Novel Compound Heterozygous TULP1 Mutations in a Family With Severe Early-Onset Retinitis Pigmentosa

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Objective: To describe the clinical characteristics and determine the genetic defect in a Surinamese family with autosomal recessive retinitis pigmentosa.

Methods: Family members underwent blood sampling and ophthalmologic examinations. After exclusion of all known mutations in all genes involved in autosomal recessive retinitis pigmentosa, a genome-wide linkage scan was performed using 11,555 single-nucleotide polymorphisms spread throughout the genome. Mutation analysis of the TULP1 gene was performed by direct sequencing.

Results: All affected family members had a severe retinal dystrophy with a history of nystagmus, low visual acuity, and nyctalopia since infancy. The scotopic and photopic responses were nonrecordable on electroretinography. A genome-wide scan suggested linkage to the chromosomal region containing the TULP1 gene. Mutation analysis of TULP1 identified novel compound heterozygous mutations (p.Arg482Trp and p.Leu504fsX140) in all affected family members.

Conclusions: The affected members of the Surinamese family have a severe early-onset form of autosomal recessive retinitis pigmentosa, which is caused by compound heterozygous mutations in the TULP1 gene.

Clinical Relevance: Clinical and molecular genetic characterization of autosomal recessive retinitis pigmentosa may help to provide a more accurate prognosis in individual patients. This study confirms that TULP1 mutations cause a severe early-onset form of autosomal recessive retinitis pigmentosa.

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Retinitis Pigmentosa (RP) is a heterogeneous group of progressive retinal dystrophies, which has a prevalence of approximately 1 in 4000 individuals. Retinitis pigmentosa is characterized by nyctalopia, a progressive constriction of the visual fields, pigment depots in the midperipheral retina, and a gradual reduction of visual acuity. The disease can be inherited in an autosomal recessive, autosomal dominant, or X-linked fashion. Mutations in 19 genes have been reported to cause autosomal recessive RP, and 5 loci have been reported for which the causative gene has not yet been identified.

Mutations in the TULP1 gene are found in approximately 1% to 2% of patients with autosomal recessive RP. To date, 14 different mutations have been found in the TULP1 gene. In comparison with other forms of RP, patients with TULP1 mutations have a very severe visual handicap, which may be better described as Leber congenital amaurosis.

TULP1 is a member of the tubby-like (TULP) family. The TULP proteins are characterized by a highly conserved C-terminal tubby domain; their expression is mainly restricted to neuronal tissues. Expression of TULP1 is confined to the retina, where it localizes primarily to the inner segments and connecting cilium of the photoreceptor cells. TULP1 is involved in protein trafficking and is essential for the transport of rhodopsin from its site of synthesis in the inner segments through the connecting cilium to the outer segments.

In this study, we describe a Surinamese family with multiple members who were affected by a severe early-onset form of autosomal recessive RP. The involvement of all known mutations in all known autosomal recessive RP genes was excluded.
A genome-wide linkage suggested linkage to a region on chromosome 6, which contains the TULP1 gene. Mutation analysis of the TULP1 gene identified 2 novel compound heterozygous mutations in all affected individuals.

METHODS

PATIENTS AND OPHTHALMOLOGICAL EXAMINATIONS

Five affected and 5 unaffected members of a Surinamese family with autosomal recessive RP participated in this study. All affected family members underwent complete ophthalmologic examinations, including best-corrected projected Snellen visual acuities, slitlamp biomicroscopy, dilated fundus examinations, Goldmann visual fields, and International Society for Clinical Electrophysiology of Vision–standardized electroretinography and electrooculography. Informed consent was obtained from all participating individuals, consistent with the tenets of the Declaration of Helsinki. This study was approved by the institutional review board of the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

MOLECULAR ANALYSIS

Standard protocols were used to extract DNA from peripheral blood leukocytes. The proband (II-8) of the Surinamese family was screened for 505 known mutations and sequence variants in 16 genes known to be involved in autosomal recessive RP (CERKL, CNGA1, CNGB1, MERTK, PDE6A, PDE6B, PNR, RDH12, RGR, RLBP1, SAG, TULP1, CRB1, RPE65, USH1C, and USH3A) with a genotyping microarray based on arrayed primer extension technology (AR-RP Chip; Asper Ophthalmics, Tartu, Estonia). Ten members of the Surinamese family underwent genotyping, with 11 555 single nucleotide polymorphisms spread throughout the genome (Affymetrix GeneChip Human Mapping 10K Array XbaI 2.0; Affymetrix, Santa Clara, California). Multipoint parametric linkage analysis was performed with Allegro v1.2c (Decode Genetics, Reykjavik, Iceland) in the EasyLinkage Plus v4.00b software package (University of Würzburg, Würzburg, Germany) using the Decode Genetics genetic single nucleotide polymorphism map and the white allele frequencies. An autosomal recessive mode of inheritance with complete penetrance was assumed, as none of the children (third generation) of the 5 affected siblings (second generation) are affected. The disease-allele frequency was estimated at 0.001. Haplotypes were constructed with HaploPainter V.024 (University of Cologne, Cologne, Germany).

Primers for amplification of the TULP1 coding exons and splice junctions were designed with ExonPrimer (Technical University, Munich, Germany; http://ihg.gsf.de/ihg/ExonPrimer.html) and Primer3 (Whitehead Institute, Cambridge, Massachusetts). Primer sequences and polymerase chain reaction conditions can be requested from the authors. Polymerase chain reaction products were purified with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing was performed with BigDye Terminator Chemistry, version 3 (Applied Biosystems, Foster City, California), on a 3730 or 3100 DNA Analyzer (Applied Biosystems). The p.Arg482Trp missense mutation was analyzed in control individuals by digestion of the exon 14 polymerase chain reaction product with restriction enzyme HpaII.

RESULTS

CLINICAL EXAMINATION

All affected individuals of the Surinamese family had severe retinal dystrophy. They had a history of nystagmus, low visual acuity, and nyctalopia since infancy. On the last examinations (ages, 39-63 years), their visual acuities ranged from light perception to 1/300 OU. The 3 youngest affected family members (II-4, II-8, and II-10) had myopic refractions, varying from a spherical equivalent of −2.5 to −2.0 diopters. They all had nonrecordable scotopic and photopic electroretinography responses and a flat line on electrooculogram. The visual fields showed a general and central decline in sensitivity with progression to a constricted visual field of 10°. On examination of the anterior segments, posterior subcapsular cataracts were found (Figure 1A). The oldest affected individual (II-1) already underwent cataract surgery. Retinal examination revealed narrowed arterioles, optic disc pallor, atrophy of the pigment epithelium, and diffuse bone spicule pigmentation extending to the macular region (Figure 1B).
Figure 2. Molecular analysis of the genetic defect in a Surinamese family with autosomal recessive retinitis pigmentosa. A. Pedigree structure and haplotype analysis at chromosome 6q21. Mutations in the TULP1 gene segregate with the disease in the family. Mutation 1 (M1), p.Leu504fsX140; mutation 2 (M2), p.Arg482Trp. Black bars indicate uninformative single nucleotide polymorphism alleles. B. Genomic sequence of the TULP1 gene in proband II-8 and a control individual. The proband carries a heterozygous missense mutation p.Arg482Trp in exon 14 and a heterozygous 11–base pair (bp) deletion in exon 15. C. Alignment of part of the C-terminal tubby domain of TULP1 orthologs and TULP family members and position of all missense mutations identified in the TULP1 gene to date, including the novel p.Arg482Trp mutation. Identical amino acids are indicated in black boxes, conserved residues in gray boxes. Amino acid positions are shown after mutation names.
Molecular Analyses

The proband (II-8) of the family was analyzed with the autosomal recessive RP mutation chip and did not carry any mutations in the genes known to be involved in autosomal recessive RP. Genome-wide linkage analysis revealed 2 regions with a maximum multipoint logarithm of the odds score of 2.9. haplotype analysis confirmed that the single nucleotide polymorphism alleles at both of these regions completely segregated with the disease. One region is located on chromosome 5p15 between single nucleotide polymorphisms rs4487467 and rs2008011, spans 6.6 megabase pairs (Mb) of genomic DNA, and contains 11 genes. The second region is located on chromosome 6p21 between single nucleotide polymorphisms rs3871466 and rs726108, spans 7.8 Mb of genomic DNA, and contains more than 150 genes, including the TULP1 gene (Figure 2A).

Mutation analysis of the TULP1 gene in the proband (II-8) of the family revealed a novel heterozygous missense mutation in exon 14 (c.1444C>T, p.Arg482Trp) and a novel heterozygous 11-base pair (bp) deletion leading to a frame-shift in exon 15 (c.1511_1521delTGCAGTTCGGC, p.Leu504fsX140) (Figure 2B). All affected individuals were compound heterozygous for these mutations, while the unaffected individuals were either heterozygous for p.Arg482Trp or did not carry these mutations (Figure 2A). The p.Arg492Trp mutation was not detected in 92 control individuals.

To date, 14 different mutations have been found in the TULP1 gene, including 4 splice-site mutations, 2 frameshift mutations, 1 nonsense mutation, and 7 missense mutations. All missense mutations are located in the C-terminal tubby domain, which is highly conserved between all TULP family members. In this study, we describe a Surinamese family with a severe early-onset form of autosomal recessive RP, which is caused by novel compound heterozygous mutations in the TULP1 gene. The 11-bp deletion in exon 15 (c.1511_1521delTGCAGTTCGGC) causes a frameshift and replaces the 39 C-terminal amino acids by 140 aberrant amino acid residues (p.Leu504fsX140). The missense mutation p.Arg482Trp affects a conserved amino acid residue in the C-terminal tubby domain of TULP1 orthologs and tubby-related proteins (Figure 2C). Similar to other TULP1 missense mutations, the p.Arg482Trp mutation converts a positively charged side chain to a neutral one. It has been postulated that an important biological function, such as DNA or protein binding, is dependent on the maintenance of a positive surface, which may be disrupted by this mutation.

The severe phenotype observed in the Surinamese family is in agreement with previous reports, which also describe a severe early-onset retinal dystrophy in patients with TULP1 mutations. The addition of novel TULP1 mutations to the autosomal recessive RP mutation chip will enhance its efficiency in the future.

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