MERTK mutation update in inherited retinal diseases

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Abstract

MER tyrosine kinase (*MERTK*) encodes a surface receptor localized at the apical membrane of the retinal pigment epithelium. It plays a critical role in photoreceptor outer segment internalization prior to phagocytosis. Mutations in *MERTK* have been associated with severe autosomal recessive retinal dystrophies in the RCS rat and in humans. We present here a comprehensive review of all reported *MERTK* disease causing variants with the associated phenotype. In addition, we provide further data and insights of a large cohort of 1,195 inherited retinal dystrophies (IRD) index cases applying state-of-the-art genotyping techniques and summarize current knowledge. A total of 79 variants have now been identified underlying rod-cone dystrophy and cone-rod dystrophy including 11 novel variants reported here. The mutation spectrum in *MERTK* includes 33 missense, 12 nonsense, 12 splice defects, 12 small deletions, 2 small insertion-deletions, 3 small duplications, and 2 exonic and 3 gross deletions. Altogether, mutations in *MERTK* account for ~2% of IRD cases with a severe retinal phenotype. These data are important for current and future therapeutic trials including gene replacement therapy or cell-based therapy.

KEYWORDS

inherited retinal dystrophy, *MERTK*, mutation spectrum, mutation prevalence

1 | BACKGROUND

Inherited retinal diseases (IRD) are a clinically and genetically heterogeneous group of disorders characterized by progressive photoreceptor degeneration (Hartong, Berson, & Dryja, 2006). Its prevalence is estimated from one out of 3,500 to one out of 5,000 worldwide with variable ages of onset and degrees of severity. So far, close to 250 genes have been implicated in cases of IRD (<https://sph.uth.edu/retnet/>) following all modes of Mendelian or mitochondrial inheritance with rare cases of digenism. Most of these genes encode proteins involved in various cellular functions essential for photoreceptor or retinal

pigment epithelium (RPE) homeostasis (Wright, Chakarova, Abd El-Aziz, & Bhattacharya, 2010). The most common form of IRD is rod-cone dystrophy (RCD), also known as retinitis pigmentosa (RP) (MIM# 268000), which manifests with night blindness, progressive visual field constriction and possible loss of central vision due to gradual dysfunction, and loss of rod photoreceptors followed by cone photoreceptors. On the other hand, cone and cone-rod dystrophies (CCRD) (e.g., MIM# 602093, 304020, and 120970) start by a decreased vision, photophobia with possible secondary night blindness, and peripheral visual field abnormalities leading to blindness. Of note, the distinction between RCD and CCRD is sometime clinically difficult, especially

in advanced or severe cases, and there are overlapping genetic mechanisms, mutations for some genes leading to both phenotypes (Boulanger-Scemama et al., 2015). There is currently no cure for IRD and most of them result in severe visual impairment or blindness with however promising clinical trials or therapeutic perspectives advocating for a better clinical and genetic characterization (Thompson et al., 2015).

MERTK (MER proto-oncogene, tyrosine kinase) (MIM# 604705) is one of the mutated genes in IRD. It is localized in 2q14.1 (Weier, Fung, & Lersch, 1999) and comprises 19 exons. It encodes the tyrosine-protein kinase Mer, a 999-amino-acid transmembrane protein (Uniprot # Q12866) belonging to the TAM (TYRO3/AXL/MER) receptor kinase family, a family of homeostatic regulators for the mature immune, reproductive, hematopoietic, vascular, and nervous systems mainly through its phagocytic function of apoptotic cells (Lemke, 2013). *MERTK* is highly expressed in monocyte/macrophages, ovary, prostate, testis, lung, and kidney as well as in epithelial cells including the RPE. In the RPE, it plays a critical role for shed photoreceptor outer segment (POS) engulfment prior to phagocytosis, a circadian process essential for photoreceptor homeostasis (Feng, Yasumura, Matthes, LaVail, & Vollrath, 2002; Kevany & Palczewski, 2010). *MERTK* functional domains, common to the TAM receptors, include two immunoglobulin-like-C2 (Ig-like C2 type 1 from amino acid (aa) residue 81 to 186 and Ig-like C2 type 2 aa residue 197 to 273) and two fibronectin type III (FN-III from aa residue 286 to 381 and 386 to 484) domains in its extracellular portion and an intracellular region with a highly conserved kinase domain (aa residue 587 to 858) including the KW(I/L)A(I/L)ES motif characteristic of the TAM receptors (Figure 1) (Strick & Vollrath, 2010). In addition, the kinase domain contains three autophosphorylation sites (Tyr-749, Tyr-753, Tyr-754), which are critical for its full activity (Ling, Templeton, & Kung, 1996). *MERTK* exists as a membrane-bound receptor and as a soluble form (Sather et al., 2007) through its cleavage at proline 485 by ADAM17 (MIM# 603639), at least in murine macrophages and RPE cells (Law et al., 2015; Thorp et al., 2011). This soluble form would act as a negative feedback loop limiting POS binding to RPE cell surface prior to phagocytosis (Nandrot et al., 2012). Phagocytosis of POS is a complex process which is now at least partially understood (Mazzoni, Safa, & Finnemann, 2014). The exposure of phosphatidyl serines (PS) at the surface of POS is the triggering stimulus. On one side, PS are recognized by the complex formed by the milk fat globule-EGF-factor 8 (MFG-E8) (MIM# 602281) bound to $\alpha v \beta 5$ integrin (MIM# 193210, MIM# 147561), CD36 (MIM# 173510) and possibly other unknown partners. MFG-E8/ $\alpha v \beta 5$ recognition leads to the protein tyrosine kinase 2 (MIM# 600758) (previously known as focal adhesion kinase) activation at the apical membrane of the RPE and to its autophosphorylation. This process is important for *MERTK* activation (Nandrot et al., 2004). On the other side, *MERTK* indirectly interacts with PS through its ligands, Gas6/Protein S (MIM# 600441, MIM# 176880) which results in *MERTK* autophosphorylation at the three sites above-mentioned and in cellular actin remodeling, through myosin light chain 2 (MIM# 160781). *MERTK* is then involved in POS engulfment through an interaction with its ligands. POS are subsequently internalized and degraded (reviewed in Kevany & Palczewski, 2010).

MERTK was first associated with retinal degeneration after the identification of a deletion causing the naturally occurring autosomal recessive (ar) retinal degeneration of the Royal College of Surgeon (RCS) rat (D'Cruz et al., 2000; Nandrot et al., 2000), a retinal dystrophy model secondary to a phagocytic defect of the RPE (Bok & Hall, 1971; Dowling & Sidman, 1962; Edwards & Szamier, 1977; Herron, Riegel, Myers, & Rubin, 1969; LaVail & Battelle, 1975; Li & Turner, 1988). Shortly after this discovery, mutations in *MERTK* were identified in patients with ar RP (Gal et al., 2000). More recently, an overexpression of *MERTK* was found in a canine progressive retinal atrophy, from a Swedish Vallhund breed, suggesting a gain of function mutation which still needs to be molecularly characterized (Ahonen et al., 2014). An ongoing clinical trial with gene augmentation in patients with IRD linked to *MERTK* mutations in Saudi Arabia (NCT01482195) (Ghazi et al., 2016) as well as interesting perspectives in cell therapy (Zarbin, 2016) are an incentive to conduct comprehensive studies to prepare patients for these therapies. In the present manuscript, we provide a comprehensive review on reported variants in *MERTK* associated with IRD, add further data generated by the screening of a large French cohort of more than 1000 IRD index cases, deliver new information on prevalence and discuss phenotype-genotype correlations.

2 | MATERIAL AND METHODS

2.1 | Literature search

Literature search was performed on Pubmed, with a last check on March 12th, 2018, in order to collect all reported variants on *MERTK* (NM_006343.2) in association with IRD. Additional databases were queried such as The Human Gene Mutation Database (HGMD® Professional 2017.4, last queried on March 12th, 2018), Leiden Open Variation Database (LOVD V.3.0), last queried on March 12th, 2018, Retina International Mutation Database (<https://www.retina-international.org/files/sci-news/mertkmult.htm>, last updated in March 2004). For each of the reported variants, we collected information on phenotype/genotype correlation, familial segregation and documented minor allele frequency (MAF) as well as assessed functional impact through in silico pathogenic predictions tools.

2.2 | Novel *MERTK* variants

In addition to the comprehensive literature search, we analyzed a large French cohort of 1,195 unrelated subjects with IRD. Each patient underwent a full ophthalmic examination as described elsewhere (Audo et al., 2010). After this clinical evaluation, patients and family members were asked to donate a blood sample for DNA extraction and further genetic studies. Written informed consent was obtained from each subject after explanation of the study and its potential outcomes. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee (CPP, Comité de Protection des Personnes Ile de France V, project number 06693, N° EUDRACT 2006-A00347-44).

The DNA of patients with a presumed diagnosis of autosomal recessive RCD and cone-rod dystrophy (CRD) was assessed. Microarray

FIGURE 1 Distribution of the 68 *MERTK* variants reported in the literature (sources: pubmed search up to the March 12, 2018) and the 11 novel variants identified by our group in the gene and protein. Nucleotide numbering is based on cDNA sequence of *MERTK* (RefSeq NM_006343.2) where A of the ATG initiation codon is 1. The nonsense and frameshift mutations appear in red, gross deletions appear in purple, missense variants in blue and the splice site change in green. Novel variants are circled in black. The protein is a transmembrane protein and its structure includes: in the extracellular portion, two immunoglobulin-like-C2 (Ig-like C2 type 1 and type 2) from amino acid (aa) residue 81 to 186 and residue 197 to 273, respectively, two fibronectin type III domains (FN-III) in blue from aa residue 286 to 381 and 386 to 484 and an intracellular region with a highly conserved kinase domain (in yellow from aa residue 587 to 858). The respective residues are written below the protein schematic

analysis on a commercial chip (ASPER Ophthalmics, Tartu, Estonia) (Koenekoop, Lopez, den Hollander, Allikmets, & Cremers, 2007) was initially applied to 186 index patients with IRD. Samples with no identified pathogenic variant as well as additional new index cases were subsequently investigated through a targeted next-generation sequencing (NGS) as described previously (Audio et al., 2012; Boulanger-Scemama et al., 2015). Guidelines from the American College of Medical Genetics and Genomics were used for variant classification (Richards et al., 2015). Functional impact was assessed using in silico prediction tools (i.e. Align GVGD (https://agvgd.hci.utah.edu/agvgd_input.php) (Tavtigian et al., 2006), Mutation Taster (<https://www.mutationtaster.org/>) (Schwarz, Cooper, Schuelke, & Seelow, 2014), Sorting Intolerant From Tolerant SIFT (<https://sift.jcvi.org/>) (Kumar, Henikoff, & Ng, 2009), Polymorphism Phenotyping v2 Polyphen-2 (<https://genetics.bwh.harvard.edu/pph2/>) (Adzhubei et al., 2010), Splice Site Prediction by Neural Network, NNSPLICE, https://www.fruitfly.org/seq_tools/splice.html, (Reese, Eeckman, Kulp, & Haussler, 1997); Human Splicing Finder v.2.4.1, HSF, <https://www.umd.be/HSF/#>, (Desmet et al., 2009) and evolutionary conservation (UCSC Genome Browser (<https://genome.ucsc.edu/index.html>; Human GRCh37/hg19 Assembly)). Only rare variants with a MAF below or equal to 0.005 in the Exome Aggregation consortium (ExAC, <https://exac.broadinstitute.org/>) and genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org/>) were considered. Each predicted pathogenic variant was confirmed by Sanger sequencing of the respective target (RefSeq NM_006343.2, primer sequences available

upon request). NGS data were also analyzed to detect Copy Number Variation and cases with large deletions were confirmed applying Multiplex Ligation-dependent Probe Amplification as performed before (Boulanger-Scemama et al., 2015). Familial segregation analysis with Sanger sequencing of the PCR amplified fragments including the identified putative pathogenic variant(s) was performed when possible.

3 | *MERTK* VARIANTS UNDERLYING IRD

A total of 68 genetic changes were found in the literature underlying IRD. These variants span the entire gene and therefore impact on all functional domains (Figure 1 and Table 1). In addition, the screening of a large cohort of IRD followed at our center (1,095 RCD and 100 CRD) identified 11 novel changes (Figure 1, Table 1, Supp Figure S1, and Supp. Table S1). Altogether, the mutation spectrum includes 33 missense mutations among which 4 may affect splicing and 13 are of questionable pathogenicity (based on allele frequency >0.005, functional assays or only found at the single heterozygous state, presented in italic in Table 1), 12 nonsense mutations, 12 splice site defects, 12 small deletions, 2 small insertion-deletions, 3 small duplication, 2 large exonic deletions, and 3 gross deletions. The screening of our large IRD cohort identified a total of 20 distinct mutations among which 11 are novel. The mutation spectrum in our cohort includes 3 small deletions, 2 small duplications, 1 large exonic deletion, 2 splice defects, 9 missense and 3 nonsense changes. All the mutations were

TABLE 1 Variants in MERTK reported in the literature and novel variant identified in a large French cohort in association with inherited retinal dystrophies

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
1-7	91 kb deletion	-	Most likely an absence of protein product	Ho	RP	Founder mutation in the Faroe Island which would represent 30% of RP in this island!	-	-	Ostergaard, Duno, Batbayli, Vilhjalmsson, and Rosenberg (2011)
1	c.3G>A	p.(Met1?)	Start codon loss	Ho	RP	Thailand	-	-	Jinda et al. (2016)
1	c.35T>C	p.(Leu12Pro)	Signal peptide	Ho	RP	Japan	rs75593299 ExAC no MAF gnomAD 0.00001872, only 4 het allele	Align GVGD class C0; SIFT Deleterious (score: 0.04); Mutation Taster polymorphism (P value: 1); Polyphen2 possibly damaging with a score of 0.910	Tada et al. (2006)
1	c.59G>C	p.(Arg20Thr)	Signal peptide	Ho	RP	Pakistan	rs552509122 No MAF in ExAC or gnomAD but one other variant for the same codon rs35898499 c.60A>T p.(Arg20Ser) ExAC 0.05467 gnomAD 0.03604 with 218 ho cases	Align GVGD class C0; SIFT tolerated (score 0.38); Mutation Taster polymorphism (P value = 1); Polyphen2 benign with a score of 0 (sensitivity: 1.00; specificity: 0.00) with poor conservation (polymorphic residue with a Thr present in Opossum)	(Tschernutter et al., 2006) considered this variant as most likely not disease causing since the p.Arg20Ser (rs35898499) has a MAF of 0.05467 and the residue is poorly conserved
1	c.60del	p.(Ala21Leufs*43)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment or NMD	Putative compound het with c.1796G>A; p.(Gly599Glu) Familial segregation was not available	RCD	France	-	-	Novel
IVS1	c.61+1G>A	Splicing defect	Exon skipping leading to a shorter protein product with only the N-terminus fragment or NMD	Compound het with c.1951C>T; p.(Arg651*)	Early onset RD	Caucasian	-	-	(Mackay et al., 2010)
IVS1	c.62-1G>A	Splicing defect	Exon skipping leading to a shorter protein product with only the N-terminus fragment or NMD	Compound het with c.1296+1G>A	RP/RCD	Not specified (US cohort)	-	-	(Wang et al., 2014b)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
2	c.92_98del	p.(Pro31Argfs*31)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment or NMD	Ho	RP	Not specified (US and Canadian cohorts)	-	-	(Wang et al., 2014a)
2	c.225del	p.(Gly76Gluufs*3)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment or NMD	Compound het with c.370C > T p.Gln124*	RP/RCD	Japanese	rs527236083 ExAC no MAF gnomAD 0.000004061 with only 1 het allele	-	(Oishi et al., 2014)
2	c.308_309del	p.(Ile103Asnfs*4)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment and part of Ig-like C2 type I or NMD	Ho	RP	Chinese	-	-	(Yang et al., 2018)
2	c.325A > T	p.(Lys109*)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment and part of Ig-like C2 type I or NMD	Ho	RP/RCD	Saudi Arabia	rs786205533 ExAC no MAF gnomAD no MAF	-	(Patel et al., 2015)
2	c.343T > G	p.(Cys115Gly)	Ig-like C2 type I domain	Only het change	RP	Japan	-	Align GVGD class C65; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Tada et al., 2006)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
2	c.345C > G	p.(Cys115Trp)	Ig-like C2 type I domain	Compound het with c.1530del; p.(Ala446Serfs*28)	RP/RCD	Germany	rs772421550 ExAC 0.00007 gnomAD 0.00004329, only het alleles (12 including 10 in European non-Finish)	Align GVGD class C65; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Eisenberger et al., 2013)
2	c.370C > T	p.(Gln124*)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment and part of Ig-like C2 type I or NMD	Compound het with c.225del p.(Gly76Glu fs*3)	RP/RCD	Japanese	rs527236134 ExAC no MAF gnomAD no MAF	-	(Oishi et al., 2014)
2	c.390G > A	p.(Trp130*)	Premature stop codon leading to a shorter protein product with only the N-terminus fragment and part of Ig-like C2 type I or NMD	Ho	RP	Not specified (US and Canadian cohorts)	-	-	(Wang et al., 2014a)
3-19	119 kb deletion	-	Most likely absence of protein product	Ho	CRD	France	-	-	(Boulangier-Scemama et al., 2015)
3	c.500G > A	p.(Arg167His)	Ig-like C2-type 1 domain	Single het	RP	Not specified (US cohort)	rs781470358 ExAC 0.0000033 gnomAD 0.00003612, only het alleles (10 including 7 in European non-Finish)	Align GVGD class C25; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Mandal et al., 2005)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
4	c.634A > C	p.(Thr212Pro)	Ig-like C2-type 2 domain	Compound het with c.345C > G, p.(Cys115Trp)	IRD not speci- fied	British cohort	-	Align GVGD class C35; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 0.997) Polyphen2 probably damaging with a score of 0.988 (sensitivity: 0.73; specificity: 0.96) and conserved residue	(Khan et al., 2017)
4	c.640T > G	p.(Phe214Val)	Ig-like C2-type 2 domain	Single het	LCA	China	ExAC no MAF gnomAD 0.000008124, only 2 het alleles in East Asians	Reported only as a single het but partial sequencing of the exons Align GVGD class C45; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Li et al., 2011)
4	c.665G > A	p.(Gly222Asp)	Ig-like C2-type 2 domain	Compound het with c.665_666delinsAA; p.(Gly222Glu) Phase confirmed by familial segregation	CRD	France	-	AA change from nonpolar neutral to polar negative Align GVGD class C65; SIFT deleterious score 0; mutation taster disease causing P value = 1; Polyphen2 probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)	Novel

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if spec- ified	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
4	c.665_666 delinsAA	p.(Gly222Glu)	Ig-like C2-type 2 domain	Compound het with c.665G > A; p.(Gly222Asp) Phase confirmed by familial segregation	CRD	France	-	AA nonpolar neutral changed to AA polar neutral Align GVGD class C65; SIFT deleterious score 0, no prediction on Mutation Taster; Polyphen2, probably damaging, P value = 1, (sensitivity: 0.00; specificity: 1.00)	Novel
4	c.718G > T	p.(Glu240*)	Premature stop codon leading to a shorter protein product with part of Ig-like C2 type 2 domain or NMD	ho	RP	Pakistan	-	-	(Shahzadi et al., 2010)
4	c.721C > T	p.(Gln241*)	Premature stop codon leading to a shorter protein product with part of Ig-like C2 type 2 domain or NMD	Ho	RP	India	-	-	(Srilekha et al., 2015)
5	c.785G > T	p.(Cys262Phe)	Ig-like C2-type 2 domain	Ho	RCD	Russia	-	AA able to form disulfide bound changed to an aromatic AA Align GVGD class C65; SIFT deleterious score 0; mutation taster disease causing P value = 1; Polyphen2 probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)	Novel

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
5	c.791C > G	p.(Ala264Gly)	Ig-like C2-type 2 domain	Single het	RP	Germany	rs199779970 ExAC 0.0004284 gnomAD 0.0003861, only het alleles	Align GVG D class C55; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Eisenberger et al., 2013) Reported only as a single het in a patient with two pathogenic mutations in EYS
5	c.814G > A	p.Val272Met	Ig-like C2-type 2 domain	Compound het but not specified with which variant	RP	China	rs140065278 ExAC 0.0000082 gnomAD 0.00007577, only het alleles	Align GVG D class C15; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 0.939); Polyphen2 probably damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) and conserved residue except in Lizard (Thr residue)	(Xu et al., 2014) who considered it as less likely pathogenic with additional more pathogenic variant as a disease cause
6	25 kb deletion including ex6-8	-	Most likely an absence of protein product	Ho	RP/RCD	Newfoundland, Canada	-	-	(Evans et al., 2017)
6	c.933_935 delinsTT	p.(Pro313Argfs*15)	Premature stop codon leading to a shorter protein product with part of the first FN-III domain or NMD	Compound het with c.2180G > A p.(Arg727Gln)	RP/RCD	Not specified (British cohort)	-	-	(Ellingford et al., 2016)
7	c.985_988dup	p.(Gly330Glu fs*8)	Premature stop codon leading to a shorter protein product with part of the first FN-III domain or NMD	Compound het with c.1450G > A p.(Gly484Ser) Phase confirmed by familial segregation	RCD	France	rs777350533 ExAC or dbSNP no MAF gnomAD 0.000008122, two het allele in European non-Finish	-	Novel

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
7	c.986A > G	p.(Asn329Ser)	First FN-III domain	Ho	RCD	Madagascar	rs34943572; ExAC 0.0002553 gnomAD 0.0003210 found once ho in 'other' populations	Both AA are polar neutral Align GVGD class C0; SIFT deleterious score 0.03; mutation taster disease causing <i>P</i> value = 0.995; Polyphen2 possibly damaging with a score of 0.472 (sensitivity: 0.89; specificity: 0.90)	Novel
7	c.992_993del	p.(Ser331Cysfs*5)	Premature stop codon leading to a shorter protein product with part of the first FN-III domain or NMD	Ho	RP/RCD	Spain	-	-	(Lukovic et al., 2015)
8	~9 kb deletion		Shorter protein product or NMD	Ho	Early onset RD	Middle East		-	(Mackay et al., 2010)
8	c.1179dup	p.(Leu394Serfs*3)	Premature stop codon leading to a shorter protein product with part of the second FN-III domain or NMD	Ho	RP/RCD	The Netherlands		-	(Collin et al., 2011)
8	c.1186G > T	p.(Glu396*)	Premature stop codon leading to a shorter protein product with part of the second FN-III domain or NMD	Ho	RP	China	-	-	(Xu et al., 2014)
IVS8	c.1296+1G > A	Splicing defect	Exon skipping leading to a shorter protein product with part of the second FN-III domain or NMD	Compound het with no specified variant in Xu et al., 2014 and with c.62-1G > A in Wang et al., 2014a	RP/RCD	Not specified (US cohort)	rs774577413 ExAC 0.00003296 gnomAD 0.00002889, only het alleles	-	(Xu et al., 2014) who questioned its pathogenicity (Wang et al., 2014b) who considered it as pathogenic

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
IVS8	c.1297-2A > G	Splicing defect	Exon skipping leading to a shorter protein product with part of the second FN-III domain or NMD	Ho	RP	Spanish	-	-	(Mendez-Vidal et al., 2014)
9	c.1301_1302del	p.(Glu434Alafs*40)	Premature stop codon leading to a shorter protein product with part of the first FN-III domain or NMD	Ho	RCD	North Africa	rs776644374 ExAC or dbSNP no MAF gnomAD 0.000004064, only one het allele in European non-Finish	-	Novel
9	c.1335_1336del	p.(Ala446Serfs*28)	Premature stop codon leading to a shorter protein product with part of the second FN-III domain or NMD	Ho	RP/RCD	Saudi Arabia	-	-	(Aldahmesh et al., 2009)
9	c.1441C > T	p.(Pro481Ser)	At the end of the second FN-III domain	Single het	RP	China	rs781442827 ExAC 0.00003295 gnomAD 0.00004692, only het alleles	Align GVGD class C35; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1) probably damaging with a score of 1 (sensitivity: 0; specificity: 1) and conserved residue Predicted to affect splicing	(Xu et al., 2014)
9	c.1450G > A	p.(Gly484Ser)	Most likely affect splicing with exon skipping leading to a shorter protein product with the absence of the transmembrane domain and the kinase domain or NMD	Ho	RP	Italy	rs527236084 ExAC no MAF gnomAD 0.00001219, only het alleles	Align GVGD class C0; SIFT tolerated (score 0.29); Mutation Taster disease causing (P value = 1) Polyphen2 benign with a score of 0.444 (sensitivity: 0.89; specificity: 0.90) and conserved residue Predicted to affect splicing	(Eisenberger et al., 2013)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
10	c.1530del	p.(Cys510Trpfs*5)	Premature stop codon leading to a shorter protein product missing the kinase domain or NMD	Compound het with c.345C > G (p.Cys115Trp)	RP/RCD	Germany	-	-	(Eisenberger et al., 2013)
IVS10	c.1604+2T > G	Splicing defect	Exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho on presumed uniparental paternal disomy	RP/RCD	Saudi Arabia	rs786205534 ExAC no MAF gnomAD no MAF	-	(Patel et al., 2015)
IVS10	c.1605-2A > G	Splicing defect	Exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho	RP/RCD	Not specified (Germany?)	rs730880273 ExAC no MAF gnomAD 0.00006456, only het alleles	-	(Gal et al., 2000)
IVS11	c.1690+1G > A	Splicing defect	Exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho	RP	China	rs761251269 ExAC 0.000000082 gnomAD 0.000004063, only one het allele	-	(Huang et al., 2015)
IVS11	c.1691-1G > A	Splicing defect	Exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho	RP	China	rs746108786 ExAC 0.000000082 gnomAD 0.000004062, only one het allele	-	(Xu et al., 2014)
12	c.1744_1751delinsT	p.(Ile582*)	Shorter protein product missing the kinase domain or NMD	Ho	LCA	Turkey	-	-	(Eisenberger et al., 2013)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
12	c.1786G > A	p.(Gly596Arg)	Most likely affect splicing with exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho	RP/RCD	Germany	-	Align GVG D class C15; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Eisenberger et al., 2013)
IVS12	c.1787-2A > C	Splicing defect	Exon skipping leading to a shorter protein product missing the kinase domain or NMD	Ho	RP	Not specified (US cohort)	No MAF in ExAC or gnomAD but one other variant c.1787-2A > T reported only in gnomAD with a MAF of 0.000004061, only one het alleles	-	(Ge et al., 2015)
13	c.1796G > A	p.(Gly599Glu)	Kinase domain	Putative compound het with c.60del; p.(Ala21Leufs*43) Familial segregation was not available	RCD	France	-	AA nonpolar neutral changed to AA polar neutral Align GVD class C0; SIFT deleterious score 0, mutation taster disease causing P value = 1, Polyphen2 probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)	Novel

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
13	c.1866G > C	p.(Lys622Asn)	Kinase domain May affect splicing	Ho	RP/RCD	Not specified (British cohort)	rs775460185 ExAC 0.000000082 gnomAD 0.000004061, only one het allele	Align GVGD class C0; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Ellingford et al., 2016)
14	c.1893C > G	p.(Ile631Met)	Kinase domain	Single het	LCA	Unspecified, (Belgium cohort)	rs543947091 ExAC no MAF gnomAD 0.000004061, only one het allele	Align GVGD class C0; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Coppie et al., 2012)
14	c.1951C > T	p.(Arg651*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Single het in Gal et al. but compound het with c.61+1G > A in Mackay et al., 2010	RP	Unspecified (Germany for Gal et al.?)	rs119489105 ExAC 0.00004945 gnomAD 0.00005686, only het allele	-	(Gal et al., 2000) (Mackay et al., 2010)
15	c.1961G > T	p.Gly654Val	Exon 15 skipping as demonstrated from patient's mRNA which may lead to an abnormal kinase domain	Ho	RP/RCD	Spain	-	Align GVD Class C0; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Riera et al., 2017) Demonstrated to affect splicing on patient's mRNA with small amount of normal length mRNA

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
15	1732-bp deletion	p.Gly654Alafs*41 or NMD	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RP/RCD	Indonesia	-	-	(Siemiakowska et al., 2011)
15	c.2070_2074del	p.(Gly691Lysfs*42)	Premature stop codon leading to a shorter protein product with truncated kinase domain or NMD	Ho	RP	Unspecified (Germany for Gal et al.?)	-	-	(Gal et al., 2000)
IVS15	c.2079+2T > G	Splice defect	Most likely exon skipping leading to a shorter protein product with a truncated kinase domain or NMD	Putative ompound het with c.1951C > T p.(Arg651*) Familial segregation was not available	RCD	Lebanon	-	-	Novel
16	c.2105dup	p.(Phe703Valfs*32)	Premature stop codon leading to a shorter protein product with truncated kinase domain or NMD	Putative compound het with c.345C > G; p.(Cys115Trp) Familial segregation was not available	RCD	France	-	-	Novel
16	c.2163T > A	p.(His721Gln)	Kinase domain	Ho	RCD	Albania	rs749472520 ExAC 0.00008237, gnomAD 0.000004061, only one het allele in European non-Finish	AA polar positive changed to an AA polar neutral Align GVG D class C15; SIFT deleterious score 0; mutation taster disease causing P value = 1; Polyphen2 probably damaging with a score of 1 (sensitivity: 0; specificity: 1)	Novel

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
16	c.2164C > T	p.(Arg722*)	Premature stop codon leading to a shorter protein product with truncated kinase domain or NMD	In cis with c.2593C > T p.Arg865Trp; Compound het with p.Arg844Cys And also reported with c.2219C > T p.(Ala740Val)	CRD	US cohort	rs541717028 ExAC 0.0000247 gnomAD 0.00002165, only het alleles	-	(McHenry et al., 2004)
16	c.2171del	p.(Leu724*)	Premature stop codon leading to a shorter protein product with truncated kinase domain or NMD	Compound het with c.1296+1G > A	RP	China	-	-	(Xu et al., 2014)
16	c.2179C > T	p.(Arg727*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Compound het with c.2530C > T p.Arg844Cys	RP/RCD	Not specified (US cohort)	ExAC no MAF gnomAD 0.000004061, only het alleles	Align GVGD class C35; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Al-Kharsan et al., 2017)
16	c.2180G > A	p.(Arg727Gln)	Kinase domain	Ho	RP/RCD	Turkey	No MAF in ExAC or gnomAD but one other change in the same codon (c.2179C > G, p.Arg727Gly, rs746238212, not found in ExAC, gnomAD 0.000004061, only one het allele	-	(Coppieters et al., 2014)
IVS16	c.2189+1G > T	Splicing defect with p.(His694Valfs*4)	Exon 16 skipping leading to a shorter protein product with a truncated kinase domain or NMD	Ho	CRD	Morocco	rs371956016ExAc 0.000008237gno- mAD 0.00002165, only het alleles	-	(Ebermann et al., 2007)
17	c.2194C > T	p.(Arg732*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RP	Unspecified (British cohort)	-	-	(O'Sullivan et al., 2012)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
17	c.2214del	p.(Cys738Trpfs*32)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RCD with early onset macu- lar involve- ment	Middle East Recurrent mutation in Saudi Arabia (Patel et al., 2015)	-	-	(Tschernutter et al., 2006)
17	c.2219C > T	p.(Ala740Val)	Kinase domain	Compound het with c.2164C > T p.(Arg722*)	RP/RCD	Not specified (US cohort)	ExAC no MAF gnomAD 0.000004066, only one het allele	Align GVG D class C65; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Wang et al., 2014b)
17	c.2237A > G	p.(Lys746Arg)	Kinase domain	Single het	LCA	Unspecified (Belgium cohort)	-	Align GVG D class C25; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Coppieters et al., 2012)
17	c.2262C > G	p.(Tyr754*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RP/RCD	Saudi Arabia	rs786205535 ExAC no MAF gnomAD no MAF	-	(Patel et al., 2015)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
17	c.2287C > A	p.(Pro763Thr)	Kinase domain	Compound het with c.390G > A; p.(Trp130*)	RP/RCD	Not specified (US cohort)		Align GVGD class C35; SIFT deleterious (score 0); Mutation Taster disease causing (<i>P</i> value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Ge et al., 2015)
17	c.2302G > A	p.(Ala768Thr)	Kinase domain	Ho	RP/RCD	Not specified (British cohort)	ExAC no MAF; gnomAD 0.00002445, only het alleles	Align GVGD class C55; SIFT deleterious (score 0); Mutation Taster disease causing (<i>P</i> value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Ellingford et al., 2016)
17	c.2323C > T	p.(Arg775*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RP/RCD	Morocco	rs387907314 ExAC 0.000008246 gnomAD no MAF	-	(Ksantini, Lafont, Bocquet, Meunier, & Hamel, 2012)
18	c.2377del	p.(Ile793*)	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	CRD	Not specified (US cohort)	ExAC no MAF gnomAD 0.000004061, only one het allele		(Consugar et al., 2015)

(Continues)

TABLE 1 (Continued)

Exon/ intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic missense changes	Reference of the first report
18	c.2435A > C	p.(Tyr812Ser)	Kinase domain	Single het	LCA	Saudi Arabia	rs141361084 ExAC 0.0000247 gnomAD 0.00006903, only het allele	Align GVGD class C65; SIFT deleterious (score 0); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Eisenberger et al., 2013) Reported only as single het in a patient with ho pathogenic mutation on RPGRIP1
IVS18	c.2487-2A > G	Splicing defect	Premature stop codon leading to a shorter protein product with a truncated kinase domain or NMD	Ho	RP/RCD	Indonesia	-	-	(Siemiatkowska et al., 2011)
19	c.2530C > T	p.Arg844Cys	Kinase domain	Compound Hz with complex allele p.(Arg722*) p.Arg865Trp And also reported with c.2179C > T p.(Arg727*)	CRD	US cohort	rs746291728 ExAC 0.00001650 gnomAD 0.000008123, only het alleles	Align GVGD class C15; SIFT deleterious (score 0.01); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(McHenry et al., 2004)
19	c.2531G > C	p.(Arg844Pro)	Kinase domain	Ho on possible uniparental disomy	RP/RCD	The Netherlands	-	Align GVGD class C0; SIFT deleterious (score 0.02); Mutation Taster disease causing (P value = 1); Polyphen2 probably damaging with a score of 1 (sensitivity: 0.00; specificity: 1.00) and conserved residue	(Collin et al., 2011)

(Continues)

TABLE 1 (Continued)

Exon/ Intron	cDNA (NM_ 006343.2)	Protein change	Protein domain involved/putative functional consequence	Status	Phenotype if speci- fied	Country of origin	RS # MAF in ExAC/gnomAD if available	Remarks and pathogenic prediction for missense changes	Reference of the first report
19	c.2593C > T	p.Arg865Trp	Kinase domain	Complex allele in cis with p.Arg722* and trans with p.Arg844Cys	CRD	US cohort	rs2230516 ExAC 0.001837 gnomAD 0.001984 with one ho case	Align GVGD class 0; SIFT deleterious (score 0.02); Mutation Taster polymorphism (p value = 1); Polyphen2 probably damaging with a score of 0.958 (sensitivity: 0.78; specificity: 0.95) and poorly conserved residue)	(McHenry et al., 2004) who performed functional analysis and considered it likely benign
19	c.2726G > A	p.(Arg909His)	C-terminus	Single het	RP/RCD	Japan	rs149131360 ExAC 0.0000095 gnomAD 0.00009022 with only het alleles	Align GVGD class C0; SIFT tolerated (score: 0.19); Mutation Taster polymorphism (P value = 1) Polyphen2 benign with a score of 0 (sensitivity: 1.00; specificity: 0.00) with same change in nonhuman primate	(Tada et al., 2006)
19	c.2873C > T	p.(Pro958Leu)	C-terminus	Single het	LCA	China	rs201460398 ExAC 0.0000033 gnomAD 0.00003251, only het alleles	Align GVGD class C0; SIFT tolerated (score: 0.7); Mutation Taster polymorphism (p value = 1) Polyphen2 benign with a score of 0.001 (sensitivity: 0.99; specificity: 0.15) and low conservation with same change in dog	(Li et al., 2011)

Variant mentioned in *italic* are those considered as nonpathogenic based on allele frequency, functional assay or only found at the single heterozygous state; NMD, nonsense-mediated mRNA decay; RP, retinitis pigmentosa; RCD, rod-cone dystrophy; CRD, cone-rod dystrophy; LCA, Leber congenital amaurosis; in the phenotypic column we mentioned the phenotype reported in the respective paper. RCD is most of the time considered as a synonymous for RP, with the symptom at onset characterized by night blindness followed by peripheral visual field constriction and secondary loss of vision; on the contrary, CRD usually starts by central retinal involvement with decreased central vision, photophobia and secondary loss of peripheral vision; this phenotypic distinction is however somewhat artificial, sometime based only on retrospective data when patients present with advance retinal disease: het, heterozygous; ho, homozygous.

unique except two: the known c.2189+1C>T splice variant (Ebermann et al., 2007) was found in four independent families including three of North African descent and may represent a founder effect in this population and the large exonic deletion encompassing exon 3 to exon 19 that we reported earlier (Boulanger-Scemama et al., 2015), which was found in three unrelated cases including one from African descent (Burkina Faso) and 2 from French descent. Novel mutations include five missense changes, one 2-bp-insertion-deletion also resulting in an amino acid substitution. Three of these amino acid substitutions are located within the Ig-like C2 type 2 domain, one within the first FN-III domain and two within the kinase domain (Figure 1). Amino acid residues involved by these mutations are highly conserved (Figure 2) and the changes induced by the amino acid substitutions are predicted to be pathogenic by prediction programs (Table 1). We found one novel splice site variant (c.2079+2T>G), the sequence being conserved across species (Figure 2). This change is predicted to affect the wild type donor site most probably affecting splicing of intron 15 (Desmet et al., 2009). This may result in the continuation of protein translation after the lysine encoded by the last codon of exon 15 with the addition of 8 amino acid residues before reaching a stop codon. An alternative cryptic site in intron 15 could also be unmasked with also part of intron 15 being translated. In both instances, this would result in a truncation of the protein missing part of the kinase domain or in nonsense-mediated mRNA decay (NMD). Additional novel changes include one 1-bp-deletion, one 2-bp-deletion, one 1-bp-duplication, and one 4-bp-duplication, all resulting either in a frameshift with premature stop codon and a truncated protein product or NMD. Our findings therefore add 11 novel changes to the 68 variants reported in the literature for a total of 79 reported variants (Table 1) spanning the entire coding and exon-flanking region (Figure 1).

All mutations identified by our comprehensive literature review and described herein, were submitted to the existing Locus Specific Databases (LSDB) on *MERTK* (<https://www.LOVD.nl/MERTK>).

4 | PREVALENCE DATA

The screening of our cohort including 1,095 RCD and 100 CRD index cases lead to the identification of 25 affected subjects belonging to 20 unrelated families (Supp. Figure S2) who carried likely pathogenic mutations in *MERTK* including 21 cases of RCD and four cases of CRD. Therefore, mutations in *MERTK* would account for a prevalence of ~1.7% IRD families in this large cohort with nearly 4% in the CRD subgroup. These numbers are comparable with previous reports with prevalence in the literature ranging from 1% (Huang et al., 2015; Strick & Vollrath, 2010; Tschernutter et al., 2006), ~2% (Ge et al., 2015), 3% (Patel et al., 2016) and up to 30% in the Faroe Islands with a common 91 kb founder deletion encompassing exons 1 to 7 (Ostergaard, Duno, Batbayli, Vilhelmsen, & Rosenberg, 2011). Of note, the 4% prevalence in our CRD should be taken with caution as the distinction between RCD and CRD is often difficult especially in advanced cases and may have been made retrospectively in some cases. This higher prevalence

may only reflect the early macular involvement characterizing IRD associated with *MERTK* mutations.

5 | CLINICAL CHARACTERISTICS OF PATIENTS CARRYING *MERTK* MUTATIONS

Mutations in *MERTK* underlying IRD was first reported by Gal et al. (2000), following the genetic characterization underlying retinal degeneration in the RCS rat (D'Cruz et al., 2000). The associated IRD phenotype is usually severe characterized by an early onset of the disease and rapid macular involvement. The screening of our large IRD cohort (i.e., 1,095 RCD and 100 CRD, with a distinction made retrospectively based on the initial symptoms) identified 25 patients with *MERTK* mutation whose clinical findings are summarized in Supp. Table S2 with representative retinal images presented in Figure 3. The 25 patients carrying mutations in *MERTK* were examined including 13 male and 12 female patients, with an age at time of examination ranging from 13 to 60 with an average of 28.76 and a median of 25. They all experienced onset of symptoms, consisting primarily of night vision disturbances, before 18 with a majority of them during their early teens. Of note, none of the patients had nystagmus, which was only reported once in association with *MERTK* mutations (McHenry et al., 2004). They all presented with severe retinal abnormalities and early macular involvement consistent with previous reports on IRD associated with *MERTK* mutations (Charbel Issa et al., 2009; Ebermann et al., 2007; Gal et al., 2000; Jinda et al., 2016; Ksantini, Lafont, Bocquet, Meunier, & Hamel, 2012; Mackay et al., 2010; McHenry et al., 2004; Thompson et al., 2002; Tschernutter et al., 2006).

Visual acuity ranges from light perception to 20 out of 32 for the youngest patient. Color vision was abnormal in all tested patients (24 out of 25) with 32 eyes showing severe dyschromatopsia, 10 eyes with tritan, two with deutan, and one with protan defects. Visual fields were constricted in all patients with a majority (92%) reduced to the 20 central degrees or below at the binocular III4e stimulus. All patients had none-detectable full field and multifocal ERG responses in keeping with severe generalized retinal dysfunction for both the rod and cone system with additional severe macular dysfunction. In this respect, the distinction between RCD (21 patients) and CRD (4 patients) could not be made based on functional assessment but mainly on retrospective elements, patients reporting initial decreased central vision, possible photophobia followed by night vision disturbances as inaugural symptoms, and macular atrophy, being classified as CRD. This distinction is however questionable and combined photoreceptor dystrophy could in these cases with severe functional involvement be a better term.

On fundus examination, 20 out of 25 patients had waxy pallor of the optic discs (e.g., Figure 3A and B), almost all had narrowed retinal vessels (e.g., Figure 3A, B, and D but not C), 13 out of 25 had a beaten-bronze appearance with very few pigment migrations (e.g., Figure 3A and C) among which three had subtle white dots in the retinal periphery (e.g., Figure 3A and C) with one also in the macular area. Along with the early onset macular involvement, the

	222	262	329	599	721
	E/D	F	S	E	Q
Human	TCQAVGPPPEPV	MAVFSCEAHND	ADPLSNGSVMI	CAGGEFGSVMEG	NRNFLHRDLAA
Orangutan	TCQAVGPPPEPV	MAVFSCEAHND	ADPLSNGSVMI	CAGGEFGSVMEG	NRNFLHRDLAA
Rhesus	TCQAVGPPPEPV	MAVFSCEAHND	VDPLSNGSVMI	CAGGEFGSVMEG	NRNFLHRDLAA
Marmoset	TCQAVGPPPEPV	MAVFSCEAHNE	VDPLSNGSVMI	CAGGEFGSVMEG	NRNFLHRDLAA
Dog	TCQAVGPPPEPV	TAVFSCEAHND	VDPLSNGSVMV	CAGGEFGSVMEG	NRNFLHRDLAA
Cat	TCQAVGPPPEPV	MAVFSCEAHND	VDPLSNGSVMV	CAGGEFGSVMEG	NRNFLHRDLAA
Mouse	TCQAVGPPPEPV	TAVFSCEAHND	ADRLSNGSVMV	CAGGEFGSVMEG	NRNFLHRDLAA
Rat	TCQAVGPPPEPV	TAVFSCEAHND	ADQLSNGSVMV	CAGGEFGSVMEG	SRNFLHRDLAA
Horse	TCQAVGPPPEPV	AAVFSCEAHND	VDPLSNGSVMI	CAGGEFGSVMEG	NRNFLHRDLAA
Opossum	TCQAVGPPPEPV	AAVFSCEAHND	VSPLTNASVLI	TAGGEFGSVMEG	NRNFLHRDLAA
Platypus	TCHAVGPPPEPV	SAVFSCEAHND	ADPLNNASVMV	CAGGEFGSVMEG	SRNFLHRDLAA
Chicken	TCQAVGPPPEPV	-----	AVPQSNVSLLL	-----	SRNFLHRDLAA
Zebrafish	-----	-----	-----	-----	SRNFLHRDLAA
Lizard	TCQAVGPPPEPV	TAVFSCEAHNE	VVVGDNASVMV	CAGGEFGSVMEG	NKMFHLRLAA
X-Tropicalis	TCEAVGPPPEPV	PAVFSCEAHNK	M-LQSNENTRY	-----	SRNFLHRDLAA

	2079+2
	g
Human	AAAGGtaaatga
Rhesus	AAAGGtaaatga
Orangutan	AAAGGtaaatga
Marmoset	AAAGGtaa---
Dog	CAAGGtaattta
Cat	CAAGGtaattta
Mouse	CAAGGtaaacag
Rat	CAAGGtaaacag
Horse	AAAGGtaagga
Opossum	GAAGGtaattta
Platypus	CCAGGtaggga
Chicken	CCAGGtaaatga
Zebrafish	TGTGgtaataaa
Lizard	TCAGGtaaat--
X-Tropicalis	TCAGGtac--aa

FIGURE 2 Evolutionary conservation of likely pathogenic novel missense and splice-site variants identified by our group

beaten-bronze appearance of the fundus and the presence of white dots have been reported before (Charbel Issa et al., 2009; Ebermann et al., 2007; Jinda et al., 2016; Ksantini et al., 2012; Mackay et al., 2010). These white dots are not specific of IRD with *MERTK* mutations since they have also been reported in association with *TULP1*, *LRAT*, *RLBP1*, *RPE65*, or *RHO* mutations among others (Lewis et al., 1999; Littink et al., 2012; Mataftsi et al., 2007; Schatz et al., 2011; Souied et al., 1996). Some of them may be hyperautofluorescent and hyper-reflective on SD-OCT, located at the level of the outer retina (see below).

Short-wave length fundus autofluorescence revealed patchy loss in the periphery for all patients. In addition, none of the patients display the classical ring of increased fundus autofluorescence reported in patients with RCD and preserved macular function (Robson et al., 2003). Instead, all patients had abnormal autofluorescence at the macula with two distinct patterns (Figure 3): 14 out of 25 patients had foveal increase of autofluorescence (e.g., Figure 3A), whereas the rest had loss of autofluorescence in the macula (e.g., Figure 3C and D). In addition, 12 out of 25 patients had fine hyperautofluorescent dots including eight with increased autofluorescence of the fovea (e.g., Figure 3B). Consistently, patients with loss of macular autofluorescence had severe outer central retinal thinning on SD-OCT unlike those with increased foveal autofluorescence. Of note, four out of 25 patients presented with mild epiretinal membranes but no obvious vitreomacu-

lar tractions on SD-OCT requiring surgery. Besides these four patients, we did not notice the wrinkled appearance of the inner retina on fundus examination reported by Charbel Issa et al. (2009) associated to a peculiar wave-like appearance of the innermost neurosensory retina on SD-OCT. None of the patients with *MERTK* mutations had preservation of the outer retinal hyper-reflective bands (i.e., external limiting membrane, ellipsoid, and interdigitation zones). They all display hyper-reflective dots above the RPE/Bruch's membrane complex (e.g., zoom in Figure 3A and C). In addition, 24 out of 25 patients had evidence of hyper-reflective dots (e.g., zoom in Figure 3A and C) in the choroid which has, to our knowledge, never been noticed before although they are visible on SD-OCT figures from recent papers (Charbel Issa et al., 2009; Jinda et al., 2016). Some of the dots located in the outer retina may correspond to the hyperautofluorescent dots on FAF seen on some of the patients and maybe to the occasional white dots seen on fundus examination. These dots may represent outer segment debris, RPE cell migration or/and phagocytes (i.e., macrophages and activated microglia) also seen in RCS rats (Dowling & Sidman, 1962; LaVail, Pinto, & Yasumura, 1981) and *Mertk* knockout mice (Duncan et al., 2003). In keeping with this, a recent study applying two photon microscopy to various *Mertk*^{-/-} mutant models suggested that microglial cells could be recruited to transport byproducts of the phototransduction cascade to the RPE and be involved in the elimination of dying photoreceptor cells (Palczewska et al., 2016). Macrophages may also account for the

FIGURE 3 Retinal images illustrating the phenotypic variability of inherited retinal diseases associated with *MERTK* mutations. **A:** Left eye of CIC00568: color fundus photograph (left) reveals a moderately pale optic disc; narrowed retinal vessels, a beaten-bronze appearance of the retinal periphery with only very few bone spicules; fundus autofluorescence (FAF) imaging (middle) shows a foveal increase of autofluorescence surrounded by a patchy loss of autofluorescence and some hyperautofluorescent dots; Spectral domain-optical coherence tomography (SD-OCT) (right with close-up) reveals a thinning of the outer nuclear layer, hyper-reflective dots above the RPE/Bruch complex and within the choroid (white arrows) with no visible external limiting membrane (ELM), ellipsoid zone (EZ), and interdigitation zone (IDZ). **B:** Right eye of CIC04456: color fundus photograph (top) reveals a pale optic disc, narrowed retinal vessels, an albinoid fundus with very few bone spicules and cystoid macular changes; FAF imaging (bottom left) shows severe diffused patchy loss of FAF including the macula with fine hyperAF dots; SD-OCT (bottom right) reveals intraretinal cysts with no visible ELM, EZ, or IDZ associated with hyper-reflective dots above the RPE/Bruch complex and in choroid (white arrows). **C:** Left eye of CIC03112: color fundus photograph (top left) reveals a well colored optic nerve, no narrowed retinal vessels, beaten-bronze appearance in the periphery with only few bone spicules, pigment clumping in the macula; FAF imaging (top right) shows severe diffused patchy loss of FAF including the macula with fine hyperautofluorescent dots; SD-OCT (bottom with close-up) reveals a severe outer retinal thinning with hyper-reflective dots above a thinned RPE/Bruch complex and in choroid (white arrows). **D:** Right eye of CIC04949: color fundus photograph (top left) reveals a well colored optic disc, narrowed retinal vessels and diffuse pigment clumping; FAF imaging (top right) shows a diffuse patchy loss of FAF including the fovea; SD-OCT (bottom) reveals severe thinning of the outer retina with some hyper-reflective dots above the thinned RPE/Bruch complex but a thinned choroid with no obvious dots

hyperreflective dots seen in the choroid. These hyper-reflective dots are not specific of IRD linked to *MERTK* mutations. They have also been reported in association with Stargardt disease (Ge et al., 2015), Bietti crystalline dystrophy, independently of crystal location (Saatci, Doruk, Yaman, & Oner, 2014), and RCD (Kuroda et al., 2014). In the latter, Kuroda et al. (2014) suggested that these hyper-reflective dots were associated with disease progression. However, no genotype was available in this publication therefore the occurrence of outer retinal hyper-reflective dots in correlation with specific gene defects in IRD remains to be established. If they indeed represent phagocytic cells, these dots may have some incidence in a putative inflammatory response to treatment such as cell therapy.

Altogether, the association with early onset IRD with macular involvement associated with a bronze-beaten appearance, several white spots on funduscopy, abnormal macular FAF, lack of preservation of outer retinal hyper-reflective bands with hyper-reflective dots in the outer retina and the choriocapillaris may be suggestive but not

specific of a *MERTK*-related IRD in a sporadic or autosomal recessive case.

Although the present cohort is one of the largest reported in the literature on patients with retinal dystrophy related with *MERTK* mutations, we were not able to draw any specific conclusion on phenotype/genotype correlations, most likely due to the diversity of the mutation spectrum in a yet limited number of patients. Intriguingly, patients from two distinct families (F751 and F3928) were carrying the same large deletion. Individuals for F751 had a CRD phenotype with early onset decreased vision and photophobia whereas the individual from F3928 had more a RCD phenotype. In addition, one of the patients with the most severe phenotype (CIC00239) was carrying two missense mutations. There may be additional factors, genetic or environmental that may also impact on the phenotype as suggested by Vollrath et al. (2015). Larger cohort studies and natural history of the disease may help better define such correlations.

6 | FUTURE DIRECTIONS

Our work expands the mutation spectrum of *MERTK* in IRD which spans the entire coding and splice regions of the gene. It sets basis for therapeutic intervention aiming at gene augmentation (Ghazi et al., 2016; LaVail, et al., 2016) or cell therapy (Jones, Lu, Girman, & Wang, 2017) for this genetic group linked to mutations in a selectively RPE-expressed gene. Mutations in *MERTK* are however associated with early onset retinal degeneration with rapid macular involvement urging for early diagnosis and intervention in strategies aiming at rescuing photoreceptors. Natural history studies with longitudinal follow-up may help better define therapeutic strategies and window of intervention.

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DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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