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## Novel Gene Discoveries

# *WDR19*: An ancient, retrograde, intraflagellar ciliary protein is mutated in autosomal recessive retinitis pigmentosa and in Senior-Loken syndrome

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Autosomal recessive retinitis pigmentosa (arRP) is a clinically and genetically heterogeneous retinal disease that causes blindness. Our purpose was to identify the causal gene, describe the phenotype and delineate the mutation spectrum in a consanguineous Quebec arRP family. We performed Arrayed Primer Extension (APEX) technology to exclude ~500 arRP mutations in ~20 genes. Homozygosity mapping [single nucleotide polymorphism (SNP) genotyping] identified 10 novel significant homozygous regions. We performed next generation sequencing and whole exome capture. Sanger sequencing provided cosegregation. We screened another 150 retinitis pigmentosa (RP) and 200 patients with Senior-Løken Syndrome (SLS). We identified a novel missense mutation in *WDR19*, c.2129T>C which lead to a p.Leu710Ser. We found the same mutation in a second Quebec arRP family. Interestingly, two of seven affected members of the original family developed 'sub-clinical' renal cysts. We hypothesized that more severe *WDR19* mutations may lead to severe ciliopathies and found seven WDR19 mutations in five SLS families. We identified a new gene for both arRP and SLS. WDR19 is a ciliary protein associated with the intraflagellar transport machinery. We are currently investigating the full extent of the mutation spectrum. Our findings are crucial in expanding the understanding of childhood blindness and identifying new genes.

### Conflict of interest

We have no conflict of interest to disclose in the work presented in this article.

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Retinitis pigmentosa (RP) is a well known inherited retinal dystrophy that leads to progressive loss of vision, and eventually complete blindness due to photoreceptor cell death. RP is restricted to the eye, specifically the retina and photoreceptors. Its prevalence is estimated to be at 1/4000 (1–3). RP is caused by progressive loss of rods followed by the loss of cones. Affected individuals start experiencing night blindness in the first two decades of life. Peripheral visual field constriction leads to 'tunnel vision', progressive loss of visual acuity (VA) leads to complete loss of all vision (4). On fundus exam, RP is classically characterized by retinal atrophy, optic nerve head pallor, vascular narrowing and a variety of peripheral retinal pigmentary deposits including bone spicules. RP can be restricted to the eye (non-syndromic) or a retinal degeneration indistinguishable from RP can be associated with a syndrome. The syndromic features can be very obvious or very subtle. Also, the syndromic features can be present at the time of diagnosing the retinal degeneration, or they may appear much later. Examples are Bardet–Biedl syndrome (BBS), Usher Syndrome (USH) and Senior-Loken Syndrome (SLS). The mode of inheritance of RP may be autosomal dominant [autosomal dominant retinitis pigmentosa (adRP); 5–25%], autosomal recessive [autosomal recessive retinitis pigmentosa (arRP); 5–20%], rarely digenic, mitochondrial or X-linked [X-linked retinitis pigmentosa (xlRP); 5–15%] (5). There are currently 36 genes implicated in arRP that only explain ~50% of the cases (RetNet), illustrating that many mutations and new genes still need to be discovered. *RPE65*, which is expressed in the retinal pigment epithelium (RPE), *PDE6A* and *PDE6B*, which are both phosphodiesterase subunits in the phototransduction cascade, *EYS* and *USH2A*, are among the most well-known or common genetic etiologies (5, 6).

Interestingly, several genes associated with RP (in patients without systemic disease) can also be mutated in syndromic disorders such as BBS1 and USH2 (7, 8) and SLS which is an autosomal recessive disease affecting primarily the kidney and retina, leading to nephronophthisis (NPHP) and RP, respectively (9). Of particular interest in RP and these syndromes is the emergence of a group of genes and proteins that function in and are localized to the cilium, the so-called ciliary genes. In photoreceptor outer segments (OS, which represent modified cilia), the stacked discs house the proteins for visual function with several important RP ciliary gene products, e.g. RPGR, RPGRIP1, USH2A, CEP290, LCA5, and TULP1.

We identified an atypical form of arRP in a French-Canadian family (Fig. 1) and excluded mutations in all the known arRP genes. We hypothesized that this family carries mutations in a novel RP specific gene and subsequently identified homozygous mutations in *WDR19*. At the time of discovery (August 2011), there was no published article in the literature linking this gene to any human disease and specifically to arRP. On re-evaluation of this family, we discovered that several of the RP patients had sub-clinical renal cysts, which prompted us to hypothesize that this gene may be mutated in RP patients with frank kidney failure, a disorder coined SLS. We then performed mutation analysis in this gene in a cohort of patients with RP in Quebec and a cohort with the syndromic form of RP, namely SLS and identified more RP families and several SLS families with mutations. At this time, both Bredrup et al. in their original article and Huber et al., in their review article, described the involvement of *WDR19*, a ciliary gene, in a variety of ciliopathies including Sensenbrenner, Jeune syndrome and isolated NPHP (10, 11). Similarly, Halbritter et al. reported the first case of Caroli disease, which is a rare inherited disorder most commonly characterized by congenital cystic dilatation of the intrahepatic biliary ducts secondary to ectasia, caused by mutations in WDR19. However, there is yet no study confirming clearly the involvement of *WDR19* in arRP as well as in SLS (12).

In the process of identifying a novel gene for RP and SLS, we also identified a novel genotype–phenotype correlation in the arRP families. We illustrate that the combined use of Arrayed Primer Extension (APEX) technology, homozygosity mapping by single nucleotide polymorphism (SNP) genotyping and next generation sequencing (NGS), can rapidly and reliably lead to new gene discovery.

## Materials and methods

This study was approved by the Institutional Review Board (IRB) committee at the McGill University Health Centre (MUHC), according to the tenets of Helsinki. Informed consents were obtained from all patients. Ophthalmic examinations included best-corrected VA, slit-lamp biomicroscopy, and funduscopy. Goldmann kinetic perimetry [Goldmann visual fields (GVF)] was performed with four targets (IV/4e, III/4e, I/4e and I/3e) testing from non-seeing retina to seeing retina. *In vivo* retinal imaging was performed in a subset of patients by optical coherence tomography (OCT) (Heidelberg Engineering) and lipofuscin metabolism was mapped using the fundus autofluorescence (FAF) module (Heidelberg Engineering).

To identify the causal gene of the proband in family A, we used a novel four-step sequential process. First, we excluded all known arRP mutations (700 mutations in 28 arRP genes, version 2012) using APEX technology (Asper Ophthalmics). We then performed SNP Homozygosity Mapping (Illumina Infinium HD 660K technology, San Diego, CA) followed by whole exome capture (WEC, Agilent SureSelect V3, Santa Clara, CA) and NGS (Illumina Hiseq, San Diego, CA) with a particular focus on the identified homozygous regions. We finally confirmed the causal mutations using Sanger sequencing.

We then used a pipeline approach to exclude variants and we excluded the found mutation from 500 normal controls. Cosegregation of the mutations was then performed in family A and B by Sanger sequencing. We then screened ~150 RP patients (mostly from Quebec), 96 SLS patients, and 96 patients with more severe

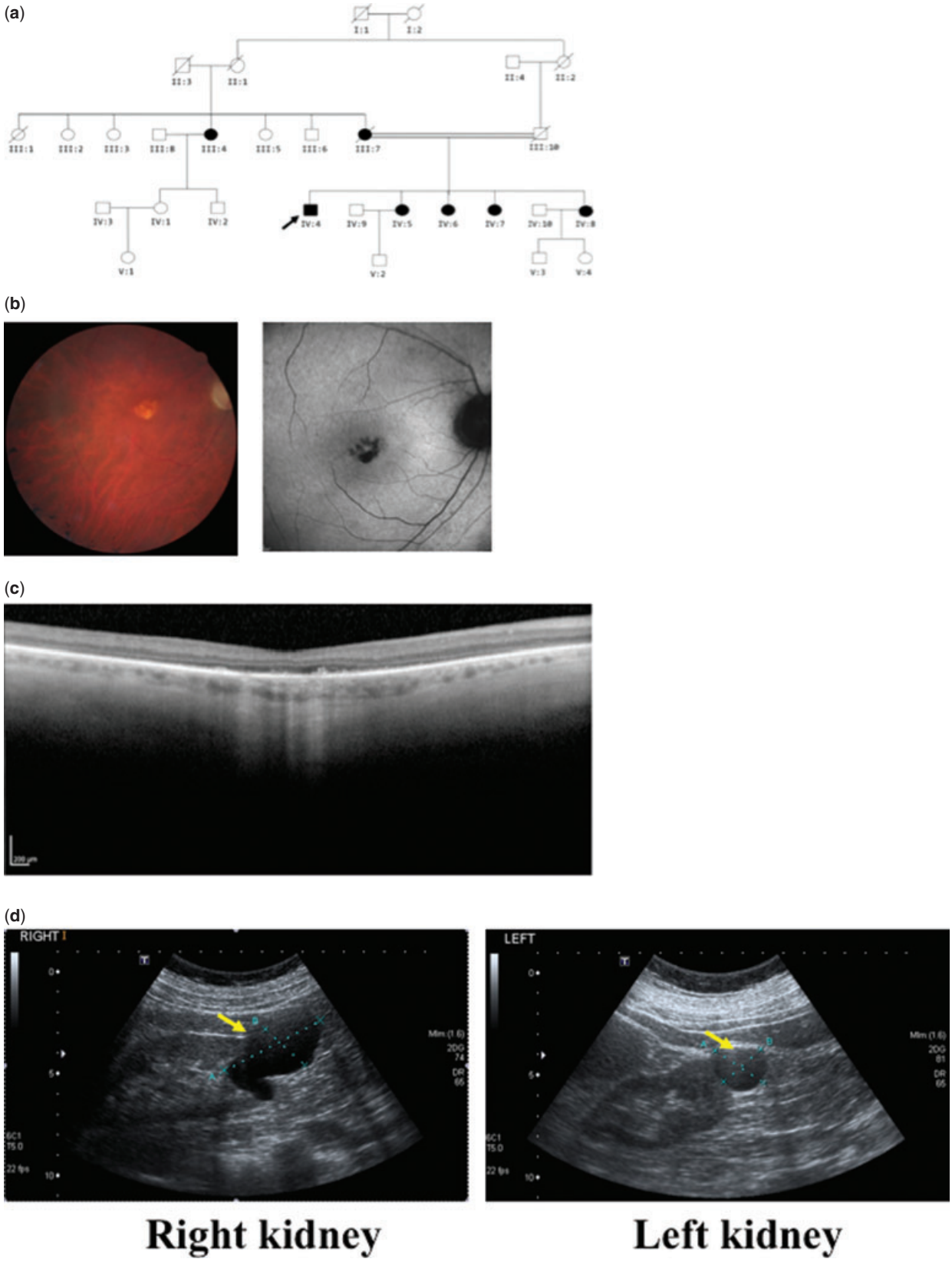


*Fig. 1.* (**a**) Family A pedigree. (**b**) Retinal photo showing an atrophic maculopathy and severe narrowing of the blood vessels and temporal pallor of the optic nerve head. (**c**) Fundus autofluorescence showing the 'bear claw' maculopathy and hypo-fluorescence in the fovea. (**d**) Optical coherence tomography of the macula showing extensive loss of the inner segment/outer segment junctions and photoreceptors, with inner retinal changes, retinal thinning and foveal debris. (**e**) Renal ultrasound showing the renal cyst of the probands of family A.

ciliopathy by applying a CEL1 endonuclease based heteroduplex DNA cleavage procedure (13) followed by Sanger sequencing in an attempt to find other families and to delineate the full mutation spectrum of the gene.

### SNP genotyping

Whole-genome SNP genotyping (Affymetrix, Santa Clara, CA) was performed to identify homozygous regions in family A (6). The DNA samples for SNP analysis were purified (QIAamp DNA Mini Kit; Qiagen, Valencia, CA) and then genotyped. The SNP genotypes were analyzed for homozygous regions (ExcludeAR sheet; Excel, Microsoft, Redmond, WA). Chromosomal segments were accepted as having significant homozygous regions if they contained $\geq$300 consecutive homozygous SNPs, as the likelihood that this would occur by chance is less than 1:100 (14).

### Whole exome capture and next generation sequencing

The pre-capture library was prepared following the manufacturer's protocol (Illumina); 2 µg of link-ligated DNA from the proband of family A was used for the capture reaction. Agilent SureSelect V3 exome capture was performed according to the company's standard protocol. The amplified capture product was sequenced on Hiseq 2000 (Illumina). We removed adaptor sequences and quality trimmed reads using the Fastx toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and then used a custom script to ensure that only read pairs with both mates present were subsequently used. Reads were aligned to hg19 with BWA 0.5.9, and indel realignment was carried out using the Genome Analysis Toolkit (GATK) (15). Duplicate reads were then marked using Picard (http://picard.sourceforge.net/) and excluded from downstream analyses. We assessed coverage of consensus coding sequence (CCDS) bases using the GATK, which showed that >93% of CCDS bases were covered by at least 10 reads. Single nucleotide variants (SNVs) and short insertions and deletions were called using samtools pileup, and were then quality filtered to require at least 20% of reads supporting the variant call. Variants were annotated using both Annovar (16) and custom scripts to identify whether they affected protein coding sequence, and whether they had previously been seen in dbSNP132, the 1000 genomes dataset (November 2011), the NHLBI GO exomes, or in approximately 500 exomes previously sequenced at our center.

### Sanger sequencing and cosegregation

Direct polymerase chain reaction (PCR) and Sanger sequencing were used to validate the mutations in the proband, their parents and siblings. Primers were designed using primer design tool 'Primer3' (17). Each amplicon was 300–500 bp in length and was sequenced directly with an ABI 3730 (Life Technologies Corporation, Carlsbad, CA) machine in both forward and reverse directions. Each read was aligned to the reference sequence and the base changes were identified with the SEQUENCHER software program.

Table 1. Result of homozygosity mapping in family A: proband (IV:4)

| CHR | Size (millions BP) | NSNP | Rank |
|---|---|---|---|
| 3 | 20.6 | 4307 | 1 |
| 18 | 19.8 | 5165 | 2 |
| 8 | 19.6 | 4265 | 3 |
| 1 | 14.6 | 3716 | 4 |
| 19 | 14.2 | 3631 | 5 |
| 20 | 12.5 | 4415 | 6 |
| 15 | 12.3 | 3281 | 7 |
| 22 | 11.7 | 3270 | 8 |
| 9 | 10.3 | 3225 | 9 |
| 4 | 7.3 | 1589 | 10 |
| 4 | 7.2 | 2126 | 11 |

CHR, chromosome; NSNP, number of single-nucleotide polymorphism.

## Results

### Genotype

#### *Autosomal recessive retinitis pigmentosa (*arRP*)*

In the consanguineous family A (Fig. 1), after excluding known arRP mutations using APEX technology, we proceeded with whole genome SNP genotyping and homozygosity mapping, (Table 1), and we ranked the sizes of the homozygous regions in descending order. We found a total of 150 Mb of homozygosity most likely by descent in this Quebec family with a total of 11 significant regions. The largest homozygous region was found on chromosome 3 (20.6 Mb) while the smallest one was found on chromosome 4 (7.2 Mb). Assuming consanguinity and recessive mutations in the causal gene, it became evident from the non-overlap between known retinal dystrophy loci and our identified homozygous regions that we were very likely dealing with a new gene for arRP.

Without any potential lead candidate gene, we then used WEC/NGS with a particular focus on the homozygous regions identified earlier in family A. A *TMEM5* splice site mutation was found on chromosome 1, but this change did not cosegregate in the family. We then concentrated on a candidate missense mutation in *AIFM3* on chromosome 22. Unfortunately, this variant did not cosegregate either. Finally, we identified a missense variant in *WDR19* on chromosome 4, the smallest homozygous region. Using Sanger sequencing we confirmed the mutation and found cosegregation in family A (Fig. 2). The identified variant is a homozygous missense mutation c. 2129T>C, p. Leu710Ser in *WDR19* (Fig. 2). The Leu710Ser mutation is predicted to be harmful according to POLYPHEN2, damaging according to SIFT and evolutionarily highly conserved, as the Leucine is always present even in *Caenorhabditis elegans* (Fig. 2).

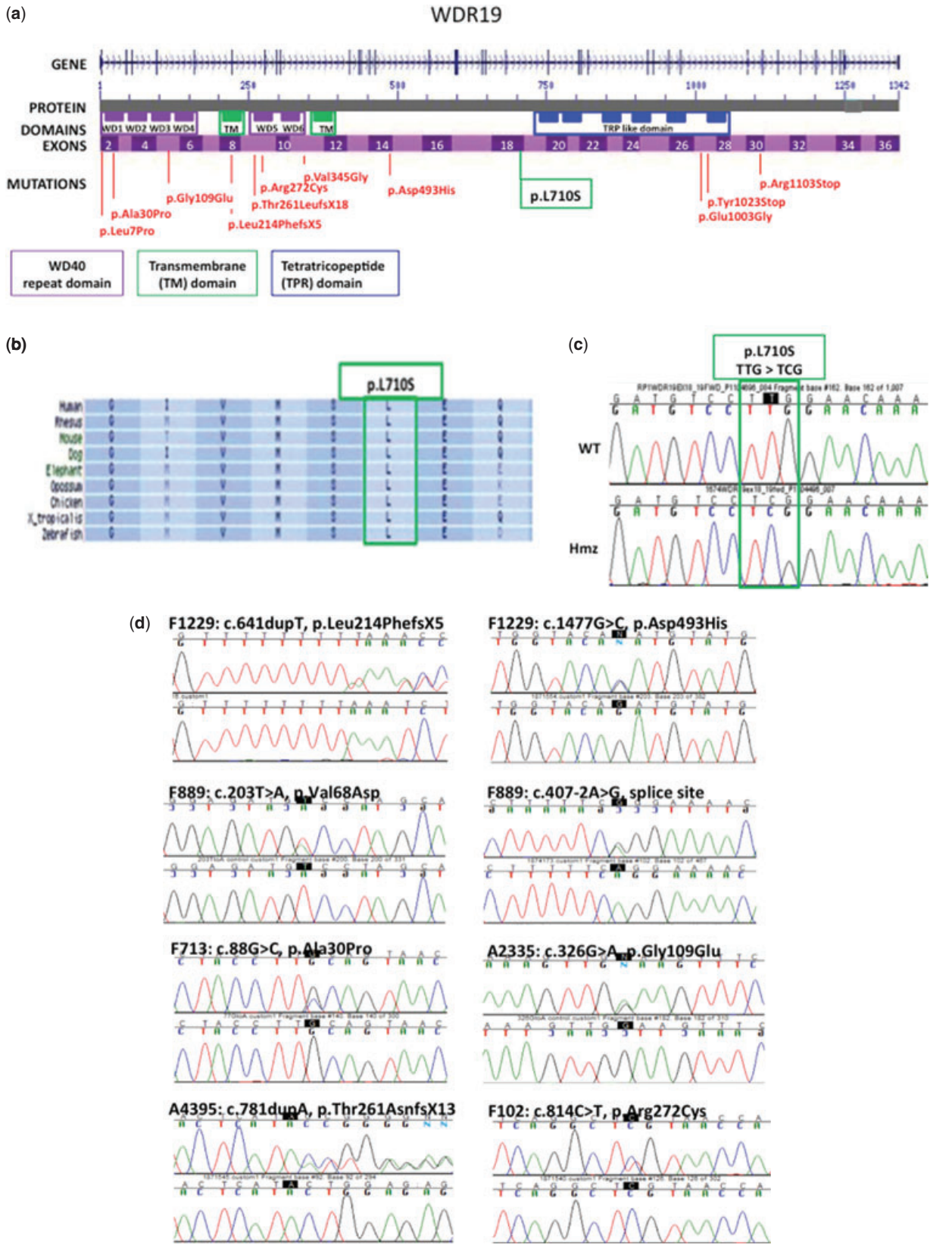


*Fig. 2.* (a) Gene and protein structure of WDR19 and IFT144, showing the protein domains and the positions of all the mutations found in this study and in Bredrup et al. showing that most mutations are in protein domains except the p. L710S mutation. (b) Conservation of the Leucine at position 710 in IFT144 (WDR19) among species. (c) Sanger sequencing results of the WDR19 mutations in Family A. (d) Sanger sequencing results of the WDR19 mutations in Senior-Loken syndrome patients.

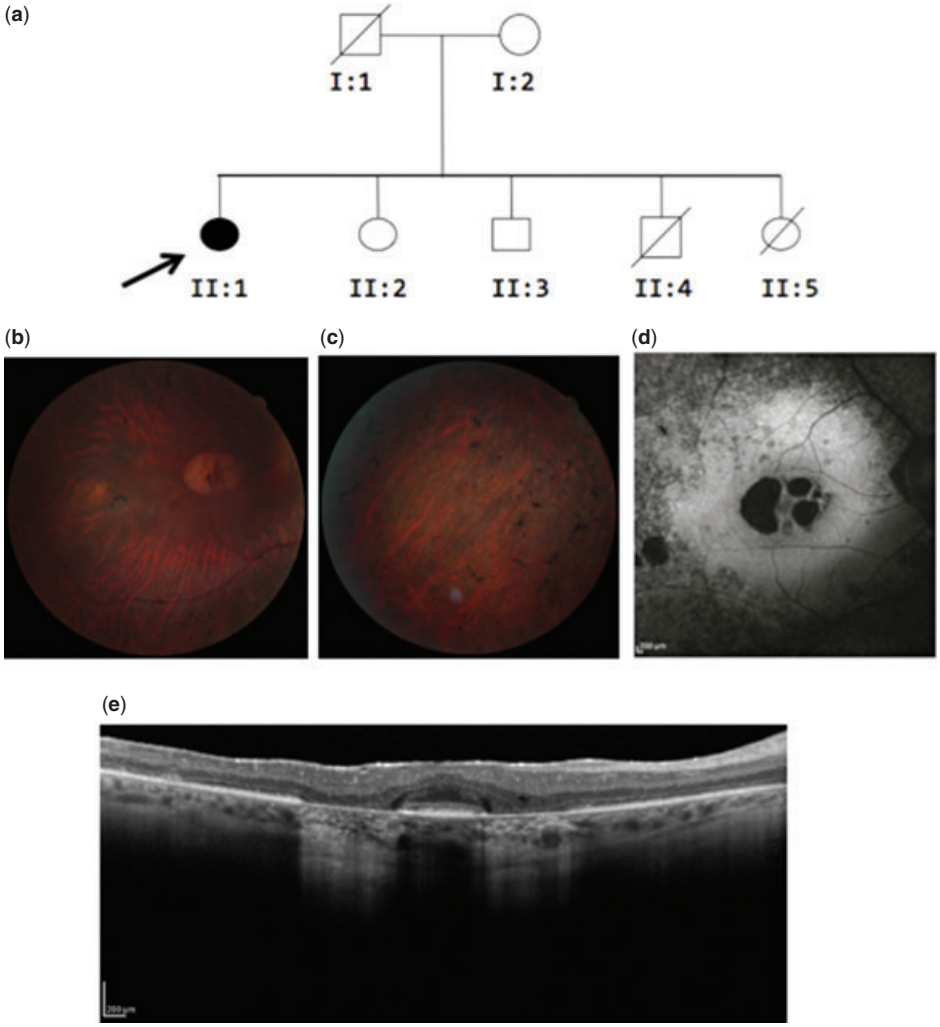


*Fig. 3.* (**a**) Family B pedigree. (**b**) Retinal photo showing an atrophic maculopathy and severe narrowing of the blood vessels and temporal pallor of the optic nerve head. (**c**) Peripheral bone spicules, extensive. (**d**) Fundus autofluorescence showing the 'bear claw' maculopathy and the hypofluorescence in the fovea. (**e**) Optical coherence tomography of the macula showing extensive loss of the inner segment/outer segment junctions and photoreceptors, with inner retinal changes, retinal thinning and foveal debris.

*WDR19* is a ciliary gene that encodes the intraflagellar transport 144 (IFT144) protein, and recently associated with a human disease during our study (10). In order to investigate the full mutation spectrum of *WDR19* in arRP and its frequency, we assembled an arRP cohort from Quebec. After Sanger sequencing of an unsettled cohort of 150 arRP from the Quebec province, we identified the same homozygous mutation again in a second Quebec arRP family B (Fig. 3). The mutation was not seen in 200 French-Canadian healthy control individuals.

We reassessed the phenotype of both families and discovered that two of seven family A affected members had 'sub-clinical' renal cysts (Fig. 1). This unusual finding surprised us, as arRP is not known to be associated with subclinical renal cysts. We performed renal ultrasounds and renal function by urine biochemistry. Fig. 1 shows the large cysts in both kidneys. Urine and blood biochemistry were normal, strongly suggesting normal renal function. This led us to hypothesize that more severe mutations in *WDR19* may be involved in more severe and more complex phenotypes, involving other tissues such as kidney, brain and others. First we hypothesized that our kidney cysts were a 'forme fruste' of NPHP and turned our attention to SLS, which represents a ciliopathy with retinal and kidney involvement.

### *Senior-Løken syndrome*

Using the same genetic approach, we screened 96 patients with SLS and found five families (7%) in which *WDR19* was mutated (Table 2). Again, all the mutations were predicted to be harmful. We found compound heterozygous and heterozygous mutations (Table 2), and we identified both missense and frameshift mutations in *WDR19* (Fig. 2). They were confirmed by Sanger sequencing and found to cosegregate in their respective families (Fig. 2). All mutations currently known are shown in Fig. 2.

Table 2. Mutations in WDR19 in patients with Senior-Løken syndrome[a]

| Patient ID | Eye phenotype Onset age(years) | Kidney phenotype ESRD age (years) | Other phenotypes Onset age (years) | Nucleotide change | Amino acid change | Zygosity | POLY-PHEN 2 score | Mutation taster prediction | References |
|---|---|---|---|---|---|---|---|---|---|
| MOGL 1764 | Characteristic arRP findings with hypopigmented macular atrophy and 'bear track' changes OU | Bilateral congenital subclinical renal cysts | Mild cognitive impairement | c.2129T>C | p.Leu710Ser | Hom | 1.0 | Disease causing | (10) |
| MOGL 3157 | Characteristic arRP findings with hypopigmented macular atrophy and 'bear track' changes OU | None | None | c.2129T>C<br>c.1477G>C | p.Leu710Ser<br>p.Asp493His | Hom<br>Het | 1.0<br>0.99 | Disease causing<br>Disease causing | (10)<br>(12) |
| F1229 | Retinal dystrophy | NPHP (17) | Growth retardation | c.641dupT<br>c.1477G>C | p.Leu214PhefsX5<br>p.Asp493His | Het<br>Het | NA<br>0.99 | Disease causing<br>Disease causing | (12)<br>(12) |
| F889 | Retinal dystrophy (8) | NPHP (12) | None | c.203T>A<br>c.407-2A>G | p.Val68Asp<br>Splice site | Het<br>Het | 0.12<br>NA | SNP<br>Disease causing | Novel<br>(12) |
| F713 | Retinal dystrophy | NPHP (12) | Bilateral hip dysplasia, ventricular septum defect | c.88G>C | p.Ala30Pro | Het | 1.0 | Disease causing | Novel |
| A2335 | Retinal dystrophy, optic nerve atrophy, nystagmus (4 months) | NPHP (10) VUR, proteinuria | Gestational polyhydramnios, intellectual disability | c.326G>A | p.Gly109Glu | Het | 0.17 | Disease causing | Novel |
| A4395 | Night blindness | NPHP (5) | JATD (3), brachydactyly, scoliosis | c.781dupA | p.Thr261AsnfsX13 | Het | NA | Disease causing | (12) |

arRP, autosomal recessive retinitis pigmentosa; ESRD, end stage renal disease; het, heterozygosity; hom, homozygosity; JATD, Jeune's asphyxiating thoracic dystrophy; NA, not applicable, SNP, single nucleotide polymorphism; VUR, vesicoureteral reflux.

[a] All patients in this study, with phenotypes and their *WDR19* genotypes. Two homozygous mutations were associated with arRP and five heterozygous mutations were associated with a variety of NPHP (kidney failure phenotypes) and retinal dystrophy (Senior-Loken syndrome) associated with a variety of other systemic diseases. In these five patients we identified heterozygous WDR19 mutations. We show the wide range of WDR19 mutations and their phenotypic effects. Mutation numbering is based on cDNA position according to the reference sequence of *WDR19* (NM_025132.3) with +1 corresponding to the A of the ATG translation initiation codon.

### Phenotype of the arRP families carrying the *WDR19* missense mutation (p.Leu710Ser)

#### *Family A*

The proband (IV:4) in family A is a 50-year-old man from a consanguineous French-Canadian family (Fig. 1). His acuities are hand motions only (HM) and his GVF are abnormal with severe constriction down to $5^{O}$. Electroretinograms (ERGs) were undetectable. His fundus examination showed $360^{O}$ fine bone spicules in the periphery and severely attenuated vessels occulus uterque (OU) (Fig. 1). Both optic discs showed slight temporal pallor. There is a striking and significant macular atrophy (Fig. 1). On FAF this maculopathy has a 'bear claw' appearance (Fig. 1). The *in vivo* retinal architecture by OCT reveals considerable thinning of the fovea, a poorly delineated photoreceptor layer with extensive loss of the inner segments (IS)/OS junctions as well as foveal debris, which are likely attributed to the maculopathy described earlier (Fig. 1). When we reviewed the phenotype of family A, we discovered that two of seven members reported having 'kidney problems'. A pelvic ultrasound scan (US) was performed and confirmed the presence of a sub-clinical large exophytic cysts (Fig. 1). The urine analysis and blood biochemistry were within normal ranges. The patient also presented with mild cognitive impairment.

#### *Family B*

The proband of family B (II:1) is a 43-year-old female also from a French-Canadian family not known to be related to family A (Fig. 3). We found the same homozygous p. Leu710Ser mutation in *WDR19*, suggestive of a founder mutation in Quebec. Her VA was 20/100. The GVF show bilateral large central scotomas. On fundus examination (Fig. 3), bone spicules, vessels attenuation and optic disc pallor can be appreciated with a very similar atrophic foveal lesion in both eyes as was seen in family A. Furthermore, the atypical 'bear claw' shaped maculopathy can be again clearly visualized on FAF (Fig. 3). The OCT shows marked photoreceptor loss and IS/OS junction loss with atrophy with mild foveal edema (Fig. 3). There were no renal cysts or any other renal abnormalities in this proband.

### Phenotype of the *SLS* families carrying the *WDR19* mutations

All of the reported five patients exhibited retinal dystrophy and NPHP. Additionally, most showed specific phenotypic characteristics involving different organ systems. These included growth retardation and cognitive impairment, hip dysplasia and scoliosis, ventricular septum defect as well as gestational polyhydramnios and Jeune asphyxiating thoracic dystrophy (Table 2).

## Discussion

Cilia and flagella are subcellular 'antenna-like' organelles that project from the cell surface. They are highly conserved in evolution as they play important roles in cell motility, sensory stimuli reception, and early developmental processes. In humans, cilia are almost ubiquitously present on many different cell types. Photoreceptor OS in the retina represent modified cilia. Defects in their structure or function lead to a wide range of developmental problems and diseases, including RP and SLS (18–21). The assembly and further structural and functional maintenance of cilia and flagella are dependent on the process of IFT, which brings the proteins to the place of work (in the photoreceptor this is the OS). The phylogenetically highly conserved IFT represents a bidirectional machinery required for cilia development, maintenance and signaling. The anterograde (from the base to the tip) and retrograde movement (from the tip back to the base) requires three components; motor proteins, IFT particles and the cargo (proteins destined for the OS). For the anterograde movement, kinesin motors and the IFT-B particles are used, while for retrograde movement, dynein motors and IFT-A particles are utilized. Retrograde movement not only recycles the proteins but also participates in sonic hedgehog signaling (22). How the IFT A and B particles are assembled at the ciliary base, how the correct cargo is chosen and bound to the IFT particles and moved to the ciliary tip, exchanged and then released at the base on the other side of the cilium is an area of intense research and current interest.

*WDR19*, also known as *DYF-2* (in *C. elegans*) is expressed in the retina, particularly in photoreceptors but also many other tissues and encodes the IFT144 protein particle. IFT144 is an IFT-A component, involved in retrograde movement of cargo in the cilium. IFT-A components (genes) have been found to be predominantly mutated in skeletal diseases. *WDR19* encodes a large protein with 1342 amino acids, 37 exons and three different types of domains in the IFT144 protein. There are six WD40 domains, which are short structural motifs of 40 amino acids ending in the tryptophan–aspartic-acid dipeptide (W–D), two transmembrane domains (TM), and six tetratricopeptide (TPR) domains. The common function of all WD40 containing proteins is the coordination of the assembly of multi-protein complexes. TPR domains mediate protein–protein interactions and multiprotein complexes.

When we started this genetic study, *WDR19* mutations were not known to be associated with human disease. During our study, a link between *WDR19* and human disease was found by Bredrup et al. (10). Because of its crucial ciliary function and conservation between man and *C. elegans* (22, 23), we argued that it was a prime candidate gene for arRP. We then identified a novel homozygous missense mutation (p. Leu710Ser) in *WDR19* in two unrelated French-Canadian RP families. This illustrates that *WDR19* is crucial to photoreceptor survival or maintenance, and suggests that p.Leu710Ser represents a founder mutation in the Quebec population. Our further studies suggest that *WDR19* mutations are a rare cause of arRP. Striking is the

unusual 'bear track' maculopathy in both families (Figs 1 and 3), suggesting a novel genotype–phenotype correlation. Finding sub-clinical renal cysts in some of the RP patients in one of the families (Fig. 1) are highly unusual, suggesting to us to investigate more severe ciliopathies. Our hypothesis that more severe *WDR19* mutations lead to more severe systemic disease including SLS was confirmed when we identified frameshift mutations in *WDR19* in patients with SLS.

To our knowledge, this is the first study reporting the direct involvement of *WDR19* in arRP as well as in SLS. In fact, both diseases are known in part to be based on ciliary pathologies. In arRP, the photoreceptors, which are the structures reported to be damaged, are derived from a ciliary ancestor. SLS is a syndrome of NPHP associated with RP. NPHP is characterized by anemia, polyuria, polydipsia, isosthenuria and cysts mainly at the corticomedullary border of the kidney. The latter is a form of ciliopathy that develops due to an excess vacuole loss of normal tissue (24).

Once we confirmed that *WDR19* mutations are a rare cause of RP and SLS, we hypothesized further that more severe human phenotypic manifestations could also result from this ubiquitously expressed gene. During our study, Bredrup et al. answered our suspicion in their study identifying *WDR19* mutations in Sensenbrenner, Jeune syndrome and isolated NPHP (10). Remarkably, their group also identified the p.Leu710Ser mutation in several different disease entities/ciliopathies. They reported compound heterozygous mutations in Sensenbrenner syndrome (p.Leu710Ser and p.Arg1103X), combining the Leu710Ser missense mutation of our study with a nonsense mutation that likely leads to pre-mature WDR19 protein truncation. Sensenbrenner is also known as cranioectodermal dysplasia-1, an autosomal recessive disorder with sagittal craniosynostosis (early closure of the scalp and orbital sutures), and facial, ectodermal and skeletal anomalies. The most prominent phenotypic features of Sensenbrenner are remarkably bony defects, with dysmorphic facies, short limbs, abnormal teeth, skin laxity and hernias. Sensenbrenner patients can have normal retinas and normal kidneys or they can develop RP or nephronophtisis.

They also found the same homozygous missense mutation in Jeune syndrome (p.Leu710Ser), a rare autosomal recessive potentially lethal congenital dwarfism, also known as familial asphyxiating thoracic dystrophy. The phenotype is again dominated by the skeletal abnormalities, and death is often due to narrow thorax and respiratory failure. Kidney disease may be seen. Finally they found compound heterozygous mutations in isolated NPHP (p. Val345Gly and p. Tyr1023X), again combining a missense and nonsense mutation (10).

Among the seven patients reported in this study, three had novel mutations in *WDR19* (Table 2). In particular, p.Val68Asp resulted in isolated case of retinal dystrophy and NPHP while p.Ala30Pro and p.Gly109Glu lead to severer phenotypes involving skeletal and cardiac anomalies as well as gestational complications and cognitive impairment, respectively. The remaining four patients had their mutations previously reported by various groups (10–12). Besides their different severity and syndromic manifestations, all the mutations share a common pathophysiology related to a dysfunctional ciliopathy in a specific organ system. What dictates the involvement of a specific organ system *vs* another could be related to the mutation itself as well as the presence of modifying genes/mutations. Functional studies need to be undertaken to fully understand this phenomenon.

It is of great scientific interest that the p. Leu710Ser mutations in our two arRP families are the same as those identified by Bredrup et al. in families with Sensenbrenner and Jeune syndromes (10). Aside from the important clinical fact that this mutation can easily be tested as the first genetic test in RP patients with cysts and in new children diagnosed with the much more severe and deadly Jeune and Sensenbrenner syndromes, is the suggestion of modifier effects. These data of a phenotype severity spectrum (RP, RP with cysts, isolated nephronophtisis, SLS, Sensenbrenner, and lethal Jeune syndrome) associated with the same missense mutation (p. Leu710Ser) strongly suggest the presence of modifying genes/mutations, additional ciliary mutations, genetic load or micro-environment variations (25–28). For instance, a change in the penetrance of retinal degeneration among ciliopathies were associated with mutations in *RPGRIP1L*. Similarly, mutations in *AHI1* were reported to cause retinal phenotypic variations in NPHP16 (25, 29, 30). In fact, Hildebrandt et al. suggested the possible presence of a modifier gene in patients with NPHP-associated ciliopathies (22). Additionally, in their studies in *Dync2h1* knockout mice, Ocbina et al. showed the presence of a 'rescue route' based on mutations in *Ift172* and *Ift122* (31). Such mutations could serve as modifier effects in gene encoding for IFT-associated proteins and thus contribute to the phenotypic severity spectrum. The p. Leu710Ser mutation is not found in a WDR19 protein domain but the Leucine is conserved all the way down the evolutionary ladder to the nematode *C. elegans* (Fig. 2). However, its effects are severe, as Bredrup et al. found that in fibroblast cultures of Sensenbrenner patients, there was no IFT144 protein in the cilia, thus indicating a complete loss of function of IFT144 due to the p. Leu710Ser mutation (10). This missense mutation may therefore be acting as a null allele.

## Conclusion

In conclusion, we show that *WDR19* mutations are a rare cause of arRP and SLS. Our results suggest that there is a large phenotypic disease and severity spectrum associated with *WDR19* mutations. These manifestations range from isolated cases of arRP, arRP with cysts, isolated NPHP to syndromal manifestations seen in SLS and even very severe manifestations such as those in Sensenbrenner syndrome and the lethal Jeune syndrome. In the process of re-evaluating and delineating the WDR19 mutation phenotype in arRP, we discovered a novel genotype–phenotype correlation,

a very distinct 'bear claw maculopathy' (Figs 1 and 3). It will be interesting to investigate the maculopathies referred to in SLS patients and in those noted in the Bredrup et al. study. It is important to re-evaluate patients after the genotype has been determined, and to search for subtle subclinical phenotypes. Syndromic disease genes may also cause isolated diseases without the systemic manifestations.

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