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Complete List of Authors:	Coppieters, Frauke; Ghent University Hospital, Center for Medical Genetics Lefever, Steve; Ghent University Hospital, Center for Medical Genetics Leroy, Bart; Ghent University Hospital, Department of Ophthalmology; Ghent University Hospital, Center for Medical Genetics De Baere, Elfride; Ghent University Hospital, Center for Medical Genetics
Key Words:	CEP290, locus-specific database, genotype-phenotype correlations, modifiers, ciliary proteome

CEP290, a gene with many faces: mutation overview and presentation of CEP290*base*

Frauke Coppieters[1*], Steve Lefever[1*], Bart P. Leroy[1,2], Elfride De Baere[1]

[1]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

[2]Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium

[*]These two authors contributed equally to this work.

Address for Correspondence: Elfride De Baere, MD, PhD, Elfride.DeBaere@UGent.be, Center for Medical Genetics, Ghent University Hospital, De Pintelaan 185, B- 9000 Ghent, Belgium, Phone: 32-9-332.5186 Fax: 32-9-332.6549.

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Abstract

Ciliopathies are an emerging group of disorders, caused by mutations in ciliary genes. One of the most intriguing disease genes associated with ciliopathies is *CEP290*, mutations in which cause a wide variety of distinct phenotypes, ranging from isolated blindness over Senior-Loken syndrome (SLS), nephronophthisis (NPHP), Joubert syndrome (related disorders) (JS[RD]), Bardet-Biedl syndrome (BBS) to the lethal Meckel-Grüber syndrome (MKS). Despite the identification of over 100 unique *CEP290* mutations, no clear genotype-phenotype correlations could yet be established and consequently the predictive power of a *CEP290*-related genotype remains limited. One of the challenges is a better understanding of second-site modifiers. In this respect, there is a growing interest in the potential modifying effects of variations in genes encoding other members of the ciliary proteome which interact with CEP290.

Here, we provide an overview of all *CEP290* mutations identified so far, with their associated phenotypes. To this end, we developed CEP290*base*, a locus-specific mutation database that links mutations with patients and their phenotypes (medgen.ugent.be/cep290base).

Key words: *CEP290*, locus-specific database, genotype-phenotype correlations, modifiers, ciliary proteome

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Background

Cilia are highly conserved organelles that are essential for many cell types. Apart from their obvious role in motility and transport of fluids and particles over epithelial surfaces, they have numerous other functions such as signal transduction (Berbari et al., 2009). The extensive presence of cilia throughout the whole body might explain the wide range of phenotypes associated with mutations in genes encoding ciliary proteins (Gerdes et al., 2009; Nigg and Raff, 2009).

One of the most intriguing disease genes associated with ciliopathies is *CEP290* (MIM[610142]), as the phenotypic spectrum of its mutations ranges from isolated blindness to the lethal Meckel-Grüber syndrome (MKS). The gene was initially identified as disease gene for Joubert syndrome (related disorders) (JS[RD]) and Senior-Loken syndrome (SLS) (Sayer et al., 2006; Valente et al., 2006). Within a few years, Leber Congenital Amaurosis (LCA), MKS and Bardet-Biedl syndrome (BBS) expanded the list of partially overlapping yet distinct disorders caused by *CEP290* mutations (den Hollander et al., 2006; Baala et al., 2007a; Leitch et al., 2008). Although these are essentially autosomal recessive (AR) monogenic diseases, epistatic effects of modifier alleles in additional ciliary genes should not be underestimated in the development of their phenotypes.

The first clone corresponding with *CEP290*, *KIAA0373*, was identified through sequencing of 100 new cDNA clones from human brain cDNA libraries (Nagase et al., 1997). Three years later, Chen and Shou independently cloned *CEP290* as *3H11Ag,* encoding an antigen for the monoclonal antibody 3H11 which specifically recognizes cancer cells from various tissues (Chen and Shou, 2001). It was predicted that about 60% of the residues form coiled-coil (CC) structures

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and that the protein has four potential dimeric CC regions. In addition, the protein displays high similarity to myosin among different species and was predicted to have a partial structural maintenance of chromosomes (SMC) conserved domain. Several predicted motifs suggested potential modifications such as N-glycosylation, tyrosine sulfation and phosphorylation (Guo et al., 2004). In 2003, Andersen and colleagues detected the KIAA0373 gene product in human centrosomes following mass-spectrometry-based proteomic analysis. The protein was called Cep290 according to its centrosomal location and approximate relative molecular mass, and was predicted to contain nine CCs (Andersen et al., 2003). Upon the identification of *CEP290* as a novel disease gene for JS, CEP290 was further characterized. Analysis of the deduced amino acid (AA) sequence revealed 13 putative CC domains, a region with homology to SMC chromosomal segregation ATPases, a bipartite nuclear localization signal, six RepA/Rep+ protein KID motifs, three tropomyosin homology domains and an ATP/GTP binding site motif A (Sayer et al., 2006). The *CEP290* gene as currently annotated, spans 54 exons with the coding region starting in exon 2 (NM_025114.3). Given the broad allelic spectrum, the complexity of associated phenotypes and the presumed influence of modifier genes, the establishment of genotype-phenotype correlations poses a major challenge.

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Variants in *CEP290*

Mutations

So far, 112 distinct mutations have been identified (, Figure 1, Supp. Table S1, NM_025114.3). The vast majority of *CEP290* mutations are truncating, with 40 nonsense and 48 frameshift mutations reported so far. All frameshifts are caused by small deletions or insertions, with the exception of two indels (c.381_382delinsT and c.5865_5867delinsGG). Three deletions and one duplication directly lead to a premature termination codon (PTC) without the incorporation of

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novel AAs (c.1550del, c.2906dup, c.3175del and c.5046del). Taking into account the position of the PTC relative to the ultimate 3′ exon–exon junction, 85 truncating mutations are assumed to undergo nonsense mediated decay (NMD) (Nagy and Maquat, 1998). However, the p.Arg151X mutation was shown to result in alternative transcripts, lacking exon 7 or both exon 7 and 8, leaving the open reading frame intact (Littink et al., 2010). Three truncating mutations located in the last exon are expected to escape NMD and render a protein product (c.7318_7321dup, c.7341dup and c.7366_7369del).

In addition to the 88 truncating mutations caused by small variations, a large heterozygous deletion was recently identified in a patient with cerebello-oculo-renal syndrome (CORS). The deleted region spanned 76,844 bp at the genomic level, encompassing the last 13 coding exons of *CEP290*, the entire *C12orf29* gene and part of the *C12orf50* gene (CEP290:c.5709+2352_54_C12orf50:c.290–1375_77del) (Travaglini et al., 2009).

The remaining 23 mutations comprise 3 missense mutations and 20 mutations predicted to influence splicing. Of the latter, 12 mutations affect consensus donor or acceptor splice sites, whereas three mutations are located within 20 nucleotides surrounding the exon. The first two, c.103-13_103-18del and c.6271-8T>G, were considered to be likely pathogenic because they decreased the scores of the normal splice sites and because they are absent in more than 115 control individuals (Tory et al., 2007). The third one, c.1711+5A>G, was presumed to result in both abnormally and normally spliced transcripts (Perrault et al., 2007). Splice site prediction scores, however, remain unchanged for this variant (Alamut v.1.5, data not shown). In addition, an aberrant splicing pattern was predicted for the c.1824G>A mutation affecting the last nucleotide of exon 18 (Coppieters et al., 2010). Surprisingly, the most recurrent mutation, c.2991+1655A>G, represents a deep intronic mutation. It creates a strong splice donor site that

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results in the insertion of a 128-bp cryptic exon between exons 26 and 27, thereby leading to a PTC immediately downstream of exon 26 (p.Cys998X) (den Hollander et al., 2006). Apart from these 17 substitutions, two deletions and one indel overspan an intron-exon boundary (c.2218-4_2222del, c.2218-15_2220del and c.3310-1_3310delinsAA).

So far, only three missense variants have been described with a probable pathogenic effect (see "Polymorphisms and unclassified variants" for other missense variants). Two affect the start codon (c.1A>G and c.2T>A) whereas the third, p.Trp7Cys, affects a highly conserved AA and is predicted to disrupt protein function according to SIFT, PolyPhen and Grantham matrix. However, the mutant protein was correctly localized at centrosomes in mouse inner medullary collecting duct (IMCD-3) cells, suggesting a pathogenic mechanism different from mislocalization (Valente et al., 2006).

Interestingly, some mutations cluster in the same region or even codon, suggesting the existence of mutation hotspots. In nine coding regions, two or more mutations arose either with a different effect on protein level (start codon; c.381-387; c.1859-1862; c.3175-3176; c.4965-4966; c.5515-5537; c.5865-5867; c.6869-6870) or with an identical predicted protein effect (c.4114-4116; c.4962-4966). In addition, one donor and three acceptor splice sites displayed more than one mutation (c.180+1G>T and c.180+2T>A; c.2218-2A>C, c.2218-4_2222del and c.2218-15_2220del; c.3104-1G>A and c.3104-2A>G; c.3310-1_3310delinsAA and c.3310-1G>C). In general, mutations are scattered throughout the protein, with some clustering in CCs III, XI and XII (Figure 1). Coiled-coils might be involved in the overall conformation of CEP290. Therefore, mutations in them might affect the accessibility of interacting proteins (Schafer et al., 2008).

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A total of 83 mutations are unique whereas 26 mutations have been reported in less than 10 families (Supp. Table S1). A few other mutations occurred multiple times and might even represent founder mutations. The most recurrent one is c.2991+1655A>G. In northwestern Europe, this mutation occurs in up to 26% of all LCA cases (den Hollander et al., 2006; Perrault et al., 2007; Coppieters et al., 2010). In Southern Europe, Korea, Southern India and Saudi-Arabia, however, c.2991+1655A>G has a significantly lower prevalence (Simonelli et al., 2007; Vallespin et al., 2007; Seong et al., 2008; Li et al., 2009; Sundaresan et al., 2009). The second-most frequent mutation, p.Lys1575X, was so far only reported in probands originating from France (Lille) and northern Belgium (Brancati et al., 2007; Perrault et al., 2007; Coppieters et al., 2010). Haplotype analysis in seven non-consanguineous families revealed a common intragenic allele but distinct extragenic haplotypes, suggesting an ancient mutation (Perrault et al., 2007). For p.Ala1832ProfsX19, allele sharing analysis in two families with MKS originating from Kosovo and Kosovo-Albania was in favor of a common founder haplotype encompassing approximately 3 Mb (Frank et al., 2008). In contrast, different haplotypes in two MKS families of Tunisian and French origin point to a mutation hotspot for p.Asp128GlufsX34 (Baala et al., 2007a). All mutations segregated in the parents (as far as this could be assessed), with the exception of p.Gln2111X, which was reported to arise *de novo* in a patient with CORS (Sayer et al., 2006).

In 18 patients, only one mutation was identified. This might be due to an inability of standard PCR-based techniques to identify mutations such as deep intronic variants, large genomic rearrangements or regulatory mutations located in promoter or enhancer/silencer elements. On the other hand, the heterozygous *CEP290* mutation might represent a modifying allele, potentially influencing the clinical expression of two AR mutations in another ciliary gene.

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Similar arguments could also apply for five families with isolated NPHP in which no mutations could be identified at all, despite linkage to a region containing *CEP290* (Helou et al., 2007). Alternatively, another as yet unidentified NPHP gene could segregate in linkage disequilibrium with *CEP290* in these families.

Polymorphisms and unclassified variants

Several polymorphisms have been described in *CEP290*, for which we refer to dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) and Brancati and colleagues (Brancati et al., 2007). They were predicted not to alter splicing patterns or to impair protein function, and/or were present in over 2% of parents of affected individuals (Brancati et al., 2007). In addition, the pathogenic potential of some variants is indefinite (Supp. Table S2). Indeed, for several missense variants, *in silico* predictions are not conclusive (PolyPhen, SIFT and Grantham matrix). The first one is p.Asp664Gly. Despite the identification of this variant in a heterozygous state in a patient with JS and renal involvement, p.Asp664Gly also occurred in a healthy parent of another family, but not in his affected child (Brancati et al., 2007; Helou et al., 2007). Interestingly, Tory and colleagues screened this variant together with three other *CEP290* missense variants (p.Glu277Gln, p.Lys838Glu and p.Arg1746Gln) in the context of modifier identification in the following three cohorts: 1) 13 patients carrying *NPHP1* mutation(s) with neurological symptoms; 2) 77-82 patients carrying *NPHP1* mutation(s) without neurological symptoms and 3) 132-154 healthy control subjects. No significant difference in frequencies was found among these three cohorts, however (Tory et al., 2007). Other missense variants of unknown significance are p.Leu906Trp, p.Asn2228Lys, p.Ala1566Pro and p.Leu1694Pro (Brancati et al., 2007; Coppieters et al., 2010). The latter two were found in two LCA patients, in compound heterozygosity with a nonsense and splice site mutation, respectively, and segregated in healthy parents (Coppieters et

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al., 2010). Two additional unclassified variants include in-frame deletions. A p.Glu1554del variant segregated with p.Phe1950LeufsX15 in an LCA patient (Perrault et al., 2007), while a heterozygous p.Lys2437del variant was identified in a patient with isolated NPHP originating from consanguineous parents (Helou et al., 2007) (Supp. Table S2).

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Mutation database

To provide a clear overview of all mutations and variants identified so far in *CEP290*, we developed CEP290*base* (medgen.ugent.be/cep290base). This locus-specific mutation database uses a novel scheme in which both genomic and phenotypic data are available, providing the possibility to link patients and their phenotypes to detailed variant information, and vice versa. Information on variants can be retrieved using the Overview page or the Mutation Browser.

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The Overview page displays all unique variants following HGVS guidelines (http://www.hgvs.org/mutnomen) (NM_025114.3), with their occurrence and links to dbSNP, UniProt (http://www.uniprot.org) and OMIM (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim). In addition, a scaled graphical representation of variants in the CEP290 protein is shown. Variants in the Overview or image are linked to their variant-specific page which, if available, includes the protein domain (Sayer et al., 2006), as well as the frequency in control individuals, the RNA effect and the estimate of pathogenic probability (Stone, 2003). For truncating mutations, it is indicated whether mRNA might be subjected to NMD (Nagy and Maquat, 1998), and for missense mutations, the Grantham score is provided (Grantham, 1974). The database also links to SIFT (http://sift.jcvi.org) and PolyPhen (http://genetics.bwh.harvard.edu/pph) prediction servers, using the CEP290 GI number (109255233) and UniProt ID (O15078) for automated input, respectively. Links to NetGene2

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(http://www.cbs.dtu.dk/services/NetGene2) and the Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html) are available for splice site mutations. A list of patients reported to carry the selected variant completes the variant-specific page. For each patient, information regarding disease, gender, age, origin, segregation and parental consanguinity is provided. Importantly, reported mutations are displayed using the nomenclature of the original publication, allowing easy retrieval of the variant from literature. If available, phenotypic information on ocular, renal, neurological and other signs appears by selecting the patient's ID. Disease calling was performed following the classification of Valente and coworkers, and is based on the involvement of different organ systems (Valente et al., 2008). Of note, these phenotypes might be incomplete due to factors such as a clinical investigation in an early disease stage.

The Mutation Browser enables custom querying. Both a quick and a more advanced search are possible. The quick search uses a selection of variant nomenclatures or patient IDs to list all patients carrying the selected variants, or all variants present in the selected patients. In the advanced search, different parameters can be set within and between three sections: variant, patient and source information. As in the quick search, the user has the choice between two output options: a mutation or patient list. Finally, users can submit novel or known variants they have identified.

In addition to the availability of phenotypic data in CEP290*base*, it also includes variants in other genes that co-occur with *CEP290* variants, thereby providing a unique opportunity to link modifiers to associated clinical manifestations (see "Epistatic effect of other components of the ciliary proteome"). The database is running on MySQL. PHP and JavaScript were used to develop the web-based interface.

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Biological relevance

Expression

The original *KIAA0373* mRNA was found in kidney and ovary, and to a lesser extent in human thymus, prostate and testis tissue (Nagase et al., 1997). In addition, it was present in centrosomes from a human lymphoblastoid cell line (KE-37) (Andersen et al., 2003). The independently cloned *3H11Ag* mRNA was extensively distributed in embryonic tissue and in different human cancerous tissues, but not in corresponding normal human tissues (Chen and Shou, 2001). The 150 C-terminal AAs of 3H11Ag appeared to be responsible for nuclear translocation. In addition, cytoplasmic presence was also established (Guo et al., 2004). Consistent with the localization of the KIAA0373 protein, CEP290 was found at centrosomes of both ciliated and non-ciliated cells (Chang et al., 2006; Sayer et al., 2006; Valente et al., 2006). This centrosomal localization of CEP290 is dynamic throughout the cell cycle, with redistribution to the cytosol starting in prometaphase (Sayer et al., 2006). During the interphase, two or four prominent spots were observed in the G1 and G2 phase. In the G0 phase, CEP290 was found on both the daughter and mother centriole, the latter from which the primary cilium is assembled (Tsang et al., 2008). Although the centrosomal localization is microtubule- and dynein-independent (Chang et al., 2006; Sayer et al., 2006), a major fraction of CEP290 is recruited to centriolar satellites along polymerized microtubules (Kim et al., 2008).

In the rod-dominated mouse retina, CEP290 was detected in the connecting cilia, and to a lesser extent in the inner segments (Chang et al., 2006; Sayer et al., 2006). Expression was also established in primate cone photoreceptors (Cideciyan et al., 2007). In olfactory sensory neurons, CEP290 localizes to dendritic knobs (McEwen et al., 2007). A recent study on differential gene expression in the ventricular myocardium of newborn piglets identified CEP290 as being three-

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fold more enriched in the right compared with the left ventricle (Torrado et al., 2010). Knock-down experiments in zebrafish using morpholinos caused defects reminiscent of JS, comprising retinal, cerebellar and otic cavity developmental abnormalities as well as pronephric cyst formation, ectopic brain tissue in the fourth ventricle and an abnormal mid-to-hindbrain region associated with hydrocephalus (Sayer et al., 2006; Schafer et al., 2008). Alternatively spliced transcripts/isoforms were identified by us in lymphocytes (data not shown) and observed as bands of low molecular mass on immunoblot analysis in bovine retinal extracts (Chang et al., 2006).

Interaction with other (ciliary) proteins and function

Using a yeast two-hybrid screen and co-immunoprecipitation, Sayer and colleagues identified an interaction between the N-terminal third of CEP290 (exons 2-21) and the C-terminal two thirds of the Activation Transcription Factor 4 (ATF4) protein. Moreover, CEP290 was able to activate ATF4-mediated transcription (Sayer et al., 2006). ATF4 has a protective function by regulating the adaptation of cells to metabolic and oxidative stress, and is required for skeletal and lens development, and haematopoiesis (Ameri and Harris, 2008). In addition, association with $G_{\gamma 13}$ and G_{olf} was identified in mouse olfactory epithelial tissue, suggesting a role of CEP290 in G protein trafficking during olfactory perception (McEwen et al., 2007).

Moreover, CEP290 interacts with several centrosomal and ciliary proteins. Co-immunoprecipitation experiments using mouse or bovine retinal extracts showed that CEP290 is in complex with dynactin subunits $p150^{Glued}$ and p50-dynamitin, kinesin subunit KIF3A, kinesin-associated protein (KAP3), the pericentriolar components γ-tubulin and pericentrin, the centriolar marker centrin, PCM1, ninein, SMC1, SMC3, retinitis pigmentosa GTPase regulator ($RPGR^{ORF15}$) and RPGR-interacting protein 1 (RPGRIP1), but not with nucleophosmin, NPHP5

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or RP1 (Chang et al., 2006). In mouse olfactory epithelial tissue, association with p150^{Glued}, KIF3A, RPGR^{ORF15} and γ-tubulin, but not BBS4 and IFT88, was also observed (McEwen et al., 2007). Of note, a subsequent study showed that pericentrin, γ-tubulin and centrin do not exactly co-localize with CEP290 (Kim et al., 2008).

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Recently, a potential role for CEP290 in primary cilium assembly was established. Knock-down of CEP290 using siRNAs in human retinal pigment epithelial cells caused a dramatic alteration in the ability of cycling cells to assemble primary cilia on the one hand, and a disrupted migration of mother centrioles to the cell cortex and primary cilia loss in quiescent cells on the other hand (Kim et al., 2008; Tsang et al., 2008). This observation might – at least partially - be attributed to the association of CEP290 with two proteins. First, CEP290 was found to be recruited to centriolar satellites by PCM-1, which is required for the organization of the cytoplasmic microtubule network. Both depletion and overexpression of CEP290 results in redistribution of PCM-1, thereby possibly influencing the transport function of PCM-1 granules between the cytosol and centrosome (Kim et al., 2008). In addition, CEP290 recruits Rab8a, a small GTPase required for ciliary membrane elongation, at centrosomes and cilia (Kim et al., 2008; Tsang et al., 2008). The interaction with Rab8a requires the CEP290 AAs 1208-1695 (Tsang et al., 2008). In growing cells (not yet capable of ciliogenesis), the latter function of CEP290 is probably inhibited through an interaction with CP110, which prevents CEP290-dependent Rab8a ciliogenesis (Tsang et al., 2008). Truncating CEP290 mutants defined the following amino-terminal regions necessary for and sufficient to bind CP110: AAs 1-366, AAs 221-366 and AAs 362-822. The binding region of CP110 consists of AAs 1-223, with major involvement of AAs 67-82 (Tsang et al., 2008).

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Interestingly, several other CEP290-interacting proteins are also associated with ciliopathies (Table 1). A first one is RPGR. Although a yeast two-hybrid screen could not identify a direct interaction with CEP290, co-immunoprecipitation assays suggested complex formation and both proteins co-localized in IMCD-3 cells and dissociated mouse rods (Chang et al., 2006). Mutations in *RPGR* account for approximately 70-90% of X-linked retinitis pigmentosa (RP) and mainly occur in isoforms containing the carboxy-terminal exon open reading frame 15 ($RPGR^{ORF15}$), which are highly expressed in connecting cilia of photoreceptors (Hong et al., 2003; Shu et al., 2007). In the *rd16* mouse (see "Animal models"), mutant CEP290 binds $RPGR^{ORF15}$ more avidly, leading to aggregation of $RPGR^{ORF15}$ in the inner segments and redistribution of rhodopsin and arrestin throughout the plasma membrane (Chang et al., 2006).

A second example of a CEP290-interacting protein also associated with ciliary disease is NPHP5, which is associated with SLS. The protein binds calmodulin and is in complex with $RPGR^{ORF15}$ (Otto et al., 2005). Despite the absence of a complex between CEP290 and NPHP5 in retinal extracts (Chang et al., 2006), NPHP5 was shown to specifically bind a region of CEP290 encompassing CCIII and part of the SMC homology domain (AAs 696 to 896) (Schafer et al., 2008). Combined knock-down of both proteins in zebrafish embryos synergistically augmented phenotypes seen in embryos treated with morpholinos for either *NPHP5* or *CEP290*. In *Xenopus laevis*, expression of the NPHP5-binding domain of CEP290 caused substantial neural tube closure defects that were similar to the effect of *NPHP5* knock-down. Moreover, co-expression of the NPHP5-binding domain of CEP290 with NPHP5 itself rescued the phenotype, supporting a physical interaction between both proteins *in vivo* (Schafer et al., 2008).

Both TMEM67 (MKS3) and CC2D2A are also CEP290-interacting proteins involved in disease. Mutations in both genes may cause MKS, JS and COACH syndrome, which are all severe

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ciliopathies with multi-systemic involvement (Smith et al., 2006; Baala et al., 2007b; Gorden et al., 2008; Tallila et al., 2008; Brancati et al., 2009; Doherty et al., 2009). TMEM67 is involved in both ciliary structure/function and endoplasmic reticulum-associated degradation of surfactant protein C (Tammachote et al., 2009; Wang et al., 2009). Morpholino experiments in zebrafish revealed a strong genetic interaction between *cep290* and *Tmem67*, with more severe phenotypic effects following knock-down of both genes, compared with depletion of only one of both (Leitch et al., 2008). *CC2D2A* is presumed to act as a sensor for intracellular calcium and is part of the basal body complex from which the cilium is assembled, where it co-localizes with CEP290 (Gorden et al., 2008; Tallila et al., 2008). The interaction between both proteins involves the 998 N-terminal AAs of CC2D2A and a fragment of CEP290 containing CCs IV-VI (AAs 703-1130). A functional interaction was observed in the pronephros of *sentinel* zebrafish (Gorden et al., 2008).

The involvement of CEP290 in the above-mentioned ciliary complexes strongly supports a role in ciliogenesis and ciliary trafficking. The (tissue-specific) access of its binding partners is possibly regulated by conformational changes of CEP290, since both the N-terminal (AAs 1-695) and C-terminal (AAs 1966-2479) domains are involved in homo- and heterodimeric interactions (Schafer et al., 2008).

Animal models

So far, two naturally occurring animal models are known with mutations in *cep290*: a pedigree of Abyssinian cats and the *rd16* mouse. Both models display AR progressive retinal degeneration, albeit with a different age-of-onset, possibly related to a differential effect of both distinct *cep290* mutations. Interestingly, no associated cerebellar or renal abnormalities are present in both

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models (Chang et al., 2006; Menotti-Raymond et al., 2007). The cat and mouse orthologues show 92.1% and 87% AA sequence homology to human CEP290, respectively (Menotti-Raymond et al., 2007) (www.ensembl.org).

The genetic defect for the Abyssinian cat retinal degeneration (rdAc) consists of a nucleotide substitution in intron 50 that creates a strong canonical splice donor site (IVS50+9T>G), resulting in a prolongation of exon 50 with 4bp, and a frameshift with a PTC three AAs downstream. The translated protein is truncated by 159 AAs, corresponding to the loss of the KIDV and KIDVI domains (Menotti-Raymond et al., 2007). The age of onset of the first ophthalmoscopic signs is highly variable, with 12-18 months of age in the majority of animals. Full-field flash electroretinography (ERG) at the age of eight months shows a reduction of mainly a-wave amplitudes, whereas reduced sensitivity of the pupillary light reflex is observed with disease progression. (Thompson et al., 2010; Narfstrom et al., 2009). Vacuolization and degeneration of membranes in the basal part of the rod outer segments (OS) in early stages of disease point to potential defects in protein transport through the connecting cilia (Menotti-Raymond et al., 2007).

In contrast to rdAC, the *rd16* mouse displays an early-onset retinal dystrophy, caused by a homozygous in-frame deletion of 897 bp (AAs 1599-1897) that covers the majority of the myosin-tail homology region. Early fundus examinations revealed white retinal vessels and large pigment patches, in addition to severely reduced ERG responses for both rods and cones. The mutation causes early progressive degeneration of the OS and reduction in thickness of the outer nuclear layer (Chang et al., 2006). In addition, considerable thickening of the inner nuclear and plexiform layer were observed in mid- and central retinal regions, together with an enlargement of nuclei of all retinal cell types (Cideciyan et al., 2007). Interestingly, *rd16* mice displayed severe early-onset olfactory dysfunction. Although the olfactory sensory neurons have

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structurally intact cilia with correct localization of CEP290 to dendritic knobs, two components of the olfactory G protein that are in complex with CEP290, $G_{\gamma 13}$ and G_{olf}, were undetectable in the cilia of *rd16* mice, suggesting a potential role for CEP290 in the regulation of olfactory G protein trafficking (McEwen et al., 2007).

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Both models offer interesting opportunities for *in vivo* research on CEP290 function and therapeutic intervention. The progressive nature of retinal disease in *rd16* mice resembles human LCA, and therefore constitutes an excellent basis to study disease development. Moreover, the existence of a large animal model such as rdAC is an asset for the implementation of gene-specific therapy for retinal degeneration, as already shown by successful *RPE65*-replacement therapy in Briard dogs which preceded human phase I clinical trials (Acland et al., 2001; Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008; Maguire et al., 2009).

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Clinical and diagnostic relevance

CEP290-linked phenotypes

Leber Congenital Amaurosis (LCA)

Leber Congenital Amaurosis (LCA, MIM[204000]) is the earliest and most severe form of all inherited retinal dystrophies, causing profound visual deficiency, nystagmus and an undetectable or severely reduced ERG in the first year of life. Approximately 20% of all blind children are thought to suffer from this disease, which is mainly inherited in an AR manner. So far, 15 disease loci (14 genes) are known, which together account for ~70% of cases (den Hollander et al., 2008). In 2006, *CEP290* was identified as a disease gene for LCA, with the deep intronic substitution c.2991+1655A>G as causal mutation. Targeted screening of this mutation in 76

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unrelated LCA patients revealed its presence in 16 additional probands (21%), suggesting a major involvement of *CEP290* in LCA (den Hollander et al., 2006). Subsequent studies corroborate that *CEP290* mutations are highly recurrent in Northwestern Europe (Perrault et al., 2007; Coppieters et al., 2010), but contribute to only a minor part of LCA in Italy, Saudi Arabia, Spain, Southern India and Korea (targeted screening of c.2991+1655A>G in the latter three populations) (Simonelli et al., 2007; Vallespin et al., 2007; Seong et al., 2008; Li et al., 2009; Sundaresan et al., 2009). Interestingly, c.2991+1655A>G was not identified in 126 Spanish patients with early-onset RP, suggesting specificity of this mutation for LCA (Vallespin et al., 2007).

The *CEP290*-related phenotype consists of a severe cone-rod type retinal dystrophy (Perrault et al., 2007), with best-corrected visual acuity of counting fingers or worse in the majority of cases (Walia et al., 2010). Early fundus changes include white dots or a marbleized or salt and pepper aspect, and progress to midperipheral nummular or spicular pigmentation (Littink et al., 2010; den Hollander et al., 2006; Perrault et al., 2007; Coppieters et al., 2010). A detailed study of the retinal architecture of human *CEP290*-mutant retinas identified profound retinal remodeling in the peripheral rod-rich regions, which was characterized by thickening of inner retinal layers. In the cone-rich foveal region, however, no clear alterations were observed. This difference in rod and cone degeneration may point to a distinct function of CEP290 in both cell types (Cideciyan et al., 2007). Spectral-domain optical coherence tomography confirmed preservation of the outer nuclear layer in the central macula with distorted inner retina (Pasadhika et al., 2009). No abnormalities were seen in visual brain pathway anatomy (Cideciyan et al., 2007). Apart from the ocular phenotype, neurological involvement (mental retardation [MR] or autism) was observed in approximately 11-33% of cases with *CEP290*-related LCA, which is more frequent than in patients with mutations in other LCA genes (Hanein et al., 2004; Perrault et al., 2007; Coppieters

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et al., 2010). Four probands presented with either (transitory) hypotonia or ataxia (Perrault et al., 2007). Interestingly, some patients displayed signs suggestive for involvement of other ciliary processes. One patient presented with recurrent otitis media (Coppieters et al., 2010), and LCA patients homozygous for c.2991+1655A>G exhibited severe olfactory dysfunction, whereas mild to severe microsmia was observed in heterozygous carriers (McEwen et al., 2007). Obviously, MR, autism, ataxia and hypotonia may be signs of the mild end of the clinical spectrum of systemic phenotypes related to *CEP290* rather than of true LCA as isolated retinal disease.

Nephronophthisis (NPHP) and Senior-Loken syndrome (SLS)

Nephronophthisis (NPHP, MIM[256100]) is the most frequent genetic cause for end-stage renal disease (ESRD) in the first three decades of life. Infantile, juvenile and adolescent forms have been described. Key histology findings are tubulointerstitial fibrosis, tubular atrophy, tubular dilatation and cyst formation. Renal ultrasound (US) reveals increased cortical echogenicity, loss of corticomedullary differentiation and corticomedullary cysts (Salomon et al., 2009; Simms et al., 2009). In 10-15% of cases, juvenile NPHP is accompanied by retinal involvement, which is called Senior-Loken syndrome (SLS, MIM[266900]). Based on the degree of retinal degeneration, both early-onset and late-onset types have been described. NPHP and SLS are AR disorders. So far, nine genes are known to cause NPHP; some of them are associated with SLS (Hildebrandt and Zhou, 2007; Hildebrandt et al., 2009). *CEP290* mutations have been described in eight families with SLS, two of which present with intrafamilial clinical variability of neurological and/or renal involvement (Tory et al., 2007). In six families, two mutations segregated, while in two other families, one heterozygous *CEP290* mutation occurred in combination with a homozygous mutation in *NPHP1* and a heterozygous mutation in *NPHP4*, respectively (Helou et al., 2007; Tory et al., 2007). The age at which ESRD occurred ranged from

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5 to 40 years and retinal degeneration was usually severe (Sayer et al., 2006; Helou et al., 2007; Tory et al., 2007; Coppieters et al., 2010). It is presumed that *CEP290* mutations do not represent a major cause of NPHP/SLS, since large-scale mutation screening in almost 100 families with SLS (Helou et al., 2007; O'Toole et al., 2007) and 21 families with isolated NPHP revealed mutations in only two patients with SLS and a heterozygous unclassified variant in one patient with NPHP (Helou et al., 2007).

Joubert syndrome (JS) and Joubert syndrome related disorders (JSRD)

Joubert syndrome (JS, MIM[213300]) is a genetically heterogeneous disorder of which the main phenotypic hallmarks are hypotonia, ataxia, psychomotor delay and variable occurrence of oculomotor apraxia and neonatal breathing abnormalities. The most consistent feature, the "molar tooth sign" (MTS), is visible on magnetic resonance imaging (MRI) and consists of a deep interpeduncular fossa with narrow isthmus, thickened, elongated and mal-oriented superior cerebellar peduncles and cerebellar vermis aplasia/hypoplasia (Doherty, 2009). In addition to pure JS, a range of disorders exists that share the MTS and the main neurological signs of JS, yet are distinct due to the involvement of other organs. They are called Joubert syndrome-related disorders (JSRD) and consist of five major subgroups: JS associated with retinopathy, JS associated with renal involvement, cerebello-oculo-renal syndrome (CORS or JS-SLS), COACH syndrome (MIM[216360]) and orofaciodigital syndrome type VI (OFDVI, MIM[277170]) (Valente et al., 2008).

Currently, less than 50% of AR cases can be attributed to mutations in nine genes, which all encode ciliary proteins, as far as known (Valente et al., 2008; Bielas et al., 2009; Doherty, 2009; Valente et al., 2010). *CEP290* mutations are a major cause of JSRD, in particular the CORS

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subgroup. Approximately 50% of cases with CORS are associated with *CEP290* mutations. In contrast, only a small fraction (~20%) of *CEP290* mutations contributes to the other JSRD subgroups (Valente et al., 2008). So far, only two families with classic JS harbored *CEP290* mutations, one of which displayed intrafamilial variability of the renal phenotype (Valente et al., 2006; Brancati et al., 2007). In most cases, NPHP was of the juvenile type, with development of renal failure toward the end of the first decade or early in the second decade, whereas retinal degeneration mostly consisted of congenital blindness. Interestingly, several patients displayed associated features reminiscent of other subgroups or overlapping ciliopathies, including cleft palate, abnormal liver ultrasound, elevated liver enzymes, Hirschsprung disease, occipital (meningo)encephalocele, atrial/ventricular septal defects, cardiomegaly, postaxial polydactyly, *situs inversus*, lobulated tongue, retinal coloboma, empty sella, hepatic fibrosis, recurrent otitis media and hearing loss (Valente et al., 2006; Brancati et al., 2007; Helou et al., 2007; Tory et al., 2007; Coppieters et al., 2010).

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Meckel-Grüber syndrome (MKS)

Meckel-Grüber syndrome (MKS, MIM[249000]) is a neonatally lethal disorder characterized by a combination of central nervous system malformations (typically occipital [meningo]encephalocele), bilateral cystic kidney dysplasia, ductal proliferation in the portal area of the liver and postaxial polydactyly. Associated features may include cardiac abnormalities, developmental anomalies of external or internal genitalia, cleft palate, *situs inversus* and others. The worldwide incidence ranges from 1/13,250 to 1/140,000 live births (Alexiev et al., 2006). Some cases present with incomplete phenotypes that are called "Meckel-like syndrome" (MIM[208540]) and probably make up a clinical spectrum situated between JSRD and MKS. In addition, a phenotypic and genetic overlap was seen between MKS and BBS (see "Bardet-Biedl

syndrome (BBS)") (Karmous-Benailly et al., 2005; Leitch et al., 2008). MKS is inherited in an AR fashion with five genes (six loci) identified so far, all encoding centrosomal/ciliary proteins. Mutations in *CEP290* were identified in eight families with MKS and four families with Meckel-like syndrome, further supporting a role for *CEP290* in a wide range of phenotypes (Baala et al., 2007a; Frank et al., 2008).

Bardet-Biedl syndrome (BBS)

Bardet-Biedl syndrome (BBS, MIM[209900] is a complex multi-organ ciliopathy, characterized by a variable combination of retinal degeneration, obesity, hypogonadism, polydactyly, renal dysfunction and MR. Additional features consist of neurological impairment, speech deficits, craniofacial abnormalities, hearing loss, diabetes mellitus, metabolic defects, cardiovascular abnormalities, hepatic defects and Hirschsprung disease. In most cases, BBS is inherited as an AR trait, with currently 14 genes known. Notably, mutations in different genes have been described in the same patient. These modifiers affect either the expressivity or the overall penetrance of the phenotype (Zaghloul and Katsanis, 2009). In search of a genetic explanation for the partial clinical overlap between BBS and MKS, Leitch and coworkers identified *MKS1* and *CEP290* as disease genes for BBS. Interestingly, the homozygous p.Glu1903X *CEP290* mutation causing BBS was found together with a complex *TMEM67* allele, possibly influencing the phenotype (Leitch et al., 2008).

Genotype–phenotype correlations

For the majority of mutations, no clear-cut correlation could be established between the genotype and clinical expression. Despite 90 mutations reported exclusively in only one phenotype, 14 others segregated with two diseases while 8 were even associated with three or more phenotypes.

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In most cases, these phenotypes are partially overlapping, although few mutations were observed to lead to strongly divergent disorders, such as LCA and MKS. Overall, mutations causing JS(RD) tend to cluster in the second half of the gene, whereas mutations segregating with LCA, SLS and MKS are homogeneously distributed throughout the gene (Brancati et al., 2007; Valente et al., 2008) (Supp. Table S1).

For a few mutations, a genotype-phenotype correlation could be established, albeit to a limited extent. The most common mutation in JSRD, p.Gly1890X, was also described in two siblings with isolated LCA, in compound heterozygosity with c.2991+1655A>G (Cideciyan et al., 2007). Despite the severe retinal phenotype of these siblings and four other patients heterozygous for Gly1890X, a milder or even absent visual impairment was seen in eight of nine patients homozygous for p.Gly1890X, suggesting that this mutation might be less harmful for retinal function (Sayer et al., 2006; Valente et al., 2006; Brancati et al., 2007; Valente et al., 2008). Similarly, a compound heterozygous genotype c.2991+1655G>A/c.451C>T caused an early-onset retinal dystrophy phenotype less severe than most *CEP290*-related LCA, probably due to the mild effect of both mutations (Littink et al., 2010). So far, c.2991+1655A>G has only been described in patients with isolated LCA. Since a small amount of wild-type product is still present, a dosage-dependent mechanism was proposed, in which complete loss of function of both alleles would lead to JS, whereas a residual CEP290 activity would be sufficient for normal cerebellar and renal function but not for correct retinal activity (den Hollander et al., 2006). However, subsequent identification of truncating mutations on both alleles in several LCA patients countered the latter hypothesis (Perrault et al., 2007). In addition, we identified c.2991+1655A>G in two patients with features suggestive of renal dysfunction, implicating that this mutation is possibly not exclusively associated with isolated LCA (Coppieters et al., 2010).

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A similar hypothesis was proposed for certain other *CEP290* splice site mutations. It was presumed that these do not lead to truncated proteins, but rather to (partial) deletion of one of the CCs. In contrast to truncating mutations, these do not seem to hamper normal neurologic development. Examples are two first-degree cousins, each carrying p.Leu1884ThrfsX23 on one allele, but a different *CEP290* mutation on the other. One of them harbored a nonsense mutation and presented with JS without renal involvement by the age of eight, whereas the other was heterozygous for c.4195-1G>A and suffered from SLS without neurologic symptoms (Tory et al., 2007). In addition, a homozygous splice site mutation segregated with SLS in a Turkish family (Sayer et al., 2006). However, in two other unrelated patients with neurological involvement, two distinct splice site mutations were detected. Of note, in both cases, one of both mutations does not affect a consensus splice site (c.1711+5A>G and c.6271-8T>G) (Perrault et al., 2007; Tory et al., 2007).

For all *CEP290*-related phenotypes, different degrees of neurological, ocular and renal involvement were observed between unrelated patients harboring the same *CEP290* genotype. A homozygous p.Lys1575X mutation was detected in four patients with isolated LCA and normal development, one patient with LCA and autistic behavior but normal MRI, one patient with LCA and severe MR (of whom no MRI was available), and one patient with LCA-JS (proven MTS on MRI) (Perrault et al., 2007; Coppieters et al., 2010). Patients homozygous for p.Gly1890X always displayed characteristics typical of JS but associated features ranged from none over JS with NPHP to CORS (Sayer et al., 2006; Valente et al., 2006; Brancati et al., 2007). A homozygous p.Trp7Cys mutation was identified in two patients of Pakistani origin who suffered from retinal degeneration and NPHP. In only one patient, however, several features suggestive for JS were observed (Valente et al., 2006; Coppieters et al., 2010). In addition, compound

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heterozygous p.Leu1884ThrfsX23 and p.Phe1950LeufsX15 mutations were described in two French siblings with CORS (Tory et al., 2007), and two French siblings with Meckel-like syndrome, aged 18 and 29 weeks of gestation, respectively (Baala et al., 2007a). Moreover, intrafamilial variability was reported in several cases. Perrault and colleagues described two LCA families in which all affected sibs carried the same mutations, but displayed different neurological involvement (Perrault et al., 2007). In addition, kidney US in two brothers with JS (deceased at an age of four and seven months), both homozygous for p.Gln1942X, revealed cortical cysts in only one patient (Valente et al., 2006). Overall, this wide clinical spectrum is difficult to explain by the *CEP290* genotype alone.

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Epistatic effect of other components of the ciliary proteome

It is presumed that the clinical variability of *CEP290*-related disease might be caused by second-site modifier alleles. Since CEP290 forms a complex with several members of the ciliary proteome, variants in these genes are likely to affect the interaction with and function of CEP290. So far, three ciliary genes have been described in which variants co-occur with *CEP290* mutations. The first gene is *AHI1*, which is associated with JS(RD) and possibly acts in a pathway common with CEP290 (Ferland et al., 2004; Parisi et al., 2006; Kim et al., 2008; Hsiao et al., 2009). Tory and colleagues identified a heterozygous p.Arg830Trp missense variant in a CORS patient harboring a homozygous p.Leu1884ThrfsX23 *CEP290* mutation. This *AHI1* variant was not present in an affected sibling with the same *CEP290* genotype. Interestingly, no significant difference could be seen in neurological and ocular manifestation between both sibs, albeit that the one carrying p.Arg830Trp displayed renal failure earlier than the other (11 versus 25 years). In addition, a higher frequency of p.Arg830Trp was observed in patients with *NPHP1* mutations and neurological symptoms, in comparison with patients with *NPHP1* mutations

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lacking neurological involvement or with healthy controls, suggesting an influence on the neurological expression of *NPHP1*-related disease. Mutations in *NPHP1* cause NPHP or SLS, with JS-related neurological involvement in up to 12% of cases (Tory et al., 2007). Recently, a modifying effect of p.Arg830Trp has also been established on the development of retinal degeneration in patients suffering from NPHP, independent of primary mutations in *NPHP1*. *AHI1* genetically interacts with *NPHP1* in retinal development and was proven necessary for photoreceptor OS development (Louie et al., 2010). In addition to p.Arg830Trp, we identified a different heterozygous missense variant, p.Asn811Lys, in the most severely affected patient out of three with the same *CEP290* genotype and LCA but with different neurological involvement as well as variable age-of-onset of ESRD. Yet another LCA patient with MR carried a third *AHI1* missense variant, p.His758Pro, in combination with two mutations in *CEP290* (Coppieters et al., 2010). Of note, all potential modifier alleles identified in *AHI1* so far have been missense variants, located in the strongly conserved WD40 repeat, whereas most of the JS-causing mutations are truncating. Obviously, these variants in *AHI1* are not sufficient to explain all of the clinical variability, as no *AHI1* mutations were demonstrated in two large cohorts of patients with JS(RD) and *CEP290* mutations (Brancati et al., 2007; Helou et al., 2007). A second gene considered to harbor a potential modifier allele is *TMEM67* (*MKS3*). A homozygous p.Glu1903X *CEP290* mutation was accompanied by a complex p.[Gly218Ala; Ser320Cys] *TMEM67* allele in a patient with BBS. The *TMEM67* allele is likely to influence the *CEP290*-related phenotype, given the genetic interaction between *cep290* and *Tmem67* in zebrafish and the pathogenic potential of the p.Ser320Cys variant and to a lesser extent the p.Gly218Ala variant (Leitch et al., 2008). *NPHP4*, which is associated with both isolated NPHP and SLS (Otto et al., 2002), is the third gene, since a heterozygous p.Thr627Met mutation in *NPHP4* was identified in a patient with SLS who is also heterozygous for p.Arg1978X in *CEP290* (Hoefele et al., 2005; Helou et

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al., 2007). In addition to the heterozygous alleles identified in *AHI1*, *TMEM67* and *NPHP4*, both homozygosity at the *PKHD1* locus and a homozygous *CEP290* mutation were identified in a consanguineous family segregating MKS as well as AR polycystic kidney disease. As expected, a homozygous *CEP290* mutation combined with linkage to the *PKHD1* locus in one sibling with MKS caused a more severe kidney and liver phenotype, in comparison with the other siblings that carried either the same *CEP290* or *PKHD1* genotype (Baala et al., 2007a).

Conversely, mutations in *CEP290* might affect phenotypes caused by mutations in other ciliary genes. A heterozygous p.Asn96MetfsX29 mutation segregated in two siblings who also harbored a homozygous *NPHP1* deletion. In spite of a common genotype and similar retinal degeneration, these siblings differ in both renal and neurological phenotype, suggesting the involvement of additional factors (Tory et al., 2007). In addition, a heterozygous *CEP290* mutation/variant was identified in two probands that might carry a homozygous mutation in another ciliary gene, since they each originate from consanguineous parents (Helou et al., 2007).

Molecular diagnostic strategies

Given the large number of coding exons (53), sequencing of *CEP290* is laborious, expensive and requires a considerable amount of DNA. Therefore, first-step analysis might comprise screening for the most recurrent mutations, the choice of which depends on the phenotype of the patient. This approach has been applied successfully by several groups (den Hollander et al., 2006; Perrault et al., 2007; Coppieters et al., 2010). Targeted mutation screening can be performed using denaturing high-performance liquid chromatography (dHPLC) or sequencing. For a few mutations, specific detection techniques are available, such as an allele-specific PCR for c.2991+1655A>G (den Hollander et al., 2006). Moreover, 96 *CEP290* mutations are present on a

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commercially available microarray, which represents standard genetic screening for LCA by testing for 641 mutations in 13 genes (Asper Ophthalmics, v8.0). In the majority of cases however, screening of the complete coding region remains necessary, either to identify the second disease allele, or to screen for both mutations in case of negative first-pass screening. PCR primers and conditions for both sequencing (Sayer et al., 2006; Valente et al., 2006) and dHPLC (Brancati et al., 2007) have been published and an alternative pre-screening assay based on heteroduplex formation and subsequent CEL I endonuclease digest has been described (Helou et al., 2007). Brancati and colleagues first screened parental DNA using dHPLC, followed by sequencing of the identified mutations in the affected offspring and of the whole coding region in case of a heterozygous mutation (Brancati et al., 2007). Apart from detecting small variants, comprehensive *CEP290* screening should also include dosage analysis of all exons, using for instance quantitative real-time PCR (Travaglini et al., 2009).

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Future Prospects

Given the emerging importance of modifier alleles on the phenotypic expression of ciliopathies, future studies are required to understand their mechanism of action and to further elucidate the ciliary protein networks. The identification of modifiers for retinal, renal, neurological or other phenotypes might contribute to an improved predictive power of a *CEP290*-related genotype. Early management of patients with a higher risk of NPHP, for instance, may delay the progression toward renal failure and minimize secondary complications. In a diagnostic setting, mutations in ciliopathies should ideally be evaluated in the context of the entire spectrum of ciliary variants. Novel technologies such as next-generation sequencing will play a crucial role in this respect. Finally, more insights in the molecular pathogenesis of ciliopathies will eventually direct towards therapeutic options, such as gene therapy. Since all *CEP290*-related phenotypes

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are inherited in an AR manner and the majority of mutations consist of loss-of-function alleles, gene-replacement therapy might indeed be an option.

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Figure legends

Figure 1: Overview of all mutations and variants currently present in *CEP290*base.

The exact location of the mutation/variant is depicted with respect to the CEP290 protein (Sayer et al., 2006). If no protein effect is known, the c.DNA nomenclature is used. Mutations depicted twice have been reported to arise from different nucleotide changes.

Figure 2: Overlap of *CEP290* mutations between different diseases.

Abbreviations used: LCA: Leber Congenital Amaurosis; SLS: Senior-Loken syndrome; JS: Joubert syndrome; JS + RD: Joubert syndrome with associated retinopathy; JS + RF: Joubert syndrome with associated renal failure; CORS: cerebello-oculo-renal syndrome; MKS: Meckel-Grüber syndrome; MKS-like: Meckel-Grüber syndrome – like; BBS: Bardet-Biedl syndrome.

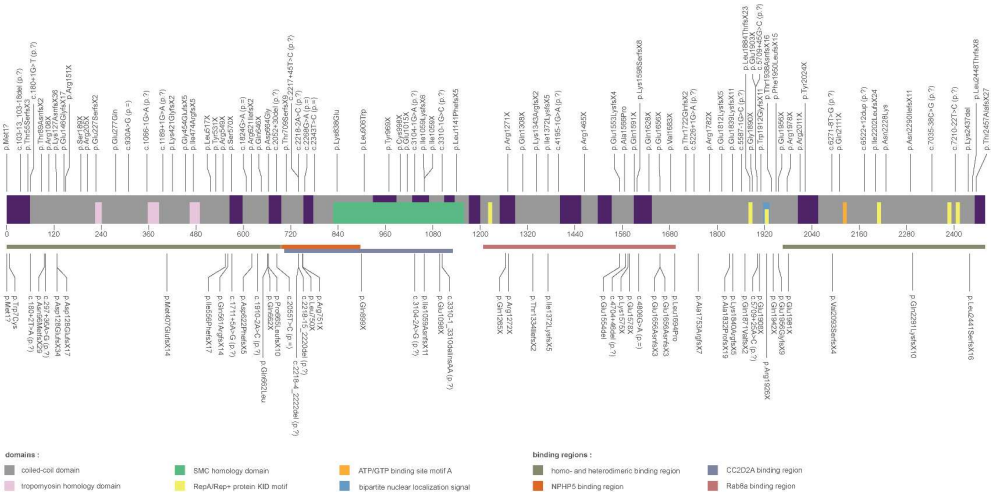
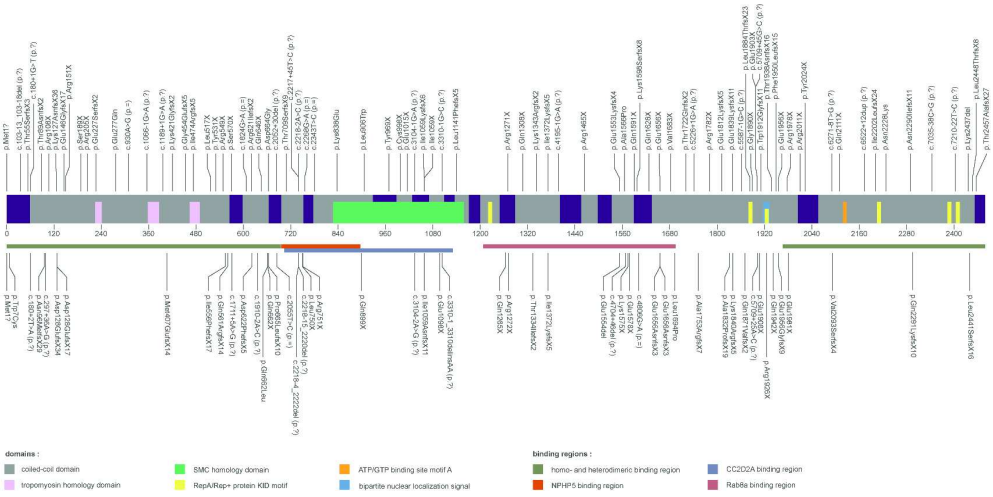


Figure 1: Overview of all mutations and variants currently present in CEP290*base*.
The exact location of the mutation/variant is depicted with respect to the CEP290 protein (Sayer et al., 2006). If no protein effect is known, the c.DNA nomenclature is used. Mutations depicted twice have been reported to arise from different nucleotide changes.
291x141mm (300 x 300 DPI)



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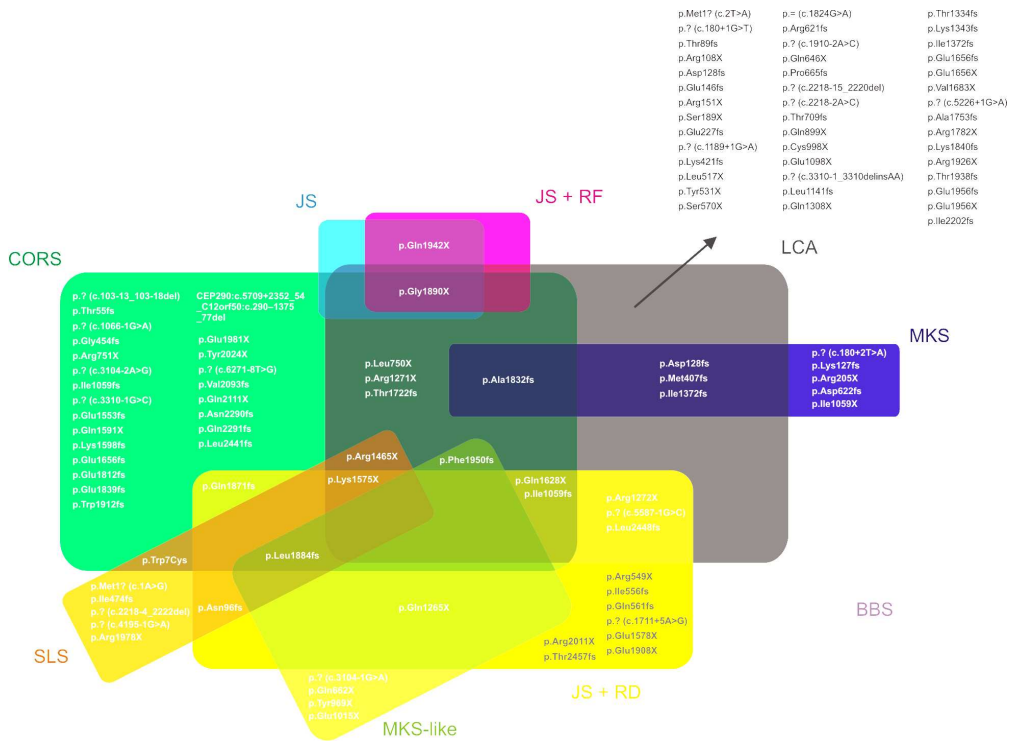

Figure 2: Overlap of CEP290 mutations between different diseases.
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165x121mm (300 x 300 DPI)

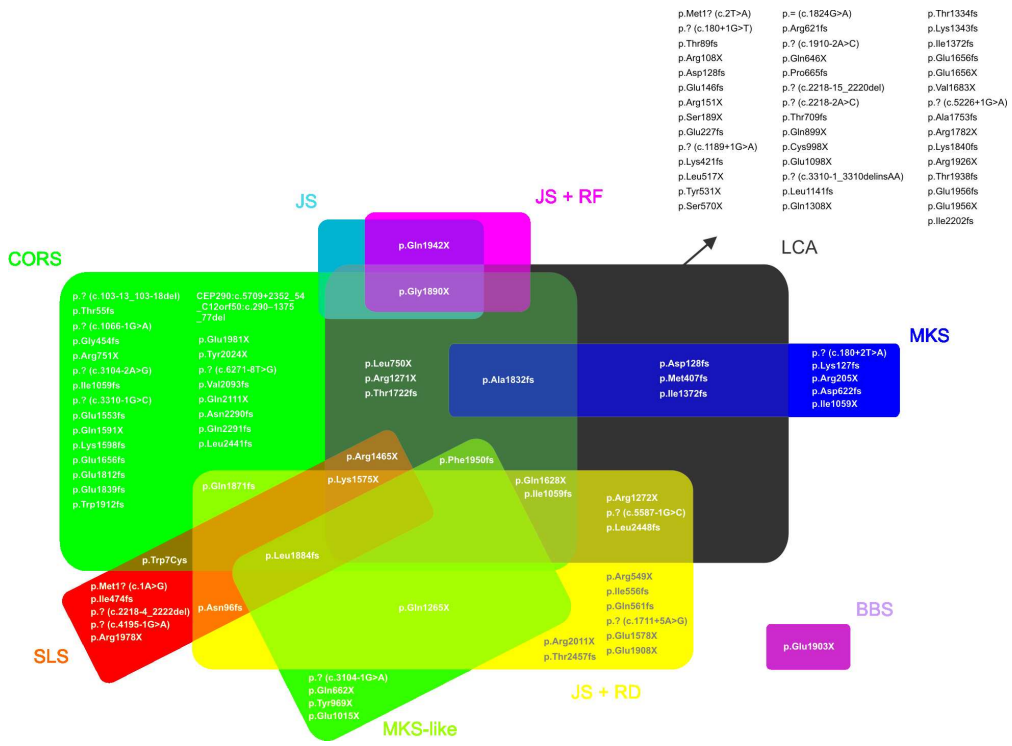

Figure 2: Overlap of CEP290 mutations between different diseases.
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Table 1: Overview of selected ciliary proteins interacting with CEP290, and their involvement in partially overlapping yet distinct ciliopathies.

Protein		Associated phenotype						
		RD	NPHP	SLS	JS(RD)	MKS	BBS	COACH
CEP290		(den Hollander et al., 2006)	(Helou et al., 2007)(?)	(Sayer et al., 2006)	(Sayer, et al., 2006; Valente et al., 2006)	(Baala et al., 2007a)	(Leitch et al., 2008)	
CEP290 interacating proteins	RPGR	(Meindl et al., 1996)						
	RPGRIP1	(Dryja et al., 2001)						
	NPHP5			(Otto et al., 2005)				
	TMEM67 (MKS3)				(Baala et al., 2007b)	(Smith et al., 2006)		(Brancati et al., 2009)
	CC2D2A				(Gorden et al., 2008)	(Tallila et al., 2008)		(Doherty et al., 2009)

Abbreviations used: RD: retinal degeneration; NPHP: nephronophthisis; SLS: Senior-Loken syndrome; JS(RD): Joubert syndrome (related disorders); MKS: Meckel-Grüber syndrome; BBS: Bardet-Biedl syndrome; COACH: cerebellar vermis hypo/aplasia, oligophrenia, congenital ataxia, ocular coloboma, and hepatic fibrosis; (?): uncertain association.

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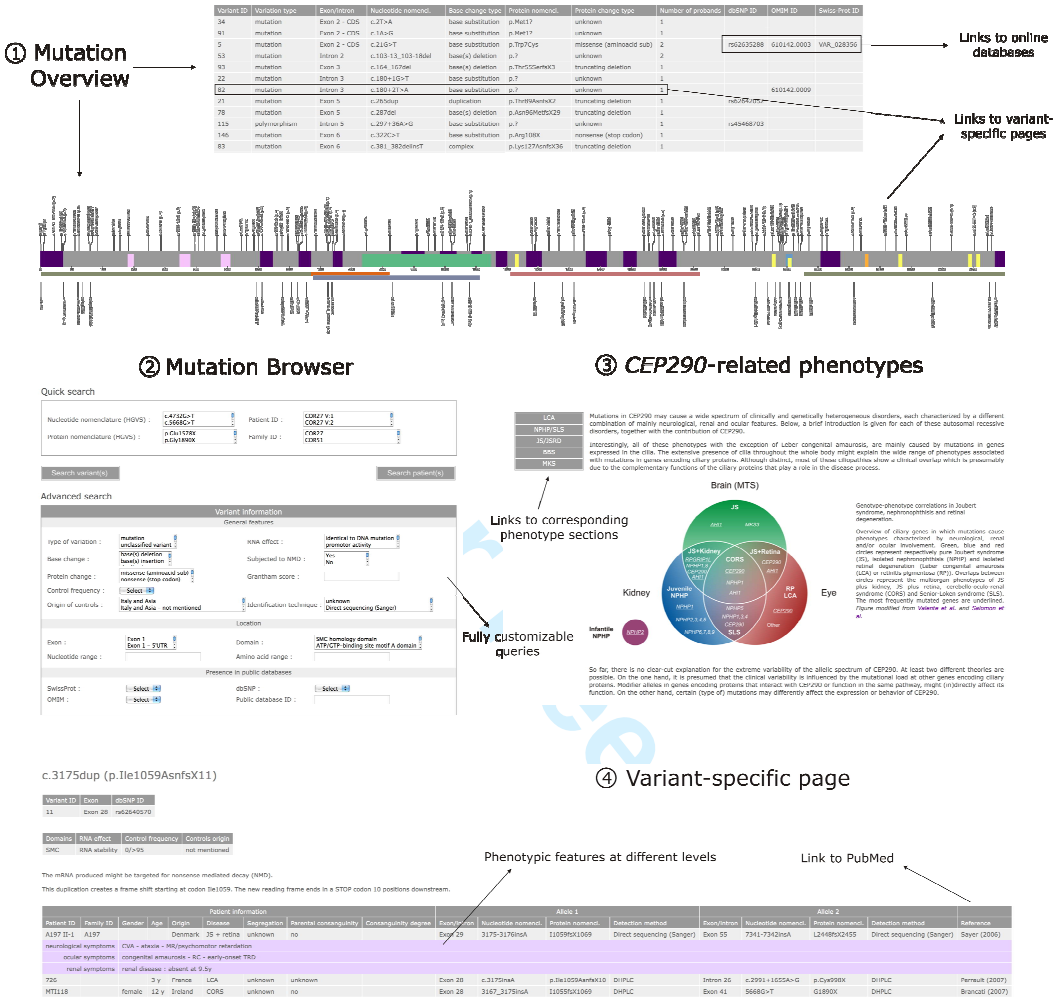
Supp. Figure S1: Overview of CEP290*base*



The database is accessible from: http://medgen.ugent.be/cep290base. Information on variants can be retrieved using the Mutation Overview (1) or the Mutation Browser (2). In addition, the Phenotype page (3) describes all phenotypes associated with *CEP290* so far. The variant-specific pages (4) are accessible from both the Mutation Overview and Mutation Browser and include detailed information on the pathogenic potential. The database links to NetGene2 and BDGP for splice site mutations and automatically fills in queries for PolyPhen and SIFT for missense changes. In addition, the database includes variants in other genes that co-occur with *CEP290* mutations, thereby providing a unique opportunity to link modifiers to associated clinical manifestations.

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_025114.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

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Supp. Table S1: Overview of all *CEP290* mutations identified so far and their occurrence in different phenotypes.

Mutation		Associated phenotype								
Nucleotide nomenclature	Protein nomenclature	LCA	SLS	JS	JS + RD	JS + RF	CORS	MKS	MKS-like	BBS
c.1A>G	p.Met1?		1							
c.2T>A	p.Met1?	1								
c.21G>T	p.Trp7Cys		1				1			
c.103-13_103-18del	p.?						1			
c.164_167del	p.Thr55SerfsX3						1			
c.180+1G>T	p.?	1								
c.180+2T>A	p.?							2 (1)		
c.265dup	p.Thr89AsnfsX2	1								
c.287del	p.Asn96MetfsX29		1*		1*					
c.322C>T	p.Arg108X	1								
c.381_382delinsT	p.Lys127AsnfsX36							1		
c.384_385del	p.Asp128GlufsX17	1								
c.384_387del	p.Asp128GlufsX34	1						4 (2)		
c.437del	p.Glu146GlyfsX17	1								
c.451C>T	p.Arg151X	1								
c.566C>G	p.Ser189X	1								
c.613C>T	p.Arg205X							2 (1)		
c.679_680del	p.Glu227SerfsX2	1								
c.1066-1G>A	p.?						1			
c.1189+1G>A	p.?	1								
c.1219_1220del	p.Met407GlufsX14	3						1		
c.1260_1264del	p.Lys421GlyfsX2	2								
c.1361del	p.Gly454GlufsX5						1			
c.1419_1423del	p.Ile474ArgfsX5		1							
c.1550del	p.Leu517X	1								
c.1593C>A	p.Tyr531X	1								
c.1645C>T	p.Arg549X				1					
c.1666del	p.Ile556PhefsX17				1					

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Page 3

Mutation		Associated phenotype								
Nucleotide nomenclature	Protein nomenclature	LCA	SLS	JS	JS + RD	JS + RF	CORS	MKS	MKS-like	BBS
c.1682_1683del	p.Gln561ArgfsX14				1					
c.1709C>G	p.Ser570X	1								
c.1711+5A>G	p.?				1					
c.1824G>A	p.=	1								
c.1859_1862del	p.Arg621IlefsX2	2								
c.1860_1861del	p.Asp622PhefsX5							1		
c.1910-2A>C	p.?	1								
c.1936C>T	p.Gln646X	2 (1)								
c.1984C>T	p.Gln662X								2 (1)	
c.1992del	p.Pro665LeufsX10	1								
c.2118_2122dup	p.Thr709SerfsX9	1								
c.2218-15_2220del	p.?	1								
c.2218-4_2222del	p.?		2 (1)							
c.2218-2A>C	p.?	1								
c.2249T>G	p.Leu750X	1					1			
c.2251C>T	p.Arg751X						1			
c.2695C>T	p.Gln899X	1								
c.2906dup	p.Tyr969X								1	
c.2991+1655A>G	p.Cys998X	92 (83)								
c.3043G>T	p.Glu1015X								1	
c.3104-2A>G	p.?						1			
c.3104-1G>A	p.?								1	
c.3175del	p.Ile1059X							1		
c.3175dup	p.Ile1059AsnfsX11	1			1		1			
c.3176del	p.Ile1059LysfsX6						1			
c.3292G>T	p.Glu1098X	2								
c.3310-1G>C	p.?						1			
c.3310-1_3310delinsAA	p.?	3								
c.3422dup	p.Leu1141PhefsX5	1								
c.3793C>T	p.Gln1265X				1				1	

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Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_025114.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. Page 4

Mutation		Associated phenotype								
Nucleotide nomenclature	Protein nomenclature	LCA	SLS	JS	JS + RD	JS + RF	CORS	MKS	MKS-like	BBS
c.3811C>T	p.Arg1271X	1					4 (2)			
c.3814C>T	p.Arg1272X	1			1					
c.3922C>T	p.Gln1308X	1								
c.4001del	p.Thr1334IlefsX2	1								
c.4028del	p.Lys1343ArgfsX2	1								
c.4114_4115del	p.Ile1372LysfsX5	1								
c.4115_4116del	p.Ile1372LysfsX5	1						1		
c.4195-1G>A	p.?		1							
c.4393C>T	p.Arg1465X	1	2				3			
c.4656del	p.Glu1553LysfsX4						1			
c.4723A>T	p.Lys1575X	12	2		1		1			
c.4732G>T	p.Glu1578X				3 (1)					
c.4771C>T	p.Gln1591X						2 (1)			
c.4791_4794del	p.Lys1598SerfsX8						1			
c.4882C>T	p.Gln1628X	2			1*		3*			
c.4962_4963del	p.Glu1656AsnfsX3	2								
c.4965_4966del	p.Glu1656AsnfsX3						1			
c.4966G>T	p.Glu1656X	1								
c.5046del	p.Val1683X	1								
c.5163del	p.Thr1722GlnfsX2	3					2			
c.5226+1G>A	p.?	1								
c.5256_5257del	p.Ala1753ArgfsX7	2 (1)								
c.5344C>T	p.Arg1782X	1								
c.5434_5435del	p.Glu1812LysfsX5						1			
c.5493del	p.Ala1832ProfsX19	1					1	4 (2)		
c.5515_5518del	p.Glu1839LysfsX11						1			
c.5519_5537del	p.Lys1840ArgfsX5	1								
c.5587-1G>C	p.?	3			2					
c.5611_5614del	p.Gln1871ValfsX2				1*		1*			
c.5649dup	p.Leu1884ThrfsX23		1*		1*		3 (2)		2 (1)	
c.5668G>T	p.Gly1890X	2 (1)		1		3	9 (7)			

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_025114.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Mutation		Associated phenotype								
Nucleotide nomenclature	Protein nomenclature	LCA	SLS	JS	JS + RD	JS + RF	CORS	MKS	MKS-like	BBS
						(2)				
¥	p.Glu1903X									1
CEP290:c.5709+2352_54_ C12orf50:c.290–1375_77del	p.?						1			
c.5722G>T	p.Glu1908X				1					
c.5734del	p.Trp1912GlyfsX11						4 (2)			
c.5776C>T	p.Arg1926X	1								
c.5813_5817del	p.Thr1938AsnfsX16	1								
c.5824C>T	p.Gln1942X			1*		1*				
c.5850del	p.Phe1950LeufsX15	3					2 (1)		2 (1)	
c.5865_5867delinsGG	p.Glu1956GlyfsX9	1								
c.5866G>T	p.Glu1956X	1								
c.5932C>T	p.Arg1978X		1							
c.5941G>T	p.Glu1981X						2			
c.6031C>T	p.Arg2011X				1					
c.6072C>A	p.Tyr2024X						2 (1)			
c.6271-8T>G	p.?						1			
c.6277del	p.Val2093SerfsX4						1			
c.6331C>T	p.Gln2111X						1			
c.6604del	p.Ile2202LeufsX24	7 (2)								
c.6869del	p.Asn2290IlefsX11						2			
c.6870del	p.Gln2291LysfsX10						2 (1)			
c.7318_7321dup	p.Leu2441SerfsX16						2 (1)			
c.7341dup	p.Leu2448ThrfsX8	1			2					
c.7366_7369del	p.Thr2457AlafsX27				2 (1)					
Total number of distinct patients (families)		**184 (167)**	**13 (12)**	**2**	**23 (20)**	**4 (3)**	**65 (53)**	**17 (11)**	**10 (7)**	**1**

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This table gives an overview of the number of patients carrying a specific mutation, for each phenotype (updating date: 14th of June, 2010). Numbers between brackets indicate the unique

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_025114.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

number of families. Abbreviations used: LCA: Leber Congenital Amaurosis; SLS: Senior-Loken syndrome; JS: Joubert syndrome; JS + RD: Joubert syndrome with associated retinopathy; JS + RF: Joubert syndrome with associated renal failure; CORS: cerebello-oculo-renal syndrome; MKS: Meckel-Grüber syndrome; MKS-like: Meckel-Grüber syndrome-like; BBS: Bardet-Biedl syndrome. ¥: nucleotide nomenclature not specified in publication. *: both patients carrying the mutation belong to the same family.

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Supp. Table S2: Overview of all *CEP290* unclassified variants identified so far and their occurrence in different phenotypes.

Unclassified variant		Associated phenotype			
Nucleotide nomenclature	Protein nomenclature	LCA	NPHP	JS +RF	Healthy
c.1991A>G	p.Asp664Gly			1	1
c.2717T>G	p.Leu906Trp				1
c.6684T>G	p.Asn2228Lys				1
c.4696G>C	p.Ala1566Pro	1			
c.5081T>C	p.Leu1694Pro	1			
c.4661_4663del	p.Glu1554del	1			
c.7311_7313del	p.Lys2437del		1		

Abbreviations used: LCA: Leber Congenital Amaurosis; NPHP: nephronophthisis; JS + RF: Joubert syndrome with associated renal failure.

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_025114.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

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c.2118_2122dup	p.Thr709SerfsX9	1								

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Supp. Figure S1: Overview of CEP290*base*.
The database is accessible from: http://medgen.ugent.be/cep290base. Information on variants can be retrieved using the Mutation Overview (1) or the Mutation Browser (2). In addition, the Phenotype page (3) describes all phenotypes associated with *CEP290* so far. The variant-specific pages (4) are accessible from both the Mutation Overview and Mutation Browser and include detailed information on the pathogenic potential. The database links to NetGene2 and BDGP for splice site mutations and automatically fills in queries for PolyPhen and SIFT for missense changes. In addition, the database includes variants in other genes that co-occur with *CEP290* mutations, thereby providing a unique opportunity to link modifiers to associated clinical manifestations.