

CEP290 Mutations Are Frequently Identified in the Oculo-Renal Form of Joubert Syndrome–Related Disorders

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Joubert syndrome–related disorders (JSRDs) are a group of clinically and genetically heterogeneous conditions that share a midbrain-hindbrain malformation, the molar tooth sign (MTS) visible on brain imaging, with variable neurological, ocular, and renal manifestations. Mutations in the *CEP290* gene were recently identified in families with the MTS-related neurological features, many of which showed oculo-renal involvement typical of Senior-Löken syndrome (JSRD-SLS phenotype). Here, we performed comprehensive *CEP290*-mutation analysis on two nonoverlapping cohorts of JSRD-affected patients with a proven MTS. We identified mutations in 19 of 44 patients with JSRD-SLS. The second cohort consisted of 84 patients representing the spectrum of other JSRD subtypes, with mutations identified in only two patients. The data suggest that *CEP290* mutations are frequently encountered and are largely specific to the JSRD-SLS subtype. One patient with mutation displayed complete *situs inversus*, confirming the clinical and genetic overlap between JSRDs and other ciliopathies.

Joubert syndrome (JS [MIM 213300]) is an autosomal recessive disease presenting with hypotonia, ataxia, neonatal breathing abnormalities, oculomotor apraxia, and psychomotor delay.^{1,2} The neuroradiological hallmark of JS is a complex midbrain-hindbrain malformation known as the “molar tooth sign” (MTS), which originates from the association of cerebellar vermis hypoplasia or aplasia, horizontally oriented and thickened superior cerebellar peduncles, and a deepened interpeduncular fossa.³ These clinical and neuroradiological features are shared by at least eight distinct syndromes termed “JS-related disorders” (JSRDs), which additionally present with pleiotropic involvement, mainly of the eyes and kidneys.⁴

The four major subgroups of JSRDs include (1) the classic form (MIM 213300), largely restricted to brain involvement and also occasionally displaying retinopathy

and postaxial polydactyly but rarely renal involvement; (2) the oculo-renal form (referred to in this article as “JSRD-SLS”), which associates JS neurological features with the Senior-Löken syndrome (SLS [MIM 266900]) phenotype of nephronophthisis (NPH [MIM 256100]) and retinal dystrophy (either Leber congenital amaurosis [LCA [MIM 204000]] or retinitis pigmentosa); (3) the subgroup with preaxial or mesaxial polydactyly and orofacial defects, such as lobulated tongue, notched upper lip, or cleft lip/palate, known as the “orofacial-digital type VI” (OFDVI, or Varadi-Papp [MIM 277170]) syndrome; and (4) the subgroup with choroidoretinal coloboma and hepatic fibrosis, referred to as the “cerebellar vermis hypoplasia/aplasia, oligophrenia, ataxia, ocular coloboma, and hepatic fibrosis” (COACH [MIM 216360]) syndrome.

The genetic bases of JSRDs are only partially under-

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stood. Two loci, *JBTS1/CORS1* and *JBTS2/CORS2*, have been mapped to chromosomes 9q34.3 and 11p12-q13.3, respectively, in a limited number of families,^{5–7} whereas mutations in the *AH1* gene have been shown to account for ~10% of patients with JSRD, who mostly display the classic form of the disease.^{8–11}

Recently, we and others identified pathogenic mutations in the *CEP290* gene, which encodes a centrosomal protein, in 13 families with JSRD.^{12,13} *CEP290* protein was localized to the base (centrosome) and stalk of primary cilia, suggesting that JSRDs constitute one of the “cilia-related” group of disorders. This point is strengthened by the clinical overlap between JSRDs and other ciliopathies, including infantile and juvenile NPH (either isolated or associated with retinal dystrophy, which is known as “SLS”), Meckel syndrome (MKS [MIM 249000]), and Bardet-Biedl syndrome (BBS [MIM 209900]).¹⁴ Further reinforcing this link, *NPHP1* homozygous deletions that are a frequent cause of isolated juvenile NPH and SLS and mutations in *MKS3* that are usually responsible for MKS have been detected in rare cases of JSRDs.^{15–17}

Among the 13 families with JSRD with *CEP290* mutations reported so far, the majority had JSRD-SLS, in line with the pleiotropic features observed in most ciliopathies. Few patients displayed incomplete phenotypes lacking either renal or retinal involvement and additional signs, such as occipital encephalocele.^{12,13} Intriguingly, mutations in *CEP290* have also been shown to be the most frequent cause of isolated LCA, in the absence of any renal and neurological involvement.^{18,19}

The prevalence of *CEP290* mutations among patients with JSRD-SLS, as well as their role in causing other MTS-associated phenotypes and genotype-phenotype correlates, are still unknown. In this study, we sought to address these issues by performing *CEP290* mutation analysis in the largest series to date of probands representative of the whole JSRD clinical spectrum.

Subjects and Methods

Patient Ascertainment

Databases located at the IRCCS CSS, Mendel Institute (Rome) (AISJAC database), at the University of California–San Diego (San Diego) (Center for Cerebellar Malformations), and at the JS Foundation were screened for patients with a definite diagnosis of JSRD and neuroradiologically proven MTS. Patients were referred to these two centers from >20 countries on all continents through European and U.S. referral centers. Samples were obtained from referring physicians or through the JS BioBank. Whenever available, detailed clinical data were collected by means of a standardized questionnaire to assess the possible involvement of all organs, including diagnostic testing for ocular, renal, hepatic, and skeletal features.

From these databases, we selected two groups of patients for *CEP290* screening. The first cohort consisted of 44 probands with definite or probable JSRD-SLS. Thirty-two patients met the following inclusion criteria for definite JSRD-SLS: (1) presence of MTS, (2) renal signs of NPH (end-stage renal disease [ESRD] and/or typical ultrasound features and/or proof of impaired urinary-

concentration ability), and (3) either LCA or progressive retinitis pigmentosa. In this group, we also included 12 children younger than 10 years presenting a cerebello-ocular phenotype with MTS and LCA but with no obvious renal involvement, who had not yet undergone proper urinary-concentration testing or ultrasound examination. In our experience, the risk of a child with LCA within this age group developing a renal disease is >50%; thus, these 12 patients were given diagnoses of “probable JSRD-SLS.”

The second cohort consisted of 84 probands representative of the complete spectrum of the remaining subgroups of JSRDs, including classic JS ($n = 42$), JS plus ocular involvement but without renal involvement by age 10 years ($n = 21$), JS plus NPH or cystic kidney disease without retinal involvement ($n = 5$), COACH syndrome ($n = 6$), and OFDVI syndrome ($n = 5$). Five patients had a proven MTS, but clinical details were not sufficient to assign them to a specific phenotypic subgroup. Informed consent was obtained from all families, and the study was approved by the local ethics committees.

Mutational Analysis

The 128 probands with JSRD were analyzed for *CEP290* mutations after a three-step strategy.¹⁰ In brief, a denaturing high-performance liquid chromatography (DHPLC)-based analysis was first performed on DNA from one parent of each proband. All exons identified as carrying abnormal elution profiles were sequenced in both directions, and, subsequently, all parental mutations were tested in the affected offspring and, in case of homozygosity, in the other parent as well. Finally, heterozygous patients underwent complete gene analysis by direct sequencing in both directions to identify the second mutation. This strategy allowed DNA from affected children (which is usually scarce and difficult to reobtain) to be preserved and DHPLC limits in identifying homozygous mutations to be overcome. PCRs were performed in a final volume of 25 μ l containing 40–80 ng genomic DNA, 1 U AmpliTaq Gold (Applied Biosystems), 15 pmol of each primer, 1.5–2 mM MgCl₂, 75 μ M of each deoxyribonucleotide triphosphate, and 1 \times PCR Buffer (Applied Biosystems), through the use of a GeneAmp PCR system 9700 (Applied Biosystems). Samples were run on a Wave DNA Fragment Analysis System (Transgenomics) at column temperatures recommended by Navigator Software version 1.5.4 (Transgenomics). Bidirectional sequencing of purified PCR products (Millipore) was performed by using BigDye chemistry and an ABI 3100 Capillary Array Sequencer (Applied Biosystems). Primers and conditions for PCR and DHPLC are listed in table 1.

The common *NPHP1* homozygous deletion, the intronic *CEP290* mutation recurring in isolated LCA (c.2991+1655A→G), and mutations in the *AH1* gene were excluded in all probands, in accordance with published protocols.^{10,16,18}

Results

CEP290 Mutations

We identified 23 distinct *CEP290* mutations in 21 families. Seventeen mutations were novel, whereas six had been reported elsewhere (tables 2 and 3).^{12,13,19} Six probands were homozygous for *CEP290* mutations, and 10 were compound heterozygous, whereas, in 5 probands, only a single mutated allele could be found. All mutations reported were either nonsense or frameshift mutations resulting in a predicted truncated protein. Most mutations

Table 1. Primers and PCR and DHPLC Conditions for CEP290 Analysis

Exon(s)	Primer Sequence (5'→3')	Amplicon Size (bp)	PCR Annealing Temperature (°C)	No. of Cycles	DHPLC Oven Temperature ^a (°C)	Buffer B Starting % ^a
2	ACCAATAACTGTGACTCTTG CAGATTGTGACAATTATAGTTG	289	58	38	53.1/57	54.4/52.1
3	CAACTATAATTGTACAATCTG GTTCCACTAATAGCCAAACC	212	58	32	55.7	50.9
4	GTGCTTACATTCCAGTATAAAG GTTAAATGAACAAATGAATTCA	186	58	32	54.4/58	53.4/49
5	ACCTTATAATCATGATGGACTC AATAACCATGATTACAATCATCC	285	58	32	51.8/53.4	54.3/54
6	TTGTTGACTCATTGAACTC AAAAAGCCAGGTAACCTGAAAC	264	58	32	53.2/56.4	55.6/54.2
7	ACTGCTGAATTTTATCTTCTC TTAGAAGACTCCAGTCTGG	208	58	32	55.7	51.2
8-9	CAAGATAATATGCATCTTTCCC ATGAAATTAAGTTTTAGGAACC	472	58	32	55	58.7
10	AGAGGACACTTATGGCTGCG GTAATGAGATAATGAAGTCTG	332	56	35	52.9/54.1	56.3/54.6
11	CACATATGTAATGAATGTATC CTAATAAACGTGTATAAACCCAG	364	56	35	52.4/55.2	55.8/54.7
12	GTATCATAAATCTACTAACGGTG ATGTTCCAGAGTCCAACCTG	284	58	35	54/55.4	55.9/55.5
13	CTTGACCACAAAGAAATATG AGAAAACTAATATTGACTTGAC	341	56	35	52.7/54.3	55.8/52.8
14	TGATTTGAAGGAATAAGTAGC CTGTGAATGGCAAGAATAATTC	282	58	35	55/56.1/58	57.2/56.1/52
15	GTACATTTTCTTTAGACTGAG ACTGTAAATCAGGTTGCGC	311	58	38	54.1/56.6	55/52.5
16-17	CATTTTTGCAGCTTATTTGAATG ATATCCAGACAACCTACTTATC	380	58	38	54.8/55.8	57.9/56.9
18	ATTAAGTGTGGAAATAGTAGG TATTTTCTTTACTCTCTTTC	218	58	38	55.3	50.1
19	ATTGATCAAACCTTTCTAACTTG ACAGAGGTAATAGGAGTAAAG	293	54	35	54	56.2
20	CCAATGATGCTTTGGTATATG AAATATCTCATCAGAAACTATGG	340	58	38	54.5/55	57.8/57.3
21	GTCCATTTTATTAAGACAGAC TTAATTCAGGGCATTCTTCTC	362	58	35	53.6/57.4	56.7/52.9
22	TATGGTTGAGGTAATAATCTCG AGTACTATCTGCATGCTTGG	390	58	38	52.6/55.8	58.9/54.2
23	TAACCTTCTATAATGTTGCAG TAAGTTCCTAACAGTAGTTACC	372	54	35	53.2	57.3
24	ATACCTCTTGTGTGAGAAAA CACAAAGACACATCATATTAC	300	54	35	53.3/54.5	56.7/54.7
25	TATGCAATATTGTACAAGTAGG TGATACCATCTCTATCTCTGC	368	58	38	54.5/55.4	58.7/57.8
26	AAAGTGGTAGTGCTTACC TGTTAAATTTATATAAATGACGGC	358	54	35	56.3	57.9
27	AACCTGGATTGTGAGTTTAAAG AGGATTATCATCTGCCTAAG	383	58	38	52.7/54.8	57.3/53.7
28	ACAGCATCTAAAATATCTGAGG AGATCCAGACAACCACTTAAC	369	58	38	54.4/56.6	57/54.4
29	AAGGCCAAGTAAAGAGGATTG TACTACTAAGAATTGTATACCTG	349	58	35	55/56.9	58/55.8
30	TAGAAAGTGTACTAATTTGTTCC CCCACTCCCAACATCTAATG	231	58	38	56.2/58	55/52
31	AATCTGTGATAAATCTTCACTGG TGTGTTGACCACTGAACCTC	604	58	35	54.7/58	61.2/56.5
32	CATTATCATCAATGGAGGAATG TAGTCATTTGTGCAATATCTTG	603	58	35	53	61.7
33	CCTGTTATGTGCTGATGTC TGAGTTAACACTCTAGACTATG	222	58	38	56.8/58.1	55.5/54
34	ATCATGTTTTATCATACAGCTG ATCATTCTATGCATTGCTCCTC	321	58	38	54.6/56.9	57.5/54.6
35	GCATTTTAAAGGGAAAAAGATAC CACTTATAGGGTAAATAATTTAG	402	54	37	53.5/55.4/57.5	58.1/56.1/54.1
36	ATATGGAGATACTGTTCTTCC GCTGAATTTAATTTACATGGTC	305	58	38	55.6	55.2
37	AATATGGAATAAGTATGGCATTG AGCAAACTATGTTTATCTTC	337	58	38	52.4/54.4/57.8	58.1/56.1/52.7
38	GTGACAGAGTGAGACTGGC ACAACACGGAGATTATACTAC	397	58	38	54.8/56.4	57.7/56
39	ATAGTAGGAAGTAATAAGCTTG TAGTGAATCTCTTCCAATAGG	305	58	38	57.5/59.2	55/53
40	GTTCCCTTTATCATTGATCTTC AAGTAGAAATAAACTACTACCTC	352	58	38	52.6/53.8	58.6/57.4
41	GTGATAGCTTCAGAAAGTTC CAGAATTAATACAGCCAGGTC	342	58	38	55.3	54.3
42	AACATATTTACATATCTCTAGG TAAAGCTATAATTTCCAGGTC	345	54	38	55.2	54.4
43	TTTGGTTTGGTAATGAGTATGC TTCAATTTCTAGGGGCAACC	301	58	38	55/56.6	56.8/55.2
44	ACACTGAACTTTCTTTTATC AGATGTAATGCTTTGGCCAG	320	58	38	54.4/57.6	56.7/52.7
45	TATCCAGTATGCTTTTATGGC ACCATCACCATGATATATTAGG	329	58	38	53.9/55.7	56.4/53.2
46	TTTGCCTTTCTTTCAATGGC TATCTAACTTTTCATTCTGGC	223	58	38	56.2/57.7	53.4/51.9
47	TGTTGATTGTTGACTTCC TTAGCCTTGCCTCTATAAG	394	56	35	53/54.9	57.9/56
48	TGGTTTCTAAAACACTTTGAAG ACTTCCAGTTTTCCAAGAGG	296	58	38	53.3/54.4/57.2	55.5/54.4/51.6
49	TAGAGCCCCAGGTTATTTTG TGTTTCATCAGGAAGAAACCAG	293	58	38	54.2/56.1/58.5	59.5/56.5/53
50	TTAGTACAGTATTTGAACTGAC ACAATGCAAGGAACATCTTGC	293	58	38	53.7/55.2	56.6/53.5
51	ACGCTTTGTTAAAATGTGTATC ATGCTTGTCTAGTTGTAGC	255	58	38	55.5/58.9	56.7/46.2
52	TCACTAGTTCATAAGAAATGCC AATTGATTTACAGGGAGAC	291	58	38	55.1/56.6	56.2/54.2
53	CCATTACCTTGAACCTATTC TAGGATACGTAGTTAAAGATGG	230	58	38	54.2/55.9	54.2/51.5
54	ATTCAGGAATACTTTGGCTTTC TTCGAGAAGCTGCTTATTTCC	418	58	38	53.6/55.5	57.7/52.9

^a Each value is a different optimized oven temperature, with the corresponding buffer B starting percent for each corresponding temperature at which the amplicon has been analyzed. For some amplicons, to ensure adequate peak separation, we needed to run at a different temperature and buffer B starting percent. In those instances, duplicate values for both are represented.

Table 2. Genotypes and Phenotypes of Patients with CEP290 Mutations

Patient	Origin	Consanguinity	Sex	Age (years)	DNA Alteration	Predicted Protein Alteration	Exon(s)	Phenotype		
								Eye ^a	Kidney ^b	Other ^c
With JSRD-SLS:										
COR083	Switzerland	No	F	2	5163delT, ^d homozygous	T1721fsX1723, homozygous	38	LCA	NPH	EC
COR145	United Kingdom	No	M	2	1657_1666delA+6031C→T	L552fsX572+R2011X	17, 44	LCA	Normal US, UCT NP	...
MTI333a	United States	No	M	3 ^e	4882C→T ^d +5610delCAAA	Q1628X+K1870fsX1872	37, 41	LCA	NA	NA
MTI333b	United States	No	F	12	4882C→T ^d +5610delCAAA	Q1628X+K1870fsX1872	37, 41	LCA, RC	NPH (7)	No MR
COR109	France	No	F	6	1682_1683delA+3814C→T ^d	A560fsX572+R1272X	17, 31	LCA	Normal US, UCT NP	...
COR003	Italy	No	F	6	5668G→T ^d +?	G1890X+?	41, ?	LCA	NPH	ASD
COR084	Russia	No	F	6	4882C→T ^d +5941G→T	Q1628X+E1981X	37, 43	LCA	NPH	...
MTI154	India	No	F	6	5668G→T, ^d homozygous	G1890X, homozygous	41	RP, VR	NPH	VSD
MTI125	United States	No	F	6	4393C→T+?	R1465X+?	34, ?	LCA	NPH (6)	...
COR004a	Italy	No	F	17	3811C→T+5734delT	R1271X+R1911fsX1922	31, 42	LCA	NPH (13)	...
COR004b	Italy	No	M	7	3811C→T+5734delT	R1271X+R1911fsX1922	31, 42	LCA	NPH	...
MTI328	United States	No	M	8	1985A→T+6277delG	Q662X+V2092fsX2096	20, 46	LCA	NPH (4)	...
MTI273	Turkey	Yes	M	10	4786_4790delTAAA, homozygous	S1595fsX1599, homozygous	36	LCA	NPH (9)	SI
COR125	United Kingdom	No	F	10	5431_5433delGA+5668G→T ^d	N1810fsX1816 +G1890X	40, 41	LCA	NPH (8)	...
MTI487	Turkey	Yes	F	10	5722G→T, homozygous	E1908X, homozygous	42	LCA, RC	US and UCT NP	LT, PP, CM
MTI118	Ireland	No	F	12	3167_3175insA ^d +5668G→T ^d	I1055fsX1069+G1890X	28, 41	LCA	NPH	...
MTI286	Brazil	No	F	13	4393C→T+?	R1465X+?	34, ?	LCA	NPH	...
MTI111a	Laos	No	M	13	6072C→A+7321dupCTCT	Y2024X+L2440fsX2456	44, 54	LCA	NPH	...
MTI111b	Laos	No	F	28	6072C→A+7321dupCTCT	Y2024X+L2440fsX2456	44, 54	NA	NA	NA
COR031	Belgium	Yes	F	15	4393C→T+4723A→T ^d	R1465X+K1575X	34, 36	LCA	NPH	...
COR001	Italy	No	F	20	5489_5493delA+?	K1829fsX1850+?	40, ?	LCA	NPH (9)	...
COR002a	Italy	No	M	34	6870delT+?	N2290fsX2300+?	50, ?	LCA	NPH (18)	...
COR002b	Italy	No	M	30	6870delT+?	N2290fsX2300+?	50, ?	LCA	NPH (11)	...
With JSRD:										
MTI012	United Arab Emirates	Yes	M	8	5668G→T, ^d homozygous	G1890X, homozygous	41	Normal	Normal US, UCT NP	...
MTI587	United Arab Emirates	Yes	M	5	5668G→T, ^d homozygous	G1890X, homozygous	41	Normal	NPH	...

NOTE.—NA = not available. All patients displayed MTS features, neurological signs typical of JS.

^a RC = retinal coloboma; RP = retinitis pigmentosa; VR = vision reduction.

^b NPH includes ESRD (if present, age at onset in years is given in parentheses) and/or typical ultrasound (US) features and/or proof of impaired urinary-concentration ability. UCT = urinary-concentration testing after 1-deamino-8-D-arginine vasopressin challenge; NP = not performed.

^c EC = encephalocele; MR = mental retardation; ASD = atrial septal defect; VSD = ventricular septal defect; LT = lobulated tongue; PP = postaxial polydactyly; CM = cardiomegaly; SI = complete *situs inversus*.

^d The six previously reported mutations (see table 3 for details). All other mutations are novel.

^e This patient died at age 3 years.

Table 3. Recurrent Mutations Identified in the *CEP290* Gene

Phenotype and DNA Mutation	Predicted Protein Alteration	Exon	No. of Families	References ^a
JSRD only:				
5668G→T	G1890X	41	10	Valente et al., ¹² Sayer et al., ¹³ PD
4393C→T	R1465X	34	3	PD
LCA only:				
2991+1655A→G	C998X	26	44	den Hollander et al., ¹⁸ Perrault et al. ¹⁹
5587-1G→C	Splice	40	4	den Hollander et al. ¹⁸
5850delT	F1950fsX1964	42	3	den Hollander et al. ¹⁸
6604delA	I2203fsX2226	48	2	den Hollander et al. ¹⁸
JSRD and LCA:				
4723A→T	K1575X	36	8	Perrault et al., ¹⁹ PD
5163delIT	T1721fsX1723	38	4	Perrault et al., ¹⁹ PD
3167_3175insA	I1055fsX1069	28	3	Sayer et al., ¹³ Perrault et al., ¹⁹ PD
4882C→T	Q1628X	37	3	Perrault et al., ¹⁹ PD
3814C→T	R1272X	31	2	den Hollander et al., ¹⁸ PD
7341-7342insA	L2448fsX2455	54	2	Sayer et al., ¹³ den Hollander et al. ¹⁸

^a PD = present data.

(20 of 23) clustered from exon 28 to the end of the gene, with a peak at exon 41 (fig. 1). The most common mutation was G1890X, which was homozygous in three families (two from the United Arab Emirates and one from Turkey) and heterozygous in three others (from Italy, Ireland, and the United Kingdom). The R1465X mutation in exon 34 recurred in three families (from Belgium, Brazil, and the United States), and the Q1628X (exon 37) mutation in two (from the United States and Russia), whereas the remaining 20 mutations were all found in single families (table 2). All mutations segregated with the disease in familial cases. *CEP290* nucleotide variants representing either polymorphisms or nucleotide changes of unknown significance are listed in table 4.

Phenotypes Associated with *CEP290* Mutations

JSRD-SLS subgroup.—*CEP290* mutations were identified in 16 (50%) of 32 probands with definite JSRD-SLS and in 3 (25%) of 12 patients with probable JSRD-SLS. All patients had neurological signs typical of JS and a neuroradiologically proven MTS (fig. 2). Fifteen had sporadic cases of disease, whereas four had one affected sib.

Among the 16 probands with definite JSRD-SLS, 15 presented with LCA and blindness within the 1st year of life, whereas only 1 (MTI154) had progressive retinitis pigmentosa, with visual reduction documented at age 6 years. One patient (MTI133b) had chorioretinal colobomas in addition to LCA. All patients had signs of infantile or juvenile NPH; the youngest was age 2 years. Eight of them had already developed ESRD at ages ranging between 4 and 18 years. Four patients with sporadic cases presented additional clinical features, including a small occipital encephalocele (COR083), atrial (COR003) or ventricular cardiac septal defect (MTI154), and complete *situs inversus* (MTI273) (fig. 3).

Three patients with mutations were given diagnoses of probable JSRD-SLS, since they had LCA but no overt signs

of renal involvement by ages 2, 6, and 10 years. However, evaluation of urinary-concentration ability after water deprivation or desmopressin challenge had not been performed, and the presence of an asymptomatic urinary-concentration defect could not be excluded. One patient presented additional clinical signs, including chorioretinal colobomas, postaxial polydactyly, lobulated tongue, and cardiomegaly (MTI487) (table 2).

Other JSRD subgroups.—The screening of the *CEP290* gene among a second cohort with MTS yielded mutations in only 2 (2%) among 84 probands, one with classic JS (1 of 42) and one with JS plus NPH (1 of 5). No mutations were found among 21 patients with JS plus ocular involvement, 6 with COACH syndrome, 5 with OFDVI syndrome, and 5 with proven MTS but limited clinical data for subgroup assignment.

Between the two patients with mutations, the one classified as having pure JS presented no overt signs of retinal or renal involvement at age 8 years, and results of kidney ultrasound examination were normal. Yet, the possibility that this child might develop a renal disease phenotype with increasing age cannot be ruled out. The second patient with mutation had urinary-concentration defect but normal vision and no signs of retinal involvement at age 5 years. Both patients were homozygous for the recurrent G1980X mutation.

Discussion

In this study, we identified 17 novel *CEP290* truncating mutations, raising to 65 the number of known deleterious alterations found in patients with JSRD and/or LCA.^{12,13,18,19} Most mutations appear to be private, with only 12 mutations recurring in two or more families (table 3). Besides the most common mutation—C998X, which is restricted to LCA—our data show that G1890X represents the second-most frequent *CEP290* mutation. Interestingly,

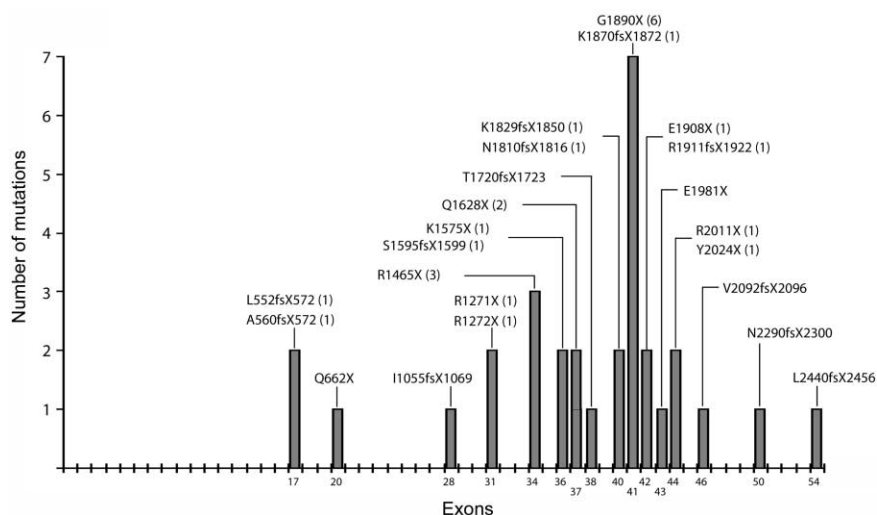


Figure 1. Schematic of distribution of mutations identified in this study across the 54 exons of *CEP290*. Numbers of patients with mutations are listed in parentheses.

this aberration seems to be strictly associated with the JSRD phenotype, since it was never reported in large cohorts of patients with isolated LCA. Yet, other recurrent mutations have been described in families with both JSRD and LCA, making it difficult to establish genotype-phenotype correlates. Of note, whereas mutations causative of isolated LCA appear to be homogeneously distributed throughout the gene,^{18,19} >80% of mutations identified in families with JSRD cluster in the second half of the gene. We, as well as others,^{13,19} failed to identify the second mu-

tation in ~20% of *CEP290* families despite extensive sequencing of the whole coding region and canonical splice sites. The possibility that other mutations reside within intronic sequences (as is the case for the recurrent C998X mutation)¹⁸ or represent large genomic rearrangements needs to be further explored.

We found that *CEP290* is a major causative gene of definite JSRD-SLS, with demonstrable deleterious mutations detected in half of the patients in this subgroup. The renal disease phenotype was characterized mostly by juvenile

Table 4. Polymorphisms and Variants Observed in the *CEP290* Gene

DNA Alteration	Reference SNP ^a	Predicted Protein Alteration	Exon or Intron	Allele Frequency ^b (n = 204 Chromosomes)
IVS5+36A→G	ss69374911	...	Intron 5	Common
c.829G→C	ss69374912	E277Q	Exon 10	Common
c.930A→G	...	V310V	Exon 11	NS
c.1991A→G	...	D664G	Exon 20	NS
IVS20+30delT	Intron 20	NS
c.2055T→C	ss69374913	A685A	Exon 21	Common
IVS21+44T→C	ss69374914	...	Intron 21	Common
c.2268G→A	rs2468255	S756S	Exon 22	Common
c.2343T→C	...	N781N	Exon 22	NS
c.2512A→G	rs11104738	K838E	Exon 24	Common
c.2717T→G	rs7970228	L906W	Exon 25	NS
IVS35+40_46delT	rs11356711	...	Intron 35	Common
c.4806G→A	...	T1602T	Exon 36	NS
IVS41+25A→C	rs17015438	...	Intron 41	Common
IVS41+45G→C	ss69374915	...	Intron 41	Common
IVS47+5_12insT	rs11405846	...	Intron 47	Common
c.6684T→G	...	N2228K	Exon 49	NS
IVS51-37C→G	ss69374916	...	Intron 51	Common
IVS53-22T→C	ss69374917	...	Intron 53	Common

^a "rs" Refers to reference SNP number; "ss" refers to submitter SNP number.

^b Common = allele frequency >2%. NS = variation found in the parent but not segregating in affected offspring.

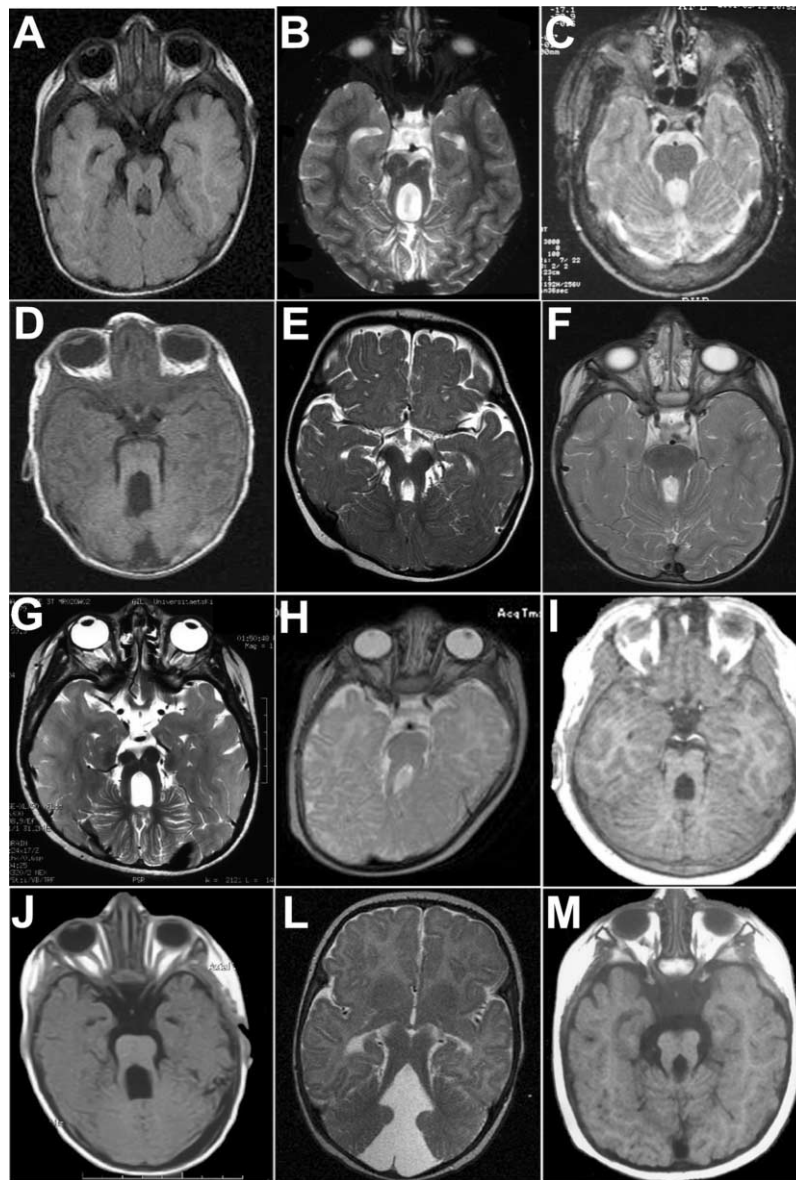


Figure 2. Brain magnetic resonance imaging results for 12 of 21 probands with mutations, showing the typical midbrain-hindbrain malformation known as the MTS. All panels display axial sections at the pontine or pontomesencephalic junction showing the MTS. A–L, respectively, patients MTI012, MTI286, COR002a, COR083, COR145, COR109, COR084, COR125, MTI333b, MTI154, MTI125, and MTI487.

NPH, with development of renal failure toward the end of the 1st decade or early in the 2nd decade. However, the age at onset of renal failure was found to be extremely variable, with the youngest patient aged 4 years. Thus, patients with *CEP290* mutations can also develop the infantile form of NPH with rapid progression toward renal failure within the first few years of life.

We also identified mutations in three children with retinal blindness but no overt clinical signs of NPH by age 10 years (probable JSRD-SLS). It will be crucial to follow up these patients to evaluate whether they will develop renal disease later in life, to define the prognostic value of *CEP290* mutations in predicting NPH. This issue carries

important implications for genetics counseling of families and for the setup of early interventional strategies aimed at delaying the progression toward renal failure and minimizing complications such as growth retardation or bone disease.

The ocular phenotype observed in patients with JSRD-SLS is usually characterized by LCA. Yet, patients with rarer cases show no detectable defects in vision within the 1st year of life but later develop a progressive phenotype of retinitis pigmentosa, usually with some residual sight. By screening patients with other non-JSRD-SLS phenotypes, we identified pathogenic mutations in two children with preserved vision and normal ophthalmologic testing at

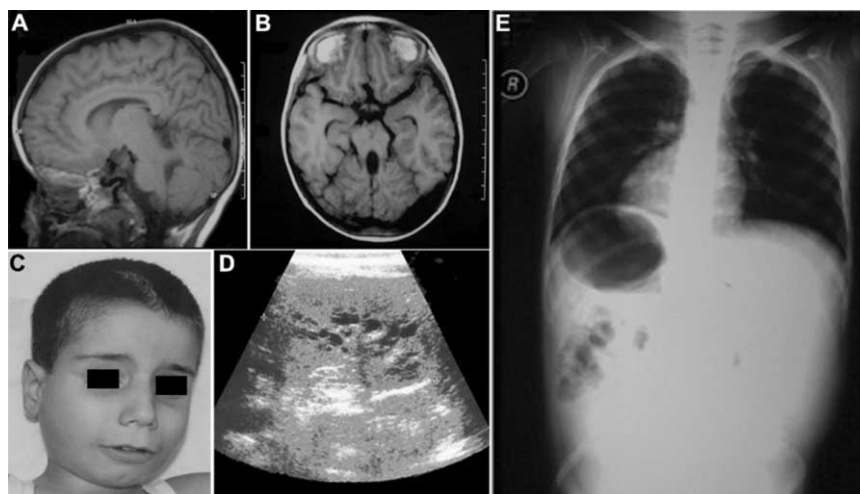


Figure 3. Patient MTI273, homozygous for the S1595fsX1599 mutation in exon 36 of the *CEP290* gene and presenting with JSRD-SLS associated with complete *situs inversus*. Sagittal (A) and axial (B) brain magnetic resonance imaging sections show the MTS malformation. C, Face showing absence of specific dysmorphic features. D, Kidney ultrasound displaying multiple renal cysts and increased echogenicity. E, Chest x-ray showing complete *situs inversus*, with stomach bubble and heart apex on the patient's right side (R).

ages 5 and 8 years (MTI012 and MTI587, respectively). Intriguingly, we noticed that these two patients, as well as the single child with JSRD-SLS with retinitis pigmentosa who was not blind at age 6 years (MTI154), were the only three to be homozygous for the recurrent G1890X mutation in exon 41. This correlation holds true also for patients described elsewhere. In fact, of nine patients homozygous for this mutation, only one was reported to have LCA,^{12,13} leading us to speculate that this truncating mutation, through unknown mechanisms, might spare the retina. Conversely, other truncating and splice-site mutations, including the recurrent C998X, are invariably associated with isolated LCA (table 3), and the *rd16* mouse, which carries an inframe 298-aa deletion of *CEP290*, shows a pure retinal degeneration phenotype.^{18–20} Therefore, there appear to be specific mutations that show predilection for one organ system over another. We excluded the possibility of alternative splicing of implicated exons as a mechanism of this selective organ sparing, because they are present in all mRNAs represented in the UCSC Genome Browser. This observation has not been reported elsewhere for any known genetic disease, to our knowledge, and warrants functional studies of these mutant proteins in specific cell and animal models.

Among the 84 patients with other JSRD subtypes, we identified no mutations other than the ones in the 2 patients without retinopathy. Therefore, it appears that the clinical spectrum of *CEP290* mutations is largely restricted to the JSRD-SLS phenotype. It is interesting to note that one patient with mutation with probable JSRD-SLS (MTI487) also presented additional clinical features reminiscent of the OFDVI and COACH subgroups, such as lobulated tongue, postaxial polydactyly of the hands and feet, and bilateral chorioretinal colobomas. Although a

definite diagnosis of one of these syndromes could not be made with the current classification system, the clinical spectrum of MTS-associated features does require further delineation.

In some patients with JSRD-SLS with *CEP290* mutations, we observed additional peculiar clinical features, expanding the phenotypic spectrum to clearly overlap with other ciliopathies. The first example is the presence in three patients (COR083 and two patients in the work of Sayer et al.¹³) of occipital (meningo)encephalocele. This neural-tube defect represents a constant feature of MKS, which has been recently shown to be allelic to JSRD at the *MKS3* gene.¹⁷ The second example is patient MTI273, a 10-year-old Turkish boy presenting a JSRD-SLS phenotype associated with complete *situs inversus*. Although this observation may be coincidental, this is unlikely because of the rarity of both conditions and the report of similar phenotypes within the spectrum of ciliopathies. Mutations in *NPHP2*, which encodes inversin, cause infantile NPH occasionally associated with retinal dystrophy or with *situs inversus*.^{21,22} Similarly, randomization of left-right body-axis symmetry was reported in 4% of Finnish patients with MKS²³ and in a BBS-affected family with mutations in *BBS8*.²⁴ Of note, two other patients with *CEP290* mutations in our cohort (COR03 and MTI154) presented with congenital heart malformations (atrial and ventricular septal defect, respectively) that are often part of the clinical spectrum of abnormalities observed in *situs inversus*.

The study of animal models with disrupted ciliary structure or function has highlighted a critical role for cilia in regulating specific developmental pathways of neurulation and left-right axis determination. In mouse embryos lacking KIF3B, a protein essential for intraflagellar transport (IFT) and ciliogenesis, the consequent lack of twirling

nodal cilia led to a complex developmental phenotype of growth retardation, randomized left-right axis asymmetry, and neural-tube formation defects.²⁵ Knockout mice for distinct IFT proteins display neural-tube defects that are consequent to disruption of the sonic hedgehog signaling pathway,^{26–28} whereas a deletion of inversin in mouse or knockdown in zebrafish resulted in renal cystic phenotype with laterality defects.^{21,29}

Since these proteins share several domains and have been demonstrated to act in large protein-protein networks,¹⁴ it will be intriguing to investigate how CEP290 possibly interacts with other ciliary proteins to regulate basic developmental mechanisms and sensory functions in multiple tissues.

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Web Resources

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

AIJSAC, <http://www.aisjac.com>

Center for Cerebellar Malformations, <http://www.ccm.ucsd.edu>

JS BioBank, <http://www.joubertsyndrome.org/BioBank.asp>

JS Foundation, <http://www.joubertsyndrome.org/RegCoordinators.asp>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for JS, JSRD, SLS, NPH, LCA, OFDVI, COACH, MKS, and BBS)

Standardized clinical questionnaire for JSRD evaluation, <http://www.ccm.ucsd.edu/PatientQuestionnaire2004.pdf>

UCSC Genome Browser, <http://www.genome.ucsc.edu/>

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