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A Novel Nonsense Mutation in *CEP290* Induces Exon Skipping and Leads to a Relatively Mild Retinal Phenotype

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Purpose. To identify the genetic defect in a family with variable retinal phenotypes. The proband had a diagnosis of Leber congenital amaurosis (LCA), whereas her two cousins had an early-onset severe retinal dystrophy (EOSRD) with useful vision. A distant family member had retinitis pigmentosa (RP).

Methods. DNA samples of the affected family members were genotyped with 250 K genome-wide SNP microarrays. Genetic defects were localized by linkage analysis and homozygosity mapping, and candidate genes were analyzed by sequencing. Patients underwent a full ophthalmic examination.

Results. Compound heterozygous mutations in *CEP290* were identified in the proband and her two cousins: the frequent c.2991+1655A>G founder mutation and a novel nonsense mutation in exon 7 (c.451C>T, p.Arg151X). The proband had nystagmus, hyperopia, a flat electroretinogram (ERG), and decreased visual acuity (20/250) from birth. The two cousins had minimal scotopic ERG responses at the age of 2. In one of these patients, visual acuity had reached a level of 20/32 at age 5, which is high for patients with *CEP290* mutations. Analysis of the *CEP290* mRNA in affected individuals revealed altered splice forms in which either exon 7 or exons 7 and 8 were skipped. In both mutant cDNA products, the open reading frame was not disrupted. Furthermore, homozygosity mapping and mutation analysis in the distant family member affected by RP revealed a homozygous mutation in *MERTK*, but no *CEP290* mutations. This *MERTK* mutation was heterozygously present in the most severely affected (LCA) patient, but was absent in the two more mildly affected cousins.

Conclusions. A novel nonsense mutation in *CEP290* results in nonsense-associated altered splicing. That the remaining open reading frame is intact may explain the less severe phenotype observed in the two affected cousins. The additional heterozygous mutation in *MERTK* may clarify the more severe phenotype in the proband. This study extends the phenotypic spectrum of *CEP290*-associated diseases at the mild end. (*Invest Ophthalmol Vis Sci.* 2010;51:3646–3652) DOI:10.1167/iovs.09-5074

Mutations in *CEP290* cause a broad spectrum of diseases, ranging from Meckel-Gruber syndrome (MKS), a lethal multisystemic disorder, and Joubert syndrome (JBTS) at the more severe end, to Leber congenital amaurosis (LCA), at the milder end of the clinical spectrum.[1–5] In addition, several families with Senior Løken syndrome (SLSN) and one case of Bardet-Biedl syndrome (BBS) have been associated with mutations in *CEP290*.[6,7] The CEP290 protein encodes a ciliary and centrosomal protein that is present in cells of different tissues, explaining the variable set of symptoms, including renal abnormalities (MKS, JBTS, SLSN, and BBS), neurologic abnormalities (MKS, JBTS, and BBS), retinal degeneration (JBTS, SLSN, BBS, and LCA), and polydactyly (MKS and BBS).

CEP290-associated LCA is a single-organ disease affecting the retina.[4,5] Mutations in *CEP290* are the most common cause of LCA, responsible for up to 6% to 22% of cases.[4,5,8,9] A founder mutation in intron 26 (c.2991+1655A>G), leading to the insertion of a cryptic exon with a premature stop codon, is the most prevalent *CEP290* mutation in LCA patients.[4,10] Since a fraction of wild-type *CEP290* mRNA remains in patients carrying the intronic mutation, it is hypothesized that this hypomorphic character of the mutation explains why the mutation leads to LCA, instead of a multiorgan disease.[4] The *CEP290*-associated retinal phenotype is classified by Perrault et al.[5] as a cone–rod type of LCA (type I as classified by Hanein et al.[11]), with visual acuity of 20/400 or lower, high hyperopia (+6 or more), slight photo aversion, and a salt-and-pepper appearance of the retina, with macular degeneration in the first decade, changing to the typical appearance of retinitis pigmentosa (RP) at later stages.[5]

In this study, we examined three patients with a LCA-like phenotype. However, two of them retained useful vision, and we therefore classified their disease as early-onset severe reti-

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nal dystrophy (EOSRD), which is considered to be milder than LCA. The less severe phenotype is caused by a novel nonsense mutation that induces exon skipping without disrupting the open reading frame. In addition, we report on the finding that one individual with the same set of *CEP290* variants showed an LCA phenotype, which may be caused by the cumulative effect of a heterozygous *MERTK* frameshift mutation.

METHODS

This study was approved by the ethics review board of The Rotterdam Eye Hospital and adhered to the tenets of the Declaration of Helsinki. Informed consents were signed by all participants and, in the case of minors, by their parents.

Clinical Evaluation

The affected individuals (VI-3, VI-4, and VI-6; Fig. 1) visited the University Medical Centre Groningen for the first time at the age of 6 months and were seen for follow-up examinations regularly thereafter for up to 11 years. The examinations included electroretinograms (ERGs), recorded according to the protocol of the International Society for Clinical Electrophysiology of Vision (ISCEV).[12] The three patients were re-evaluated at The Rotterdam Eye Hospital recently. Parents were extensively interviewed about the medical history of their children. Clinical evaluation included best corrected visual acuity using ETDRS charts and was expressed as Snellen equivalents, objective refractive error after cycloplegia, biomicroscopy, and fundoscopy. Visual fields were assessed with Goldmann kinetic perimetry (targets V-4e to I-4e for patients VI-3 and VI-4, and V-4e to II-4e for patient VI-6). Color vision was tested with the American Optical Hardy-Rand-Rittler Test (AO-HRR) and the Farnsworth Panel D-15 Test (saturated and desaturated) in all patients and by the Ishihara Test for Color Blindness in patient VI-4. Additional tests included fundus photography and spectral domain optical coherence tomography (OCT; Spectralis; Heidelberg Engineering, Heidelberg, Germany) for evaluating the in vivo retinal structure. Autofluorescence images (30°) were obtained with a confocal scanning laser ophthalmoscope with 488-nm excitation (Retinogram Angiograph 2; Heidelberg Engineering, Heidelberg, Germany) to detect alterations of the retinal pigment epithelium and lipofuscin accumulation. Kidney function was assessed by serum analyses of sodium, potassium, and creatinine levels. A magnetic resonance imaging (MRI) scan of the brain was performed in patient VI-3 to exclude brain abnormalities, including the molar tooth sign typical of JBTS. Electroretinography was repeated in patient VI-3.

Ophthalmic examination and ERG were also performed in the parents to search for clinical abnormalities in mutation carriers.

Molecular Genetic Analysis

Blood samples for molecular genetic analysis were obtained from all family members (Fig. 1). Total genomic DNA was extracted from leukocytes by a standard salting-out procedure.[12] DNA samples of the affected subjects, including a distant family member affected by RP (VI-3, VI-4, VI-6, and V-1; Fig. 1), were genotyped for 262,000 single-nucleotide polymorphisms (SNPs; GeneChip Mapping 250K *Nsp*I array; Affymetrix, Santa Clara, CA). Array experiments were performed according to protocols provided by the manufacturer. Multipoint linkage analysis was performed with GeneHunter ver. 2.1r5[13] in the easyLINKAGE-Plus software package,[14] with use of the Caucasian allele frequencies. Homozygosity mapping was performed with the software package CNAG.[15]

Mutation analysis of the *CEP290* gene was performed using previously described PCR primers and conditions.[1] Primers for the *MERTK* gene were designed by using primer3 software[16] and are listed together with the PCR conditions in Supplementary Table S1, http://www.iovs.org/cgi/content/full/51/7/3646/DC1. Direct sequencing was performed in sense and antisense direction on an automated sequencer (BigDye Terminator, ver. 3, model 3730 DNA analyzer; Applied Biosystems, Inc. [ABI], Foster City, CA). Segregation analysis of the *CEP290* and *MERTK* mutations in all family members was performed by sequencing. Eighty-five LCA patients and 123 patients affected by cone–rod dystrophy (CRD) were screened for the *CEP290* c.2991+1655A>G mutation by allele-specific PCR[17] and for the p.Arg151X mutation by restriction fragment length polymorphism analysis using *Taq*I (ABI; Supplementary Table S1).

To determine the effect of p.Arg151X on the *CEP290* mRNA level, we collected blood samples (PAX vials; Qiagen, Venlo, The Nether-

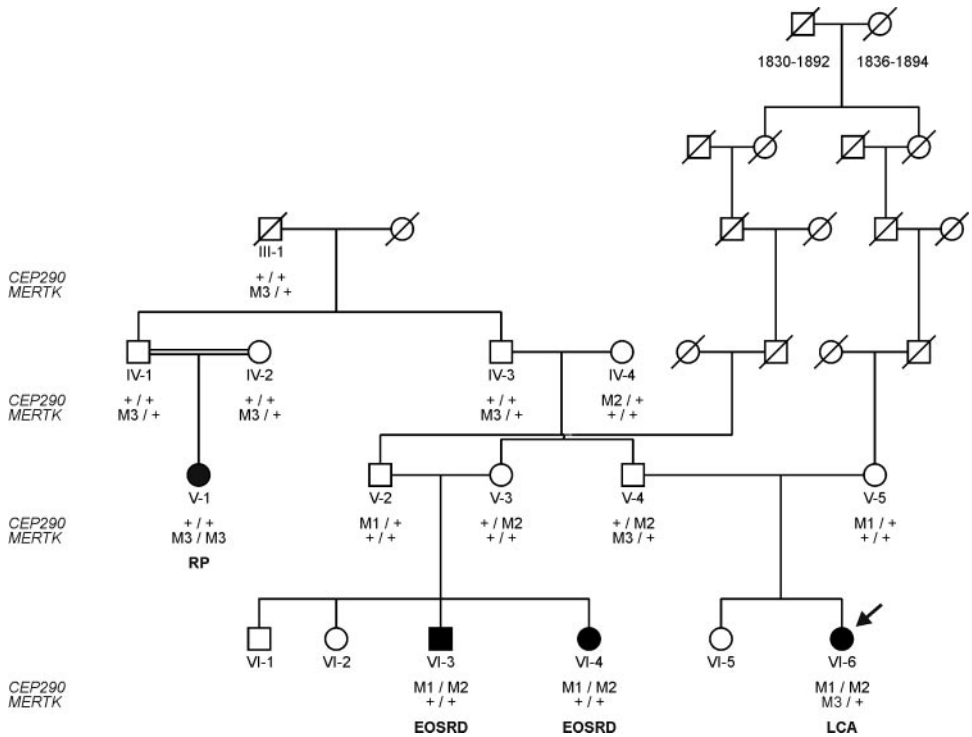

FIGURE 1. Pedigree with inherited retinal diseases and pathologic *CEP290* and *MERTK* variants. The proband (VI-6; *arrow*) is affected by LCA, two cousins (VI-3 and VI-4) are affected by EOSRD, and one distant family member (V-1) is affected by RP. Mutations in *CEP290* and *MERTK* are indicated. For reasons of privacy, mutations in the unaffected siblings are not presented. M1, *CEP290* c.451C>T, (p.Arg151X; Δexon7;Δexons7-8); M2, *CEP290* c.2991+1655A>G (p.Cys998X;wild-type); and M3, *MERTK* c.1179dup, p.Leu394SerfsX3.

lands) of the three affected children (VI-3, VI-4, and VI-6, Fig. 1) and six control individuals and isolated the RNA (PAXgene Blood RNA kit 50 ver. 2; Qiagen), according to the protocol provided by the manufacturer. Total RNA (1.5 μg) was reverse transcribed to cDNA with M-MLV reverse transcriptase (Invitrogen, Breda, The Netherlands). RT-PCR experiments were performed on 2.5 μL of synthesized cDNA or human retina Marathon-Ready cDNA (Clontech, Temecula, CA), with primers in exon 5 and 10 (35 cycles), followed by nested PCR on 0.5 μL, with the same forward primer in exon 5 and a reverse primer in exon 9 (15 cycles; Supplementary Table S1).

RESULTS

Clinical Features

Individuals VI-3, VI-4, and VI-6 visited the ophthalmologist within 6 months after birth because of nystagmus and lack of fixation. Ophthalmic assessment revealed a vertical nystagmus, normal pupil responses, a high hyperopia, and essentially normal-appearing fundi. In all three individuals, ERG was performed at the age of 2 years. In patient VI-6, both scotopic and photopic responses were nonrecordable. In patients VI-3 and VI-4, photopic responses were nonrecordable, whereas the scotopic responses were severely reduced, but measurable (15% of normal values). Patient VI-3 was evaluated by a pediatric neurologist because of the vertical nystagmus, but intracranial abnormalities were excluded by neurologic assessment and by an MRI scan of the brain. The patients' histories did not reveal any abnormalities about pregnancy and delivery. Beside the ophthalmic symptoms, no other abnormalities in motor or cognitive development were reported, and their intelligence was normal.

An overview of the most recent clinical data are presented in Table 1. In summary, all three patients experienced night blindness and no photophobia. Patients VI-4 and VI-6 displayed mild exotropia. Visual acuity varied from 20/40 in the best eye of patient VI-3 to 20/125 in patient VI-4 and 20/250 in patient VI-6 at ages 11, 8, and 9, respectively (Fig. 2). On funduscopy the posterior retina was relatively well preserved in all three patients, with pink optic discs with a scleral rim, attenuated retinal vasculature, and subtle retinal pigment epithelium (RPE) changes in the macular region. In patient VI-3, we found the remnants of a tapetal reflex that had a marbled appearance. This reflex was more pronounced at the age of 5 years and was documented on fundus photographs (Figs. 3A, 3B). In the far periphery, subretinal pigmentations were noted in patients VI-3 and VI-4. The periphery in patient VI-6 had a very hypopigmented appearance with intraretinal bone spicule pigmentations (Fig. 3E). Visual fields were constricted in all patients, but most severely in VI-6, who was also not able to perform a color vision test. On OCT a preserved photoreceptor layer was noted in the posterior pole in the two patients tested (VI-3 and VI-4; Fig. 3D). Autofluorescence images, which were difficult to obtain because of the nystagmus, showed a mildly reduced intensity of the signal in the posterior pole and midperiphery, with a normal decrease of intensity at the macular region in all three individuals (Fig. 3C).

Altogether, the phenotype of the proband (VI-6) was most similar to LCA. The phenotype of the two more mildly affected cousins (VI-3 and VI-4) was classified as EOSRD, to emphasize the fact that they were less severely affected.

Ophthalmic examination did not reveal abnormalities in three of four heterozygous carrier parents. In one parent, we detected a choroidal melanoma (V-2, see Fig. 1). The ERG responses were in the high range of normal in three of the parents (V-3, V-4, and V-5) and completely normal in parent V-2.

Molecular Genetics

Genome-wide linkage analysis was performed on DNA of the proband (VI-6) and her two cousins (VI-3 and VI-4). The family, originating from a northern province in The Netherlands, was reported to be nonconsanguineous. However, the patients were related through both parental lineages: They were first cousins through one parent and fourth cousins through the other (Fig. 1). Linkage analysis revealed only one significant chromosomal region, which localizes between SNPs rs10879550 and rs1579244 on 12q21.1-q23.1, with a LOD score of 2.4. The region harbors 68 genes, among which we found *CEP290*, making this the most obvious candidate gene in this family. Mutation analysis of the *CEP290* gene revealed a compound heterozygous combination of the most common *CEP290* founder mutation in intron 26 (c.2991+1655A>G)[4] and a novel nonsense mutation in exon 7 (c.451C>T; p.Arg151X). The mutations segregate with the disease in the family (Fig. 1). The deep intronic c.2991+1655A>G variant creates a functional 5′ splice site and the insertion of a cryptic exon into approximately 50% of the CEP290 mRNA.[4] The cryptic exon contains a nonsense codon (p.Cys998X), resulting in a predicted truncation of the CEP290 protein. As ~50% of the CEP290 mRNA remains wild-type, the c.2991+1655A>G variant can be considered a hypomorphic change.[4]

Because patients VI-3 and VI-4 had residual rod function and nondetectable cone ERGs at the age of 2 years, we screened 123 patients affected by CRD for both these *CEP290* mutations. In addition, 85 LCA patients in whom the c.2991+1655A>G mutation was previously excluded[4] were screened for the p.Arg151X mutation. The c.2991+1655A>G and p.Arg151X mutations were not detected in additional CRD or LCA probands. The novel p.Arg151X mutation was also excluded in 182 alleles of ethnically matched control subjects.

Usually, the combination of a hypomorphic change and a truncating change would lead to an LCA phenotype. Since the phenotypes in VI-3 and VI-4 were much less severe than typical LCA, we hypothesized that the p.Arg151X mutation should have a hypomorphic character. Therefore, using RT-PCR we analyzed *CEP290* mRNA in peripheral blood lymphocytes of the affected children (VI-3, VI-4, and VI-6) and six control individuals. Although splice site and exonic splice enhancer site prediction software[18,19] did not predict an effect on the splicing process, we identified three different RT-PCR products in heterozygous carriers of p.Arg151X when using a forward primer in exon 5 and a reverse primer in exon 10. One product represented normally spliced mRNA, in which only a minimal fraction of the mutated T-allele was present (Fig. 4C). The second PCR product (282 bp) lacked exon 7, and the third product (261 bp) lacked exons 7 and 8 (Fig. 4). The removal of exon 7 or exons 7 and 8 does not disrupt the open reading frame. These alternatively spliced products were not detected in six control individuals. It was also not present in human retina cDNA (data not shown). In patients as well as control individuals, we found a weak-intensity PCR product lacking exon 6.

Homozygosity mapping in the distantly related RP patient (V-1; Fig. 1) revealed several homozygous segments (Collin et al., manuscript in preparation). Sequence analysis of *MERTK*, a known RP gene located within the patient's largest homozygous region, revealed a homozygous frameshift mutation (c.1179dup, p.Leu394SerfsX3). This mutation was identified heterozygously in the most severely affected (LCA) patient (VI-6) and was not identified in the more mildly affected (EOSRD) patients (VI-3 and VI-4; Fig. 1).

Table 1. Clinical Characteristics of Three Patients with Mutations in the *CEP290* Gene

Patient	Age (y)	Nystagmus	Visual Acuity		Refractive Error*		Color Vision†	Ophthalmoscopy	Goldmann Perimetry	ERG	
			RE	LE	RE	LE				Rod‡	Cone§
IV-3	11	Mild horizontal with slightly rotating component	20/80	20/40	+8.75	+8.75	Sat.: normal Desat.: mild, aspecific errors	Pink optic discs with scleral rim, mild attenuation of vessels, absent macula and fovea with subtle RPE changes. Faint,white, patchy reflex in midperiphery, dot-like RPE atrophy in far periphery, no intraretinal pigment.	Horizontally constricted field up to 50–60° (15° nasally, 50° temporally)	NR	NR
IV-4	8	Horizontal with rotating component	20/100	20/100	+7.75	+7.75	Normal	Pink optic discs with scleral rim, mild attenuation of the vessels, present foveal reflex, mild wrinkling of the ILM. In the midperiphery, normal aspect of RPE. Far periphery, dot-like RPE atrophy with small nummular subretinal pigmentations, no intraretinal pigment.	Constriction up to 80° (10° nasally, 70° temporally)	NP[1]	NP[1]
IV-6	9	High frequency horizontally and vertically	20/320	20/250	+4.5	+4.5	NP[2]	Pink optic discs with scleral rim, mild/moderate attenuation of the vessels, absent foveal and macular reflexes, RPE changes in macula, hypopigmented periphery with dot-like RPE atrophy, and intraretinal pigmentations.	Severely constricted visual field up to 10°	NP[1]	NP[1]

ILM, internal limiting membrane; LE, left eye; NP[1], not performed at this age, because nonresponsive at earlier age; NP[2], not able to perform due to low visual acuity; RE, right eye; RPE, retinal pigment epithelium.

* Spherical equivalent in diopters.

† Saturated (sat.) and desaturated (desat.) AO-HRR, Panel D-15 Test.

‡ Isolated rod response.

§ Single-flash cone response.

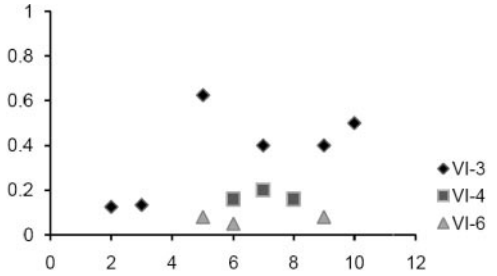

FIGURE 2. Visual acuity of patients with EOSRD and LCA. The course of visual acuity is shown in decimals (*y*-axis) with age (*x*-axis) in patients VI-3 and VI-4 (EOSRD) and patient VI-6 (LCA). The improvement in visual acuity at age 5 in patient VI-3 is most likely the effect of better performance at visual acuity assessment.

DISCUSSION

Using linkage analysis in a family with one proband affected by LCA and her two cousins affected by EOSRD, we localized the genetic defect on 12q21 and detected compound heterozygous mutations in *CEP290* (c.2991+1655A>G and c.451C>T,

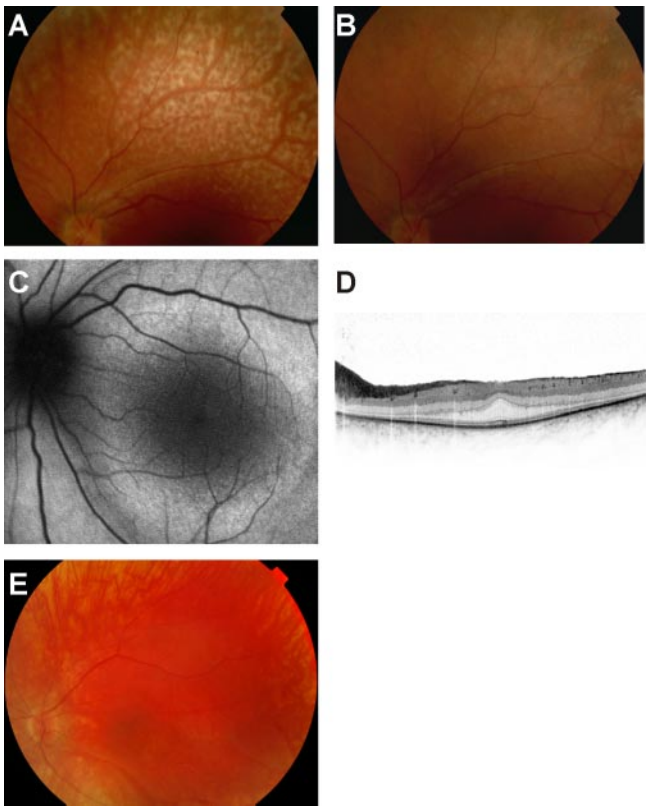

FIGURE 3. Retinal imaging of patients VI-3 and VI-6. (**A**) Photograph of the midperipheral fundus of patient VI-3 at the age of 5, showing a marbled appearance. (**B**) Fundus photograph of the same patient VI-3 at the age of 11, which shows that the marbled appearance had disappeared, and mild atrophy of the RPE was noted. (**C**) Mean autofluorescence image (30°) of the left eye of patient VI-3, calculated from 12 single images (488 nm), showing a well preserved signal in the posterior pole with decreased intensity in the macular region. (**D**) Spectral domain OCT of the left eye of patient VI-3 at the age of 11 shows a recognizable photoreceptor layer, intact retinal layers, and disappearance of the foveal dimple. (**E**) Fundus photograph of left eye of patient VI-6, showing preserved RPE in the macular region with an extremely hypopigmented area along the vascular arcade, which extends to the periphery.

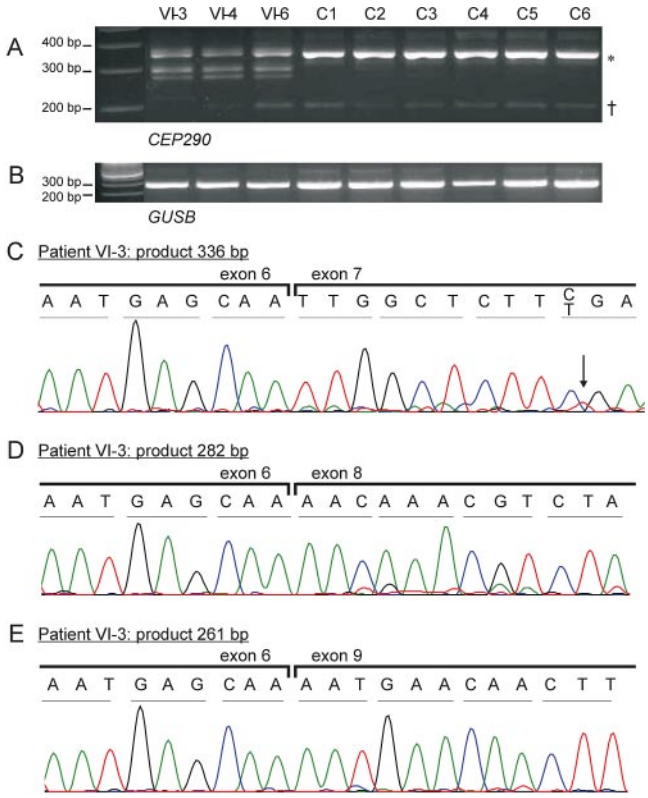

FIGURE 4. *CEP290* cDNA analysis of the effect of the c.451C>T variant on splicing. (**A**) Agarose (3%) gel electrophoresis showing three major PCR products in carriers of p.Arg151X and one major product in the six control individuals. (**B**) Agarose gel electrophoresis showing *GUSB* as the reference gene product. (**C**–**E**) PCR sequences of cDNA of patient VI-3 (CEP290 c.451C>T/c.2991+1655A>G), showing (**C**) mRNA containing all exons, but showing a low peak for the mutant heterozygous T at position 451 (*arrow*). (**D**) mRNA missing exon 7, and (**E**) mRNA missing exons 7 and 8. *Low-intensity product could not be amplified for sequence analysis. Based on the size, it could contain the *CEP290* mRNA lacking exon 8 only, which should be 315 bp; †low-intensity product ~200 bp was analyzed by sequence analysis and appeared to be the *CEP290* mRNA lacking exon 6 only (192 bp). Since *CEP290* lacking exon 6 is present in patients as well as control individuals, it appears to be a normal splice variant.

p.Arg151X). The intronic mutation is known as the most prevalent LCA-causing mutation in *CEP290*.[4,5] The nonsense mutation p.Arg151X has not been described before. The phenotype of the LCA patient was comparable to the *CEP290*-LCA patients previously described[4,5] and consisted of nystagmus, high hyperopia (+4.5 D), night blindness, reduced visual acuity from birth (20/250), fundus abnormalities, and nonrecordable electrophysiological responses. The phenotypes of the two affected cousins were comparable, but less severe, with minimal measurable scotopic responses on ERG and a more functional visual acuity. Lorenz et al.[20] coined the name early-onset severe rod–cone dystrophy to distinguish between true LCA and relatively less severe phenotypes as, for example, observed in patients carrying mutations in *RPE65*. Comparably, we decided that the classification EOSRD would be a more appropriate description of the phenotype of the two affected cousins.

That we found a remnant of rod function at the age of 2 years in these two patients suggests that *CEP290*-associated LCA is of the cone–rod type as observed by Perrault et al.[5] On the other hand, visual acuity and color vision were relatively well preserved, suggesting the opposite. An interesting finding was the marbled appearance of the fundi, which seemed to disappear with age, in patient VI-3. A similar appearance of the

fundus was seen in two other patients carrying mutations in *CEP290* (L. Ingeborgh van den Born, personal observation). Perrault et al.[5] showed a similar fundus as well and called it "white dots." The etiology of this fundus abnormality remains unclear, but tapetal reflexes can be pathognomonic for certain diseases, as has been observed in carriers of *RPGR* mutations.[21] The marbled appearance of the fundus may be pathognomonic for the *CEP290*-associated phenotype.

In contrast to the previously described *CEP290*-associated phenotype, we determined a visual acuity that is remarkably high compared with most patients carrying mutations in *CEP290*, especially in patient VI-3 (20/40 at age 11). A well-preserved visual acuity has been reported previously in 4 patients of the 77 *CEP290*-LCA patients who have been described clinically (18 patients from Yzer et al., manuscript in preparation).[4,5,10] Three patients carried the c.2991+1655A>G variant homozygously and had visual acuities of 20/80 and 20/150 at age>40[4] and 20/50 at age 20.[10] One patient carried the c.2991+1655A>G mutation together with a c.5668G>T (p.Gly1890X) nonsense mutation and had visual acuity of 20/50 at age 19.[10] In most patients, however, the visual acuity ranged from no vision at birth to a maximum of 20/400, as shown in 47 patients by Perrault et al.[5] and in the 18 patients evaluated by Yzer et al. (manuscript in preparation).[5] We hypothesize that the mild phenotype in the two cousins described in this study can be explained by the fact that they carry two mutations with a relatively mild effect on the protein. First, the c.2991+1655A>G mutation affects only part of the mRNA; part of the wild-type mRNA remains present as shown by den Hollander et al.[4] Second, the allele carrying the p.Arg151X mutation is differentially spliced, lacking exon 7 or exons 7 and 8, but remain in frame. The low peak of the mutated T nucleotide in the mRNA (Fig. 4) shows that most of the differentially spliced *CEP290* results from the mutated allele. Usually, the presence of a premature termination codon (PTC) leads to nonsense-mediated mRNA decay, a process that results in the degradation of nonsense-containing mRNAs.[22–24] We suggest that the different splice products are the result of nonsense-associated altered splicing (NAS), a putative correction mechanism that recognizes an exon with a PTC, and excludes that exon from the mature mRNA.[25–28] One form of NAS is due to the disruption of an exonic splice enhancer (ESE) by a mutation. Since the ESE finder program[19] does not predict a disruption of an ESE, NAS is likely to occur specifically due to the nonsense character of the p.Arg151X mutation. The precise mechanism is not well understood yet.[29] Exons 3 to 17 of the CEP290 protein are predicted to encode a coiled-coil domain of 506 amino acids. The protein sequences lacking the 18 or 25 amino acids encoded by exon 7 or by exons 7 and 8 are still predicted to encode a coiled-coil domain that is only slightly shorter than the wild-type coiled-coil domain. Therefore, we hypothesize that the altered spliced products encode a stable protein with residual function, resulting in a relatively mild phenotype. Because of technical limitations, it is difficult to quantify the amount of mutant mRNA that is subjected to NAS. Since the mutant splice products are smaller than the wild-type product, they will be amplified more efficiently. In addition, we cannot be certain that the analysis of lymphoblast mRNA is representative of the in vivo situation in the retinas of these patients.

The three patients in this study showed an intrafamilial phenotypic variability that led to different clinical diagnoses. Intrafamilial variability has been reported before in a family with four patients carrying homozygous c.2991+1655A>G mutations.[4] Since the amount of wild-type *CEP290* product, quantified in lymphoblast RNA, was comparable in all patients of that family (data not shown), another variant in other gene(s) may influence the phenotype and the intrafamilial variability. In the family described in this study, the intrafamilial variability between the proband and her two cousins may be explained by the heterozygous p.Leu394SerfsX3 mutation in the *MERTK* gene, which was present in the LCA patient (VI-6) and absent in the two EOSRD patients (VI-3 and VI-4). *MERTK* is associated with autosomal recessive RP[30–32] and is strongly expressed in the RPE and macrophages, where it plays a role in the RPE phagocytosis process.[33] CEP290 is localized in the connecting cilia of photoreceptors and is suggested to play a role in microtubule-associated protein transport in the cilia.[2,3,34] In view of the difference in function and localization, a physical interaction between these proteins seems unlikely. Moreover, one *MERTK* p.Leu394SerfsX3 mutation and one *CEP290* c.2991+1655A>G mutation together do not lead to symptoms of retinal dystrophy (in individual V-4). In addition, human and murine carriers of heterozygous pathogenic *MERTK* mutations do not show signs of retinal dystrophy as well. However, cultured RPE cells from the RCS rats that carry a homozygous truncating deletion in *MERTK* in vitro phagocytose only a few photoreceptor outer segments, in contrast to wild-type cells, in which many phagosomes appear.[35] Therefore, RPE cells with only 50% of the amount of functional MER tyrosine kinase receptor may have a lower turnover of photoreceptor outer segment phagocytosis, which does not have a measurable pathogenic effect in otherwise healthy individuals, but could have a cumulative (or modifying) effect in patients in whom the production of photoreceptor outer segments is disturbed, in this case by two pathogenic mutations in *CEP290*. The phenomenon of modifier alleles has been described—for example, tri-allelic mutations resulting in a more severe phenotype in Bardet-Biedl patients and a modifier *RPGRIP1L* allele that is suggested to result in a more severe retinal phenotype in ciliopathies.[36,37] Nevertheless, it remains speculative whether the *MERTK* mutation functions as a modifier allele. The a priori chance for an individual to carry a pathogenic mutation in a retinal dystrophy gene is ~10%, assuming that there are 67 autosomal recessive retinal dystrophy genes that all account for a similar proportion of cases, as calculated by Rivolta et al.[38] in 2002. Currently, 44 nonsyndromic autosomal recessive retinal dystrophy genes have been identified (http://www.sph.uth.tmc.edu/retnet/ University of Texas Houston Health Science Center, Houston, TX) that account for ~50% of cases, indicating that the calculation of Rivolta et al. could be realistic. Therefore, we estimate that 10% of retinal dystrophy patients are expected to carry a heterozygous mutation in a second gene. Eventually, the identification of combinations of mutations in more than one retinal dystrophy gene by next-generation sequencing, together with a precise description of phenotypes, may uncover the unknown influences of additional heterozygous mutations.

In conclusion, we found a novel nonsense mutation in exon 7 of *CEP290* that causes a milder phenotype than expected for an early nonsense mutation, most likely due to nonsense-associated altered splicing. Furthermore, we detected a heterozygous *MERTK* mutation in the most severely affected patient that may have a deteriorating effect on the phenotype. In this study, we extended the phenotypic spectrum of *CEP290*-associated diseases at the mildest end.

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