Segregation of a mutation in $CNGB1$ encoding the $\beta$-subunit of the rod cGMP-gated channel in a family with autosomal recessive retinitis pigmentosa

Abstract Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal diseases leading to blindness. By performing full genome linkage analysis in a consanguineous French family affected with severe autosomal recessive RP, we have excluded linkage to known loci involved in RP and mapped a novel locus to chromosome 16q13-q21 ($Z_{\text{max}} = 2.83$ at $\theta = 0$ at the $D16S3089$ locus). Two candidate genes $KIFC3$ and $CNGB1$ mapping to this critical interval have been screened for mutations. The $CNGB1$ gene, which encodes the $\beta$-subunit of the rod cGMP-gated channel, is mutated in the family presented in this study.

Introduction Retinitis pigmentosa (RP, MIM 268000) includes a number of retinal diseases primarily affecting rod photoreceptors. The clinical symptoms of RP include night blindness, loss of peripheral vision, and bone spicule-shaped pigmentary deposits in the fundus. With time, visual impairment progresses toward the center of the retina eventually resulting in blindness. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X-linked trait. Autosomal recessive RP (arRP) accounts for 5%–20% of all RP, whereas sporadic RP, presumed to be recessive in most cases, accounts for a further 40%–50% (Bird 1995). Mutations causing arRP have been found in the genes encoding rhodopsin ($RHO$), the $\alpha$- and $\beta$-subunits of the rod phosphodiesterase ($PDE6A$ and $PDE6B$), the $\alpha$-subunit of the rod cyclic GMP-gated channel protein ($CNGA1$), and arrestin ($SAG$), and in genes $RPE65$, $RLBP1$, $ABCA4$, $TULP1$, $RGR$, $CRB1$, $NR2E3$, and $MERTK$. In addition, genetic linkage studies have identified arRP loci at 2p11-p16 ($RP28$), 2q31-q33 ($RP26$), 4q32-q34 ($RP29$), 6cen-q13 ($RP25$), and 16p12.1–12.3 ($RP22$; for reviews, see Phelan and Bok 2000; Rattner et al. 1999). Each of the 18 independent loci assigned so far is responsible for only a minor portion of all arRP patients; many more RP genes have yet to be identified.

A genome-wide search for homozygosity undertaken in a consanguineous French family affected by autosomal recessive RP has localized the disease gene to chromosome 16q13-q21. A search for mutations in two candidate genes, $KIFC3$ (kinesine family C3, MIM 604535) and $CNGB1$, located in the region of interest, has demonstrated that RP, in this family, cosegregates with a homozygous missense mutation in the $CNGB1$ gene, which encodes the $\beta$-subunit of the rod cGMP-gated channel.

Materials and methods Patients

A consanguineous French family in which RP segregated as an autosomal recessive trait was studied (Fig. 1). The pedigree contained three affected individuals presenting typical and severe clinical features of RP. The proband had had night blindness since early childhood. Later, she experienced loss in the peripheral visual field. At the time of examination (30 years), the fundus showed typical bone spicule-shaped pigmentary deposits. Visual fields were reduced to 10 degrees in both eyes. Electroretinograms exhibited no rod response, whereas cone responses were severely reduced. There was no evidence for reproductive deficiency in this family as all RP patients had children. In addition, no taste or olfactory impairment was reported.

Informed consent was obtained from all patients included in this study. Total RNA and DNA were extracted from peripheral blood samples by standard procedures.

Linkage analysis

To map the disease locus, we performed homozygosity analysis. The screening set comprised 382 highly polymorphic fluorescent...
markers from the ABI PRISM Linkage Map Set Medium Density (PE Biosystems), which were chosen from the Généthon linkage map (Dib et al. 1996) and have an average spacing of 10 cM. Polymerase chain reactions (PCRs; 40 ng genomic DNA) were performed in a 7.5-µl final volume according to the conditions recommended by the manufacturer (PE Biosystems). PCR products were separated on a 5% Long Ranger denaturing urea-polyacrylamide gel in an ABI 377 DNA sequencer and analyzed by using GENESCAN 3.1 and GENOTYPER 2.1 softwares (PE Biosystems).

To refine the homozygous region, 27 additional microsatellite markers selected from the Généthon and CHLC linkage maps with respect to their informativity and location were genotyped in all individuals.

Parametric linkage analysis was performed with an optimized version of the LINKAGE package, version 5.2 (Lathrop et al. 1985). Pairwise and multipoint LOD-scores were calculated by using programs from FASTLINK 3.0P. Allele frequencies for markers were found in the GenBank Database (http://gdbwww.gdb.org/). The phenotype was analyzed as an autosomal recessive trait, with complete penetrance and a frequency of 0.002 for the disease allele.

Mutation screening of KIFC3

The coding sequence of the KIFC3 gene was amplified by reverse transcription/PCR (RT-PCR) with total RNA extracted from peripheral blood lymphocytes as a template, followed by four overlapping nested PCRs. The resulting products were directly sequenced in both directions by using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems) on an ABI 377 sequencer and analyzed with Sequence Analysis software, version 3.3 (data not shown). Mutation screening was carried out on an affected child, his mother, and a control individual.

Mutation screening of CNGB1

The genomic structure of the CNGB1 gene was deduced by using a BLAST search (Altschul et al. 1990) of CNGB1 cDNA (GenBank accession no. U58837; Ardell et al. 1996) against high throughput genomic sequence segments of GenBank. Intronic primers were designed to amplify the 33 exons and flanking introns of CNGB1 (primer sequences and PCR conditions are available on request). Recent publication of the genomic structure of CNGB1 (Ardell et al. 2000) allowed us to design primers to study