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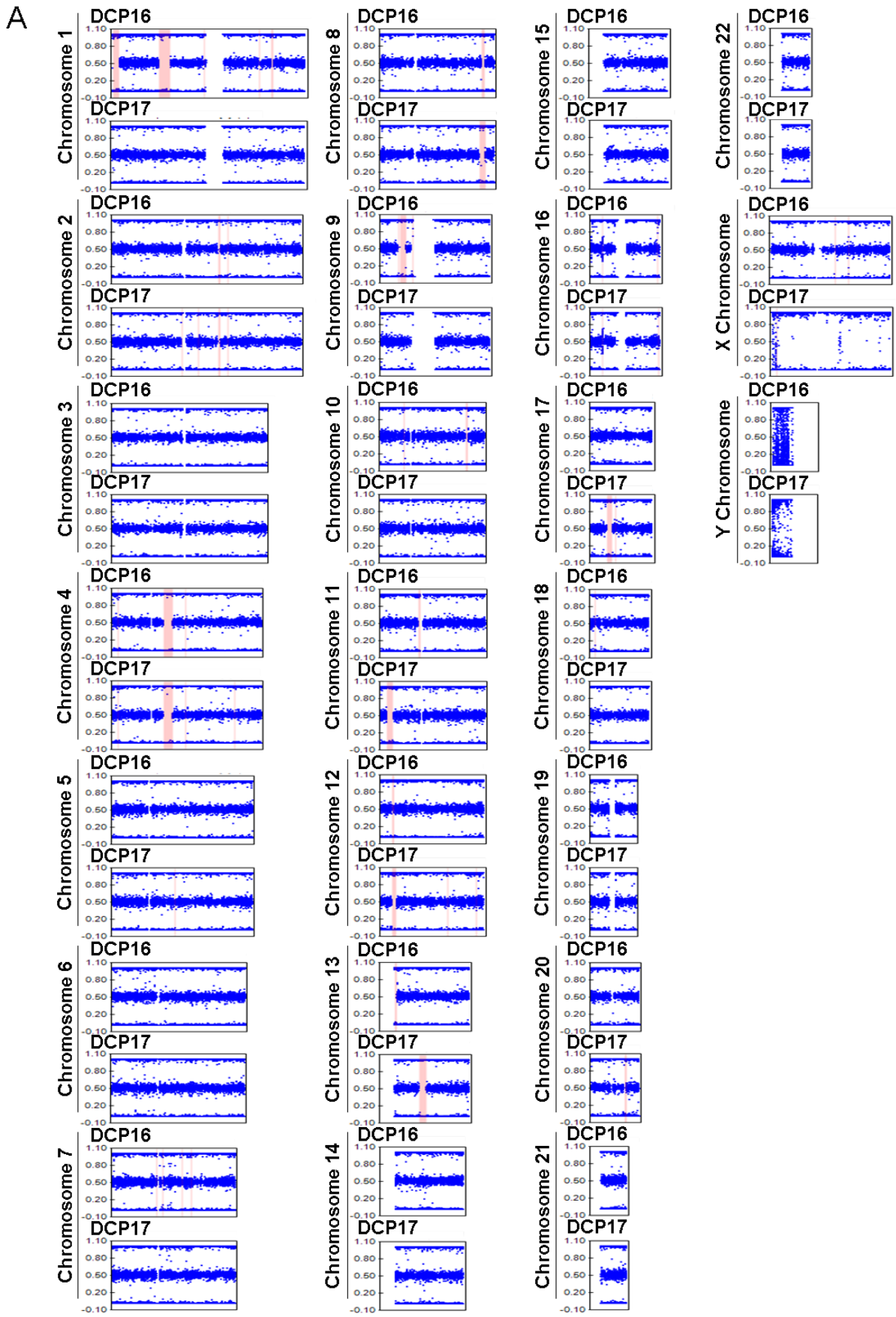
Supplemental Data

Loss-of-function Mutations in *LRRC6*, a Gene Essential for Proper Axonemal Assembly of Inner and Outer Dynein Arms, Cause Primary Ciliary Dyskinesia

Esther Kott, Philippe Duquesnoy, Bruno Copin, Marie Legendre, Florence Dastot-Le Moal, Guy Montantin, Ludovic Jeanson, Aline Tamalet, Jean-François Papon, Jean-Pierre Siffroi, Nathalie Rives, Valérie Mitchell, Jacques de Blic, André Coste, Annick Clement, Denise Escalier, Aminata Touré, Estelle Escudier, and Serge Amselem


A
Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Chromosome 11
Chromosome 12
Chromosome 13
Chromosome 14
Chromosome 15
Chromosome 16
Chromosome 17
Chromosome 18
Chromosome 19
Chromosome 20
Chromosome 21
Chromosome 22
X Chromosome
Y Chromosome
DCP16
DCP17
1.10
0.80
0.50
0.20
-0.10

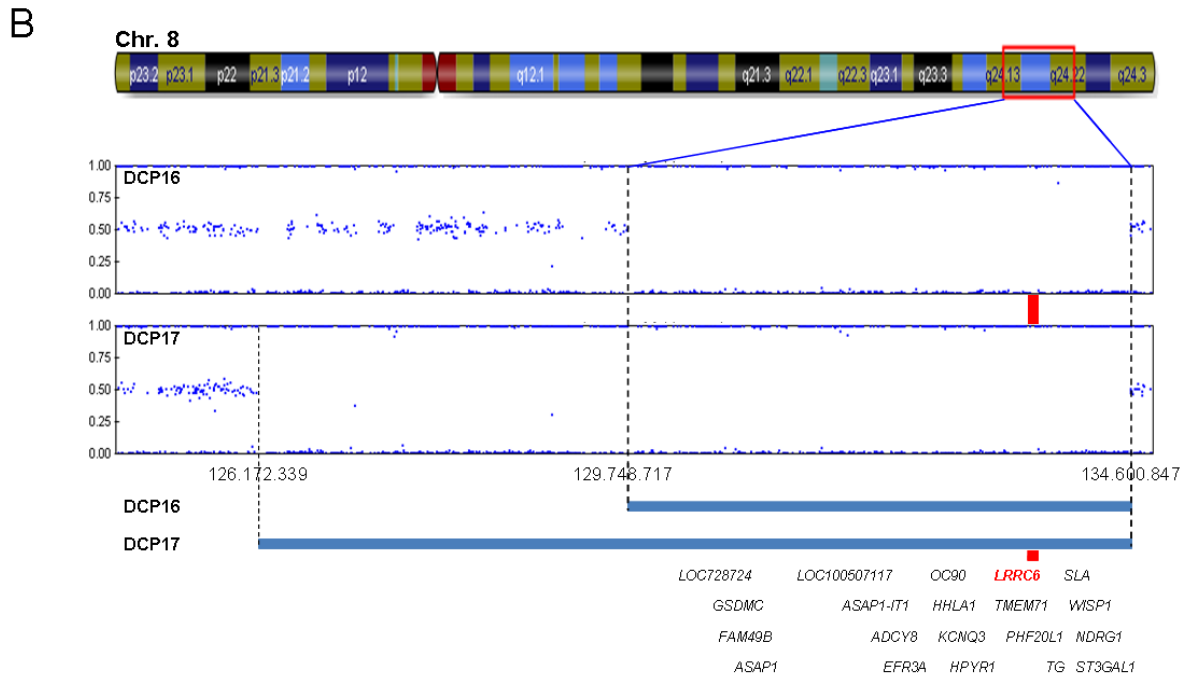


Figure S1. Homozygosity Mapping in Individuals DCP16 and DCP17

(A) DCP16 and DCP17 were genotyped with the HumanCytoSNP-12 chip from Illumina® and the data were analyzed with the Genome Studio® and CNV partition 3.1.6 softwares (Illumina) as well as with an in-house script designed to identify homozygous regions. Each blue dot represents one individual single-nucleotide polymorphism (SNP). For each SNP, a low B allele frequency indicates that the individual is homozygous for the A allele; intermediate values mean they are heterozygous and high B allele frequency means that they are homozygous for the B allele. Regions of homozygosity larger than 1 Mb are shaded in pink. The larger common region is located on chromosome 4 (between rs766818 and rs9999448 – 11.9 Mb) and contains 99 genes, among which 9 are not ubiquitously expressed; among them, only 1 is expressed in a few tissues including trachea and testis: *SCARB2 (scavenger receptor class b, member 2*). This gene is responsible, when mutated, for progressive myoclonic epilepsy (EPM4 [MIM 254900]). The second larger region is located on chromosome 8 and is the one containing *LRRC6*.

(B) Ideogram of chromosome 8, as obtained with the use of the GenomeStudio software (Illumina), showing the homozygous region identified and the localization of *LRRC6* (red) in region 8q24.22. Proximal and distal boundaries of the homozygous regions (horizontal blue bars) are indicated by vertical dotted lines. The 20 genes that are contained in the homozygous region are listed at the bottom. Among them, three have already been implicated in human pathology, i.e. potassium voltage-gated channel, KQT-like subfamily, member 3 (*KCNQ3)*, thyroglobulin *(TG)* and N-myc downstream regulated 1 *(NDRG1)* involved in familial neonatal seizures (BNFS2; [MIM121201]), thyroid dyshormonogenesis (TDH3; [MIM274700]) and Charcot-Marie-Tooth disease type 4D (CMT4D; [MIM601455]), respectively. Two sequences (*ASAP1-IT1* and *HPYR1*) are non-protein coding genes. As for *LOC78724* and *LOC100507117*, only scarce information is available from the NCBI databases. Twelve of the 13 remaining genes were not considered as good candidates because their presumed function and/or expression pattern, as reported in the NCBI databases, were obviously not consistent with the phenotypic features of PCD.

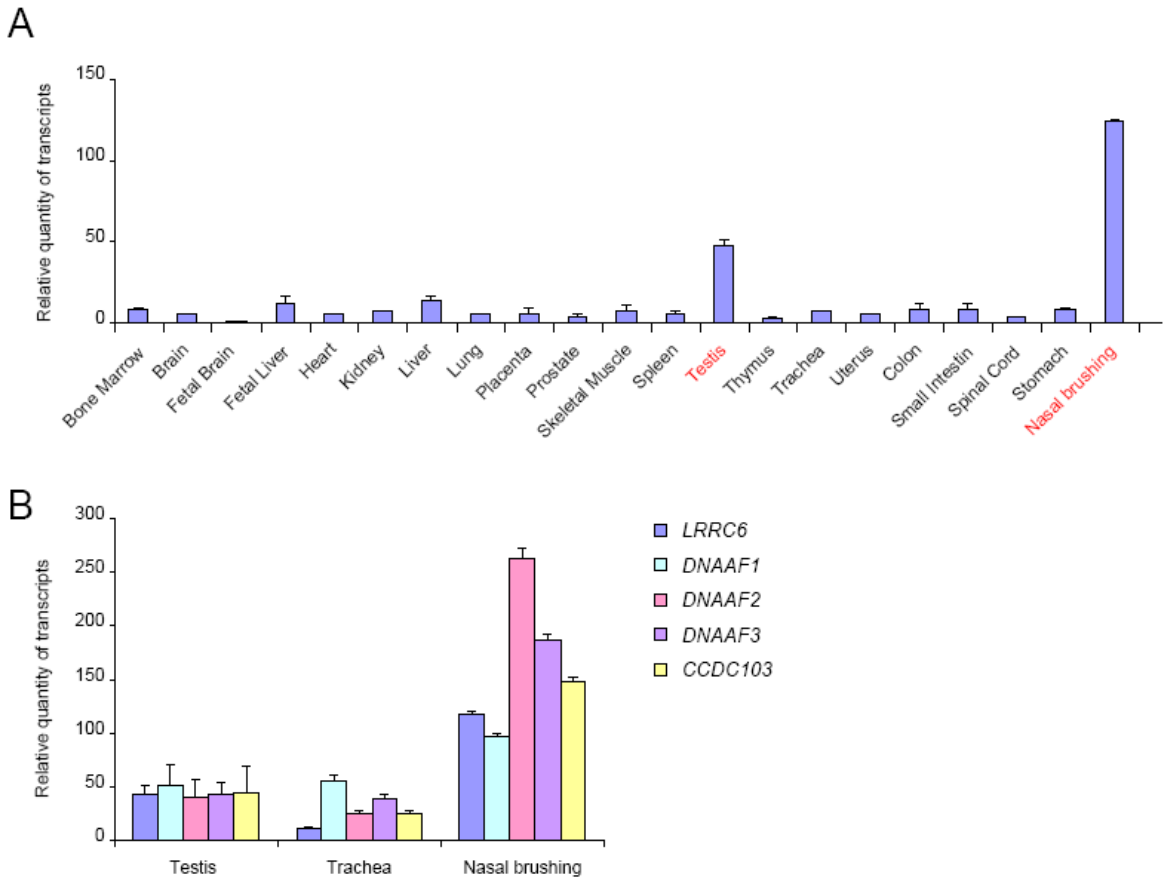


Figure S2. Expression Analysis of Human *LRRC6*, as Assessed by Means of Quantitative RT-PCR Analysis

(A) *LRRC6* transcripts are found at the highest levels in nasal brushing and to a lesser extent in testis.

(B) Expression of *LRRC6* transcripts in testis, trachea and nasal brushing, as compared with that of four other PCD genes associated with absence of both DAs.

The ubiquitously expressed *ERCC3* gene was used as an internal control to normalize the data. Values are the mean±SD of three independent experiments. 500 ng of total RNA from different human tissues obtained from Clontech (Takara Bio Europe/Clontech, Saint Germain en Laye, France) or from nasal brushings were primed with 2.5 mM of oligodT and then subjected to reverse transcription with the Reverse Transcriptor kit from Roche, following the manufacturer's conditions. cDNAs were amplified in the Light Cycler LC480 (Roche/Boehringer, Mannheim, USA) with the Mesa Blue qPCR MasterMix Plus for SYBR Assay (Eurogentec) using a forward primer in exon 5 (5' GATGGACGTTGGTACACAGACA 3') and a reverse primer in exon 6 (5' CACTGTTGTCTAATTTCTTTGTGTTG 3') for *LRRC6*.

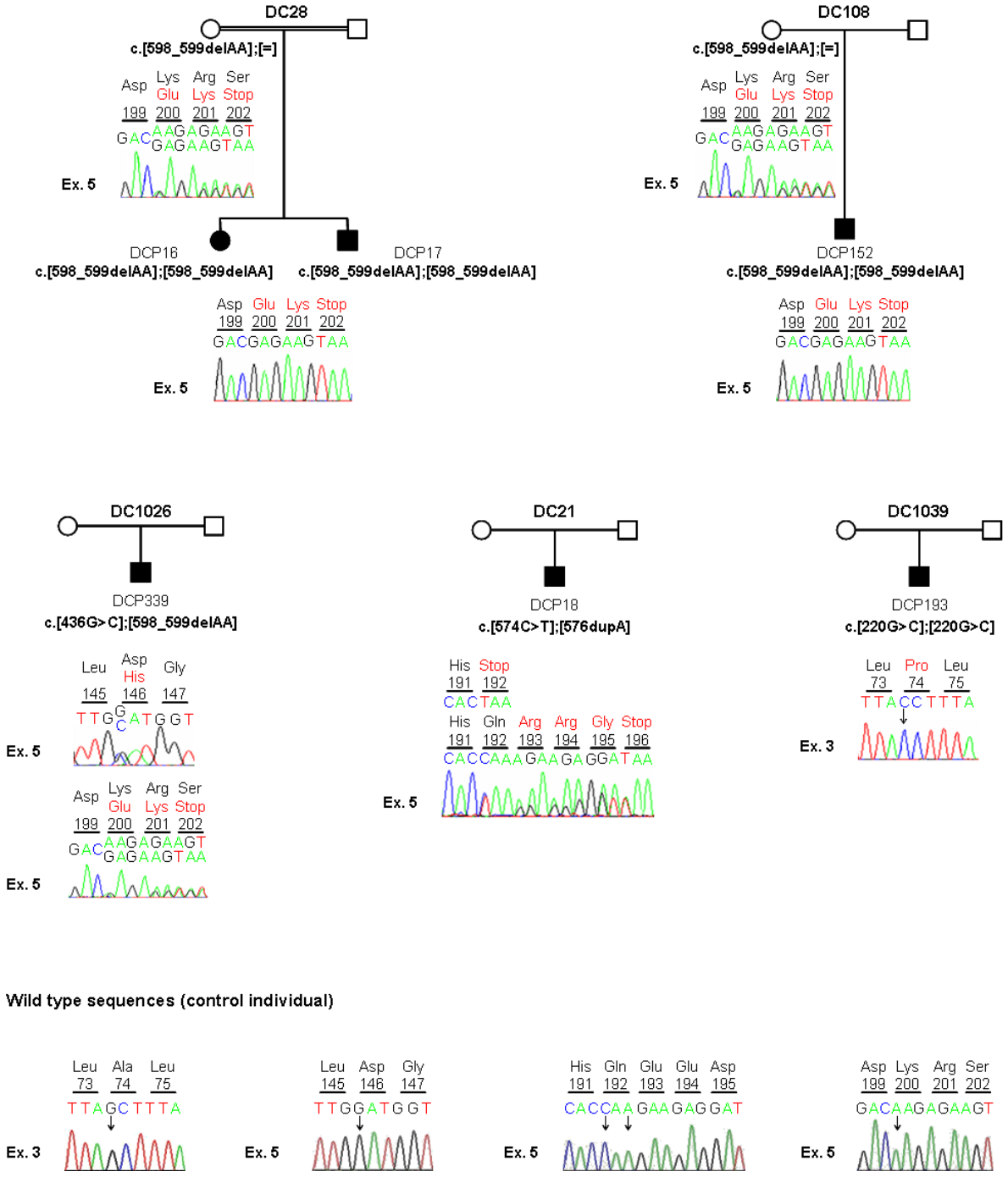


Figure S3. *LRRC6* Mutations Identified in Affected Individuals with PCD

Mutations and genotypes in families DC21, DC28, DC108, DC1026, and DC1039. The expected consequences of the identified mutations, which are detailed in text, are shown. On the wild type sequences, the arrows indicate the position of identified mutations.

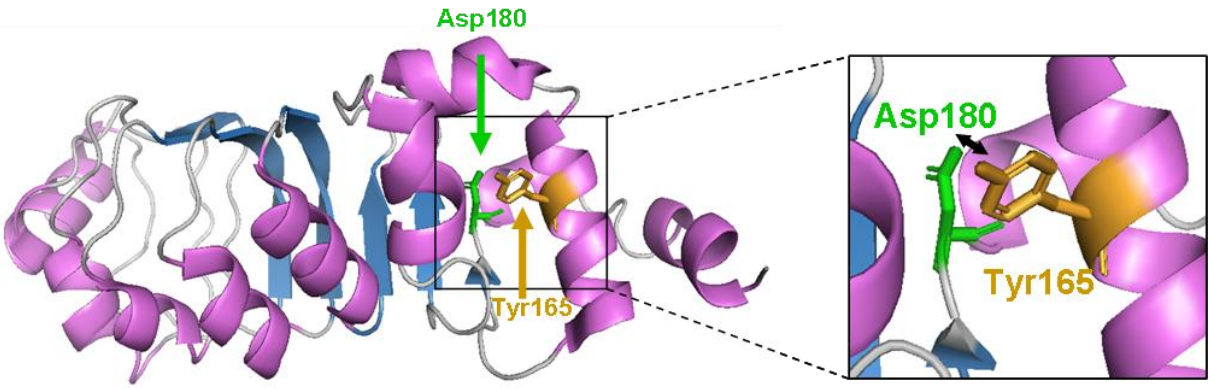
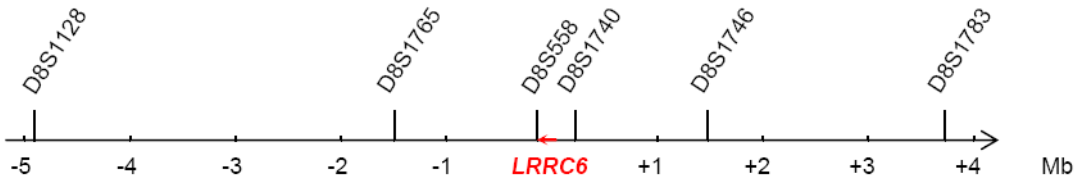


Figure S4. Predicted Impact of the p.Asp180His Amino Acid Substitution (Equivalent to Human p.Asp146His) on the Free Energy of the *Chlamydomonas* LC1 Protein Molecule

Predicted structure of wild-type LC1. Asp180 (the equivalent of Asp146 in human LRRC6) is indicated by a green arrow, and Tyr165 (the equivalent of human Tyr131) by an ochre arrow. The hydrogen bond between Tyr165 and Asp180 of the LRRcap is shown (magnification on the right, black bidirectional arrow). The p.Asp180His variation replaces an acidic amino acid by a non-charged residue with an imidazole functional group: the formation of a hydrogen bond with Tyr165 would considerably increase the free energy of this molecule (from -2843 kJ/mol to +1126 kJ/mol), a result incompatible with the existence of such structure. Solution structure of 1DS9 (*Chlamydomonas* ODA LC1) was modeled with Modeller 9.10 and tested with PROCHECK, then visualized by PyMol. Violet, α-helix; sky blue, β-sheet; grey: random coil. Free energies were calculated with Swiss-Pdb Viewer.



		D8S1128	D8S1765	D8S558	D8S1740	D8S1746	D8S1783
DC28	DCP16	241/253	**131/131**	**170/170**	**170/170**	180/186	203/207
	DCP17	253/253	**131/131**	**170/170**	**170/170**	180/186	203/207
DC108	DCP152	253/253	**131/131**	**170/170**	**170/170**	186/186	203/203
DC1026	DCP339	249/253	121/**131**	157/**170**	170/**170**	180/180	199/207

Figure S5. Genotyping of Six Microsatellites Markers that Flank *LRRC6* in Affected Individuals Sharing the c.598_599delAA (p.Lys200Glufs*3) Mutation

The c.598_599delAA (p.Lys200Glufs*3) mutation is shared by three families not known to be related. A control European population (108 chromosomes) has been genotyped. The frequencies of alleles 131, 170 and 170 of flanking microsatellites D8S1765, D8S558 and D8S1740 were found to be 27.5%, 4.5% and 45%, respectively. The frequency of the 131-170-170 haplotype was found to be very low (lower than 2.8%), thereby supporting a founder effect.