTACATTTGGAGCACTG	CCCACTTTGAAAAGAACG	1,023
IVS 3–5[a]	TTAAGGTAGACGGTACCTTTC	TAAGGTCTTACTGCGTAGATG	2,022

[a] Amplification was by long-distance PCR.

amino acid residues that shows 82% sequence identity with its paralog in rod photoreceptors (Lerea et al. 1989; Kubo et al. 1991; Morris and Fong 1993).

We screened the entire coding sequence of the *GNAT2* gene, in a collection of 77 unrelated patients with achromatopsia, in whom mutations in the *CNGA3* and *CNGB3* genes were excluded. PCR fragments covering all eight exons and flanking intron/UTR sequences of *GNAT2* were amplified from genomic DNA (for primer sequences, see table 1) and were subjected to direct DNA sequencing employing Big Dye Terminator chemistry (Applied Biosystems). Reaction products were separated on an ABI 3100 DNA sequencer, and sequences were analyzed by the Lasergene Software package (DNASTAR).

In the 77 patients with achromatopsia whom we analyzed, we were able to identify six different disease-related sequence alterations (table 2) segregating in five independent families of European descent (fig. 1*a* and *b*). The mutations comprise one nonsense mutation, c.235C→T (p.Q75X), and four small deletion and/or insertion mutations, which all cause frameshifts and lead to premature translation termination. The sixth mutation represents a large intragenic deletion of 2,019 bp (g.3407_5425del [GenBank accession number Z18859]), which includes exon 4 and flanking intron sequences. This deletion probably results from a recombination between two 229-bp direct-repeat sequences present in intron 3 and intron 4 of *GNAT2* (fig. 1*c*). BLASTN analysis revealed that this repeat is unique to the *GNAT2* locus, on chromosome 1p13. Detection of the deletion and analysis of its segregation in family CHRO22 were easily accomplished by long-distance PCR (fig.1*b* and table 1).

In four of the five families, patients apparently carry homozygous mutations. The presence of heterozygous mutations in both parents could be shown by segregation analysis in families CHRO22 and CHRO87 (fig. 1*a* and *b*). Moreover, parental consanguinity is documented in families CHRO22 and CHRO94. We also noticed that, in the remaining two families, CHRO68 and CHRO87, both parents traced their ancestry back to the same geographic region in southern Italy and Denmark, respectively. Therefore, we think that, in the end, consanguinity exists between those parents too, albeit earlier than known family history.

All the observed mutations result in premature translation termination and in mutant polypeptides that lack considerable portions of the genuine carboxy terminus. It has been shown that the conserved carboxy terminus of the rod photoreceptor paralog contains major sites of interaction with the excited rod photopigment (Cai et al. 2001). Considering the high conservation between the rod and cone transducin α-subunits, a similar structural function can be assumed to exist in the cone system. We therefore argue that the mutations represent functional *null* alleles of *GNAT2,* which prevent either the formation of the trimeric G-protein complex or its interaction with the excited photopigments.

Notably, all genes now known to be involved in achromatopsia encode crucial components of the cone phototransduction cascade. Because of the lack of cone function in patients with achromatopsia, it is reasonable to argue that, like the cone-specific cGMP-gated channel, all three different types of human cone photoreceptors utilize a common transducin α-subunit and that there is functional conservation of the phototransduction process in cones.

The present study provides convincing evidence that

Table 2

Mutations in *GNAT2* in Patients with Achromatopsia

Exon	Alteration[a]	
	Nucleotide Sequence	Polypeptide (Consequence)
3	c.235C→T	p.Q79X
3	c.285_291del7insCTGTAT	p.Y95fsX61
4	IVS3+365_IVS4+974del	Exon 4 deleted (p.A101fsX12)
5	c.503_504insT	p.L168fsX3
7	c.802_803insTCAA	p.L268fsX9
8	c.955delA	p.I319fsX5

[a] Nomenclature is according to previous recommendations (den Dunnen and Antonarakis 2001). The reference sequence is from GenBank (accession number Z18859).

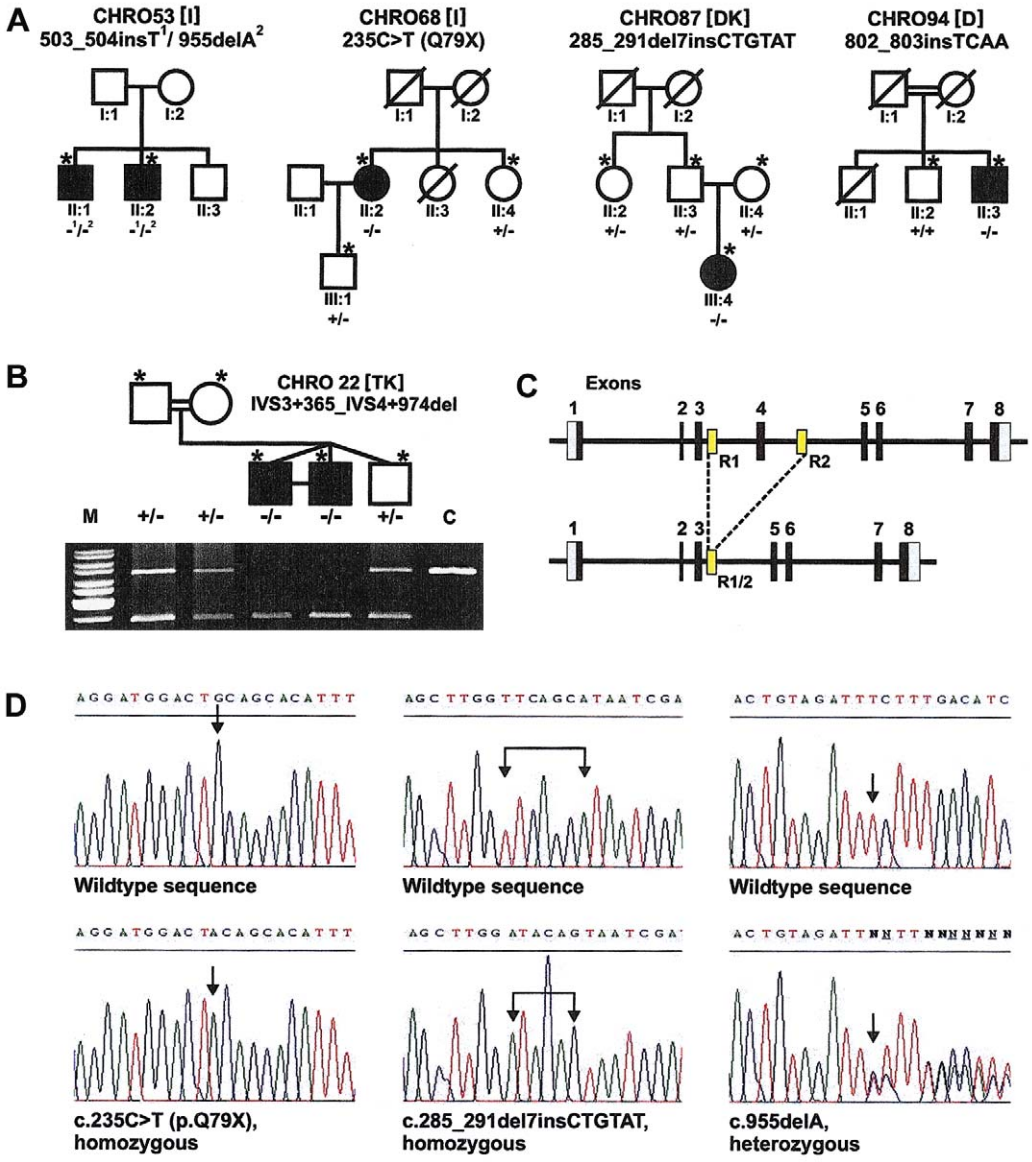

Figure 1 *GNAT2* mutations in families segregating achromatopsia. *A* and *B,* Pedigrees of the five achromatopsia families with *GNAT2* mutations, including the genotypes of available subjects (denoted by asterisks [*]) as determined by segregation analysis. + = wild type; − = mutation. The geographical origin of the families is indicated by square brackets: I = Italy; DK = Denmark; D = Germany; TK = Turkey. Panel *b* presents the results of segregation analysis of the exon 4 deletion in family CHRO22. The photograph shows the electrophoretic separation of wild-type alleles (*upper band*) and mutant alleles (*lower band*), after amplification by long-distance PCR. M = marker; C = normal control subject. *C,* Organization of the *GNAT2* gene (*top*) and location of the two repeats (R1 and R2) putatively implicated in the exon 4 deletion (*bottom*). *D,* Electropherograms of mutant sequence (*bottom*) and corresponding wild-type reverse-strand sequence (*top*), for homozygous mutations c.235C→T (p.Q79X) and c.285_291del7insCTGTAT and for heterozygous mutation c.955delA. Mutation sites are indicated by arrows (↓).

mutations in the *GNAT2* gene cause achromatopsia. Patients with mutations in *GNAT2* show a phenotype typical of achromatopsia, with low visual acuity, photophobia, nystagmus, and absent or barely detectable cone function in electroretinographic recordings and psychophysical color-vision testing. Genetic classification now allows further detailed clinical studies to investigate whether there are phenotypic differences that distinguish between *GNAT2*-associated and other forms of achromatopsia.

GNAT2 is the third gene found to be implicated in achromatopsia. Considering that our complete sample of patients consists of nearly 280 unrelated patients with achromatopsia, mutations in the *GNAT2* gene are re-

sponsible for <2% of all patients affected by this disorder. Compared with the other two genes known to cause achromatopsia—*CNGA3* and *CNGB3*, which account for ~20%–30% and ~40%–50% of the cases, respectively (Wissinger et al. 2001; authors' unpublished data)—*GNAT2* is only a minor achromatopsia locus. This emphasizes what we call the "orphan disease-gene concept," which is based on the exclusion of known common disease genes in order to define a subset of patients for subsequent identification of rare disease genes. This will not only improve genetic diagnostics but also provide examples of the effect of mutations in a particular gene and, thereby, increase our knowledge about the pathophysiological spectrum of human genetic disorders.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for human cone photoreceptor transducin α-subunit [GNAT2] [accession number Z18859] and human rod photoreceptor transducin α-subunit [GNAT1] [accession number X15088])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ACHM2 [MIM 216900], ACHM3 [MIM 262300], and GNAT2 [MIM 139340])

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