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A Novel *MERTK* Mutation Causing Retinitis Pigmentosa

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Abstract

Purpose—Retinitis pigmentosa (RP) is a genetically heterogeneous inherited retinal dystrophy. To date, over 80 genes have been implicated in RP. However, the disease demonstrates significant locus and allelic heterogeneity not entirely captured by current testing platforms. The purpose of the present study was to characterize the underlying mutation in a patient with RP without a molecular diagnosis after initial genetic testing.

Methods—Whole-exome sequencing of the affected proband was performed. Candidate gene mutations were selected based on adherence to expected genetic inheritance pattern and predicted pathogenicity. Sanger sequencing of *MERTK* was completed on the patient’s unaffected mother, affected brother, and unaffected sister to determine genetic phase.

Results—Eight sequence variants were identified in the proband in known RP-associated genes. Sequence analysis revealed that the proband was a compound heterozygote with two independent mutations in *MERTK*, a novel nonsense mutation (c.2179C>T) and a previously reported missense variant (c.2530C>T). The proband’s affected brother also had both mutations. Predicted phase was confirmed in unaffected family members.

Conclusion—Our study identifies a novel nonsense mutation in *MERTK* in a family with RP and no prior molecular diagnosis. The present study also demonstrates the clinical value of exome

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Conflict of Interest:

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Ethical Approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

sequencing in determining the genetic basis of Mendelian diseases when standard genetic testing is unsuccessful.

Keywords

Retina; genetics; retinitis pigmentosa; dystrophy

Introduction

Retinitis pigmentosa (RP) represents a genetically heterogeneous group of retinal dystrophies. The disease mainly affects rod photoreceptor cells and manifests progressively with night blindness, constriction of the visual field, and eventual blindness[1, 2]. Ophthalmoscopic findings include retinal vessel attenuation, optic disc pallor, and bone spicule pigmentary changes in the retina [3, 4]. To date, more than 80 genes have been implicated in non-syndromic cases of RP. The mutations in these genes are inherited in autosomal dominant, autosomal recessive, and X-linked patterns, accounting for approximately 20–25%, 15–20%, and 10–15% of non-syndromic cases, respectively, with the remaining attributable to sporadic cases[5, 6]. However, these mutations are only responsible for 60% of known RP cases, indicating 40% of patients do not have a molecular diagnosis [7–9]. The present study focuses on a proband with clinically established RP yet without a molecular diagnosis after commercial genetic testing. Identifying such novel mutations helps to elucidate the pathology of RP, confirm the inheritance pattern, identify potential carriers, shed insight into prognosis, and uncover novel therapeutic targets.

One of the genes implicated in the pathogenesis of RP, Mer tyrosine kinase (*MERTK* – OMIM#604705) is a receptor tyrosine kinase of the Tyro3/Axl/Mer family of tyrosine kinases[10]. This enzyme class shares a conserved sequence within the kinase domain and in the adhesion molecule-like extracellular domain[11]. The tyrosine kinase is involved in many cellular processes, among them cell proliferation, adhesion, and migration, regulation of inflammatory processes, and blood clotting[12, 13]. Within the retina, *MERTK* is involved in the retinal pigment epithelium's (RPE) role in recycling photoreceptor outer segments. Photoreceptors continually renew the light-sensitive disks in their outer segments as they bear significant light stress. These shed disks are phagocytized by the RPE [14, 15]. Prior studies have demonstrated that initial photoreceptor outer segment binding to RPE cells is facilitated by the $\alpha v\beta 5$ integrin while *MERTK* is necessary for phagocytosis of the outer segments [16–18].

Methods

Subjects

Four subjects were ascertained from a family affected by retinitis pigmentosa yet without an associated molecular diagnosis after commercial genetic testing. After IRB approval from the University of Illinois at Chicago was granted for the study, informed consent was obtained from the proband and three family members. Research was performed in concordance with the Declaration of Helsinki.

Proband Whole-Exome Sequencing

A 10mL whole blood sample was collected from the proband in an EDTA tube. A total of 3.5 µg of genomic DNA (gDNA) was extracted using the prepIT-L2P kit (Cat#PT-L2P-5, Genotek Inc., Ottawa, Ontario, Canada) according to manufacturer's instructions. Prior to library construction, the concentration of the gDNA was determined with the Qubit dsDNA HS Assay Kit (Cat#Q32851, Life Technologies, Carlsbad, CA, USA). gDNA integrity was assessed with DNA agarose gel electrophoresis (agarose Gel: 1%, voltage 150V, electrophoresis time: 40 min). Whole-exome sequencing was performed using the BGI Exome Capture Kit (59Mb) on the Complete Genomics platform using Combinatorial Probe-Anchor Ligation (cPAL) technology at 100X depth of coverage (BGI, Hong Kong, China). Quality control of whole-exome sequencing was provided by validation of 140 SNP loci with WaferGen variant validation.

Mapping and Variant Analysis

Initial mapping was performed using TeraMap. Raw read data was aligned to the NCBI human reference genome (NCBI, GRCh37)[19]. Regions found to differ from the reference were identified, and individual reads lying in these regions of interest were collected for local de novo assembly using mate alignment information. Based on the results from reference mapping and sequence assembly, a Bayesian model was applied to call variants from which only those with statistical significance—based on the best scoring hypotheses—were reported [20, 21]. Finally, ambiguous variants, found in duplicative regions or with high similarity, were rescored based on a correlation analysis. Complete Genomics in-house tools were used for the analyses (BGI, Hong Kong, China).

Candidate Gene Identification

To select candidate genes, identified single nucleotide polymorphisms (SNPs) were removed if present in the 1000 Genomes Project (www.1000genomes.org) or the Single Nucleotide Polymorphism Database (dbSNP) (NCBI). Once novel SNPs were identified, predicted pathogenicity was determined using SIFT, PolyPhen2, MutationAssessor, RadialSVM, and snpEff. Based on predicted pathogenicity and expected patterns of inheritance, two gene mutations in *MERTK* (NM_006343.2:p.Arg727*/c.2179C>T) and (NM_006343.2:p.Arg844Cys/c.2530C>T) were identified as the most likely candidate mutations. The ExAC Browser (The Broad Institute) and the NHLBI database were surveyed to search for previous reports of the two candidate gene mutations.

Sanger Sequencing

After candidate gene mutations were identified, saliva samples were obtained from the proband's unaffected mother, unaffected sister, and affected brother using the Oragene DNA Self-Collection Kit (REF OG-300, Genotek Inc., Ottawa, Ontario, Canada). gDNA was extracted using the prepIT-L2P kit (Cat#PT-L2P-5, Genotek Inc., Ottawa, Ontario, Canada) according to manufacturer's instructions. PCR amplification was performed using Green DreamTaq Master Mix (Cat#K1081, Life Technologies, Grand Island, NY) according to manufacturer's protocols and pre-designed primer sets (Primer set IDs Hs00446022_CE and Hs00360031_CE, Life Technologies, Grand Island, NY, USA). PCR products were purified

with AMPure beads (Cat #A63880, Beckman Coulter, Brea, CA, USA) and sequenced using 3730xl DNA Analyzer (Life Technologies, Grand Island, NY, USA).

Results

The proband had an established clinical diagnosis of RP. His visual acuity was light perception in both eyes. Ophthalmoscopy demonstrated retinal bone-spicule pigmentary changes in both eyes, retinal vessel attenuation, and diffuse macular atrophy (Figure 1). The proband's brother also carried the diagnosis of RP. An unaffected sister did not have any retinal disease on examination. Lastly, the proband's mother was also unaffected by RP though she had been diagnosed with age-related macular degeneration.

The proband had not demonstrated a previously reported RP gene mutation in genetic testing using the GeneDx RP panel (Cat# 368, GeneDx, Gaithersburg, MD, USA). Based on pedigree analysis, the predicted mode of inheritance was determined to most likely be autosomal recessive (Figure 2). Testing of X-linked RP genes was also performed as an X-linked pattern of inheritance could not be ruled out and given the significance of family planning for carriers (Cat# 326, GeneDx, Gaithersburg, MD, USA). No X-linked RP mutations were identified.

Whole-exome sequencing of the proband revealed 19,756 SNP variants (Figure 3). Of these, 265 SNPs were not reported in both the 1000 Genomes and dbSNP databases. Next, the candidate mutation pool was further narrowed by identifying known RP-associated genes. Eight of the 265 novel variants were located in genes previously associated with RP: 2 in *MERTK* in *RPIL1* in *CNGB3*, and 1 in *PRPF31* (Table 1). The 2 mutations in *MERTK*, a nonsense variant resulting in a premature stop (NM_006343.2:p.Arg727*/c.2179C>T) and a missense variant resulting in an arginine to cysteine change (NM_006343.2:p.Arg844Cys/c.2530C>T) were both heterozygous. The missense *MERTK* mutation was predicted to be damaging by SIFT, PolyPhen2, MutationAssessor, and RadialSVM. The nonsense mutation was analyzed by snpEff and found to likely cause loss of function and to be targeted by nonsense-mediated decay (SIFT, PolyPhen2, MutationAssessor, and RadialSVM are not well suited to predict pathogenicity in nonsense mutations).

The proband was also heterozygous for a missense mutation in *CNGB3* (NM_019098.4:p.Arg26Gln/c.77G>A) and another in *PRPF31* (NM_015629.3:p.Gly436Asp/c.1307G>A). The *CNGB3* and *PRPF31* mutations were unlikely candidates given that a single heterozygous mutation did not conform to the expected autosomal recessive disease model. Additionally, the *CNGB3* mutation was not predicted to be pathogenic. The remaining four *RPIL1* variants were all missense mutations, however, two of the *RPIL1* mutations were not predicted to be damaging (NM_178857.5:p.Gly1363Arg/c.4087G>A and NM_178857.5:p.Gly1363Ala/c.4088G>C) and thus excluded from consideration as candidate mutations. The remaining two *RPIL1* mutations (NM_178857.5:p.Glu1379Ala/c.4136A>C and NM_178857.5:p.Glu1379Lys/c.4135G>A) were only predicted to be damaging by SIFT. Though the proband could have been a compound heterozygote for these latter *RPIL1* mutations, given their lower predicted probability of pathogenicity, the *MERTK* mutations were investigated first.

Based on the predicted pathogenicity of the mutations as well as the expected mode of inheritance, the two mutations in *MERTK* were selected as the primary candidate mutations to further investigate. The proband was predicted to be a compound heterozygote with an independent mutation in each *MERTK* allele based on the pedigree. The ExAC database, which contains exome sequencing data from more than 60,000 individuals, was surveyed for previous reports of either mutation. The nonsense mutation that was identified in *MERTK* had not previously been reported. The *MERTK* missense mutation had been documented previously in just two European individuals in the ExAC database (of unknown phenotype) making it exceedingly rare. It has been previously reported in cases of RP in the scientific literature [22]. Neither variant was found in a search of the NHLBI database, which contains exome-sequencing data from 6,503 individuals recruited for common disease studies.

To establish phase and demonstrate that the *MERTK* mutations were on separate alleles as would be expected in a compound heterozygous model of inheritance, the proband's unaffected mother, unaffected sister, and affected brother's *MERTK* genes were sequenced. The proband's affected brother was also found to be heterozygous at each mutation site as expected in a compound heterozygous model (Figure 4). Meanwhile, the unaffected mother carried the nonsense *MERTK* mutation but was wild type at the missense site. The unaffected sister carried a copy of the missense *MERTK* mutation and was wild type at the nonsense site. The sequencing results from the unaffected mother and sister suggest that the *MERTK* mutations are located on separate alleles, supporting the predicted compound heterozygous model of inheritance.

Discussion

In this study, we present a family with retinitis pigmentosa with an autosomal recessive pattern of inheritance and no prior molecular diagnosis. To determine the likely causative gene mutation, whole-exome sequencing was performed on the proband. Among RP-associated genes, mutations were identified through whole-exome sequencing in *MERTK*, *RP1L1*, *CNGB3*, and *PRPF31*. Ultimately, the two mutations in *MERTK*, a novel nonsense mutation and a missense mutation, were selected as the primary candidate mutations given their predicted pathogenicity as well as their adherence to the observed inheritance pattern.

MERTK has previously been associated with RP. The initial work uncovering the role of *MERTK* mutations in the pathogenesis of RP evolved from the Royal College of Surgeons' (RCS) inherited retinal degeneration rat line, which was the first animal model with inherited retinal degeneration[23]. Subsequent studies uncovered the causative mutation, a 1.5kb deletion in *MERTK*, in the RCS rat [24, 25]. Molecular studies demonstrated that while the RPE was able to bind to photoreceptor outer segments, it could not successfully engulf them[26, 27]. The outer segment debris accumulated in the subretinal space [28]. After these initial studies, multiple *MERTK* mutations were documented in patients with RP. These patients' disease progression is thought to be more severe than typical RP cases, with early onset in the first decade of life and quickly worsening macular atrophy as seen in our family[15].

The present study also further demonstrates the clinical utility of targeted exome-sequencing in patients presenting without diagnoses on initial genetic panel testing. While many genetic panels for RP do include *MERTK*, the commercial panels used in the initial diagnosis of this patient did not (GeneDx). Targeted gene capture panels are advantageous in that they are cheaper and produce results more rapidly than WES. Therefore, targeted genetic panels are an appropriate first-line molecular diagnostic tool. However, if initial screening does not yield a molecular diagnosis, especially in genetically heterogeneous diseases like RP, WES should be considered. WES can lead to higher diagnostic yields and the discovery of novel gene-disease associations that would otherwise be missed on targeted panels at an increasingly cost-effective rate[29]. Uncovering such novel gene mutations furthers our understanding of Mendelian diseases and offers novel therapeutic targets. Understanding the role of these mutations in the function of the retina will aid in therapy design. Furthermore, as targeted gene therapy becomes available, the identification of specific genetic causes of retinal dystrophy will be critical to matching patients with appropriate therapies.

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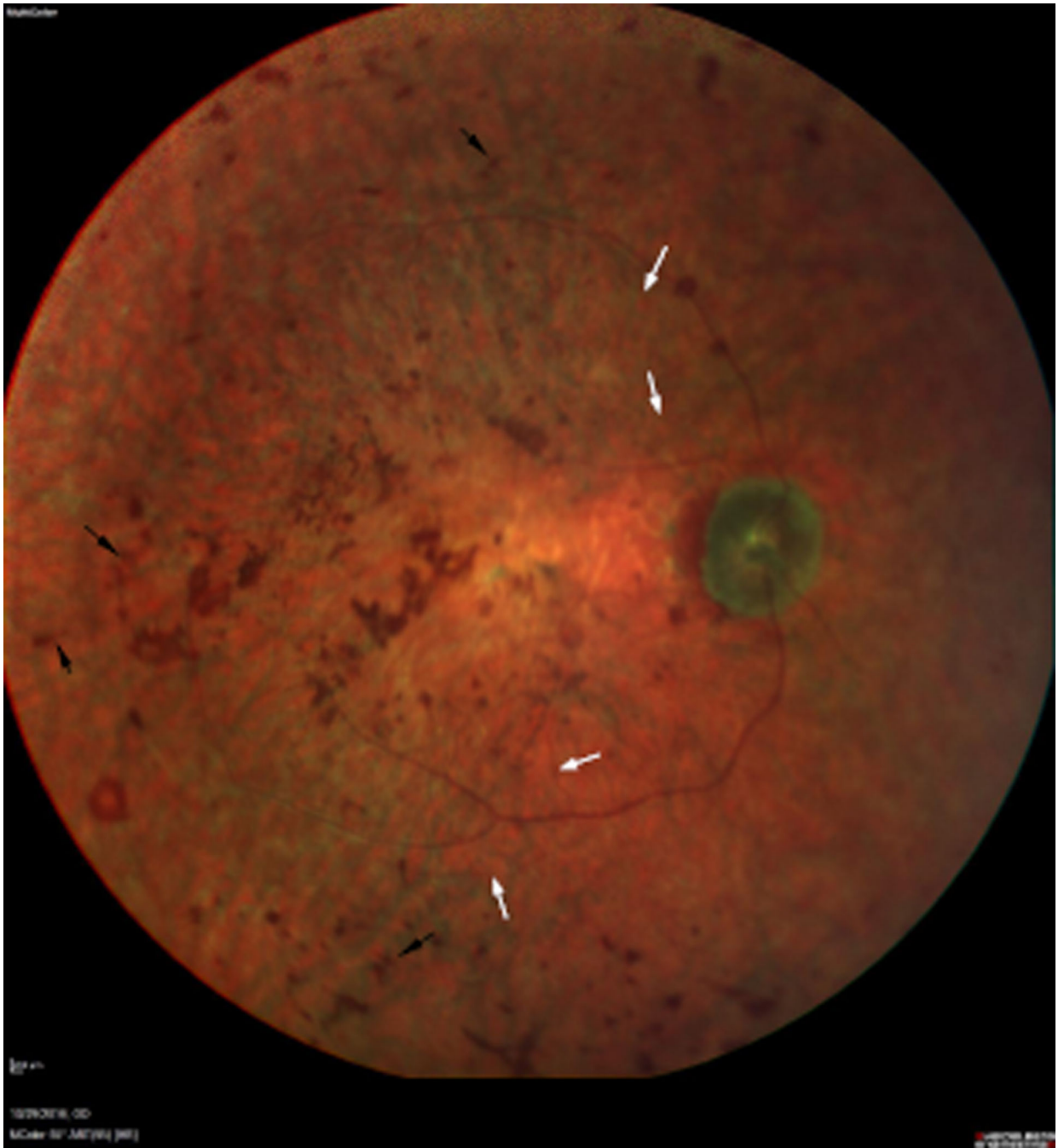


Fig 1. Multicolor 55 Degree Image of the retina (right) reveals a pallorous optic nerve with severely attenuated retinal arterioles (white arrows). Central atrophy is evident with associated coarse, placoid pigmentation in the macula. The midperiphery reveals diffuse retinal pigment epithelial atrophy with associated bone spicule-like pigmentation (black arrows). Similar findings were present in the left eye.

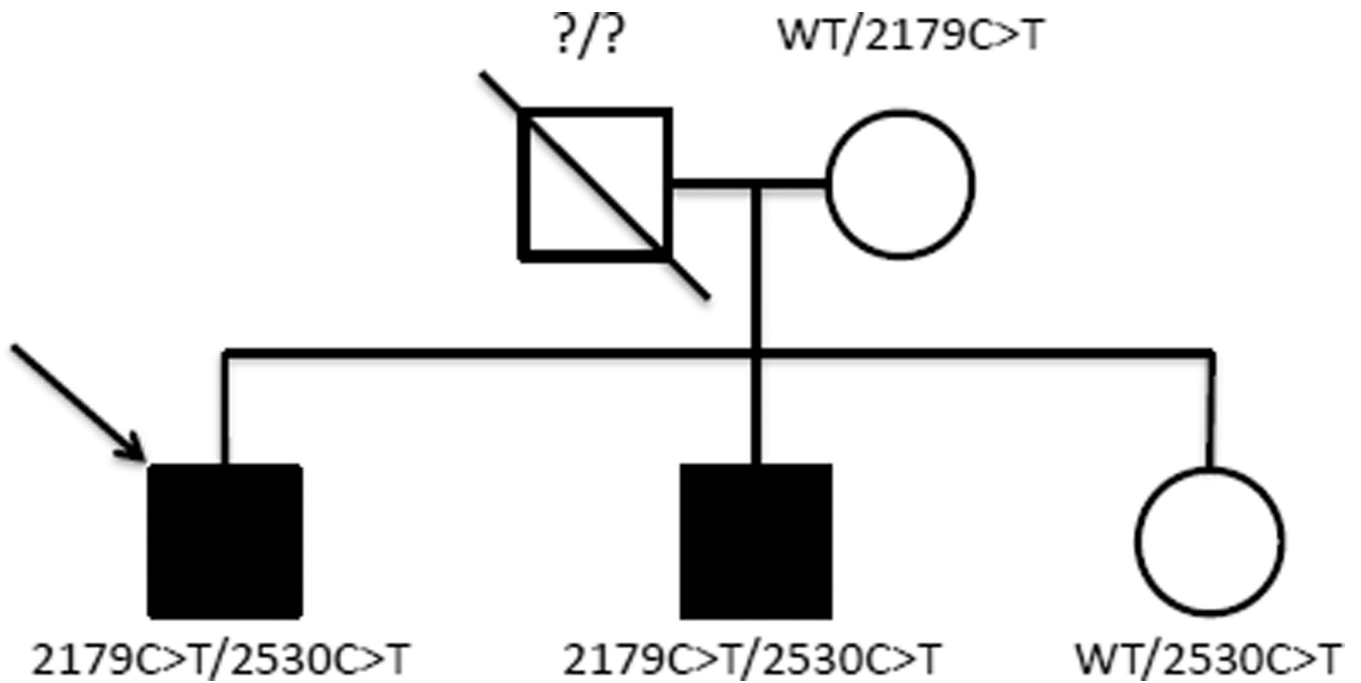


Fig 2.
Family Pedigree: The arrow identifies the proband. Shaded individuals carried established diagnoses of retinitis pigmentosa. The proband's mother and sister were unaffected, though the mother had age-related macular degeneration. Carried *MERTK* variants are shown next to each individual (WT = wild type).

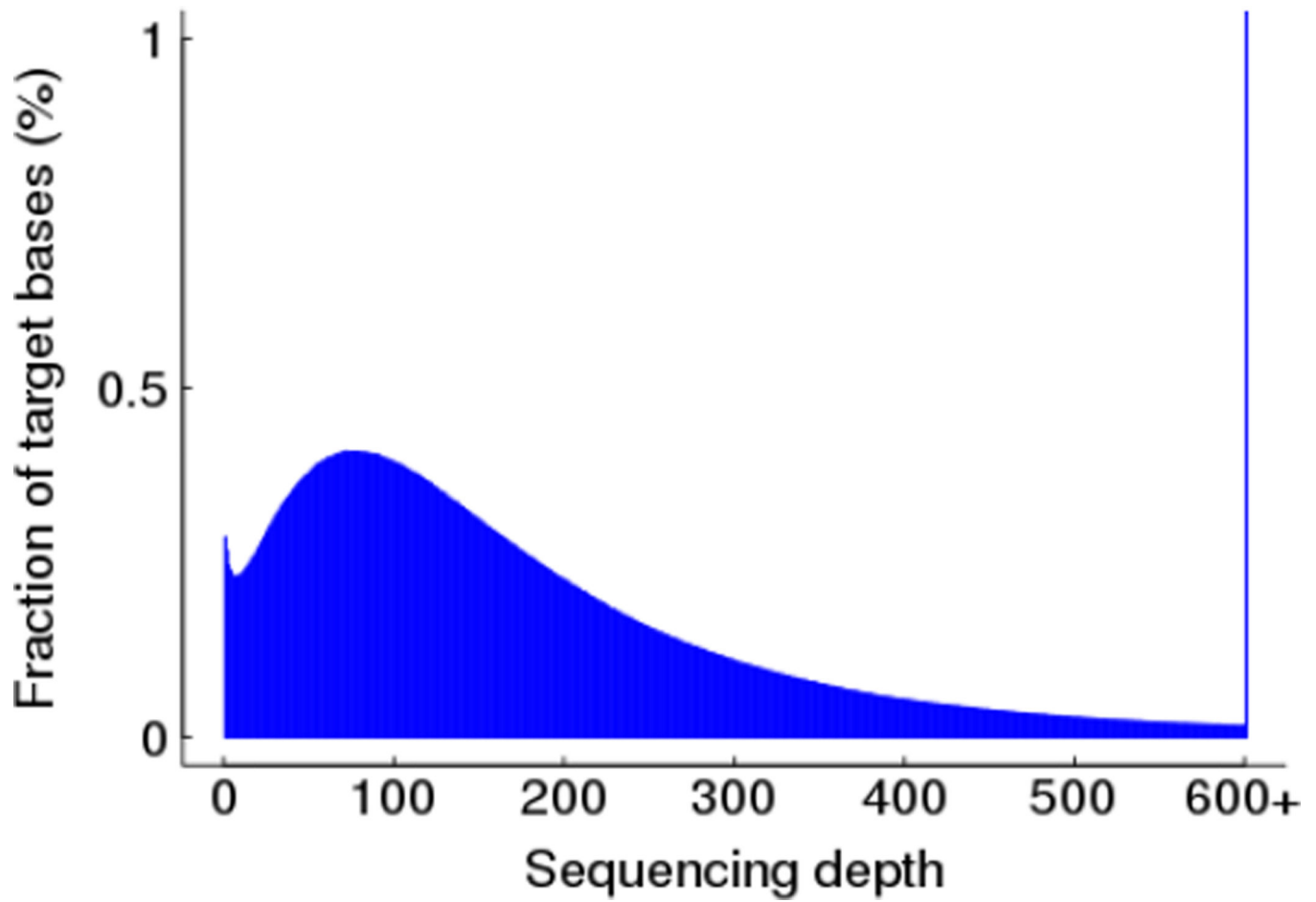


Fig 3. Whole-exome Sequencing Coverage: 97.3% of targets were covered with at least 10X coverage. 20X depth of coverage or more was achieved for 94.82% of targets. Average sequencing depth was 192X.

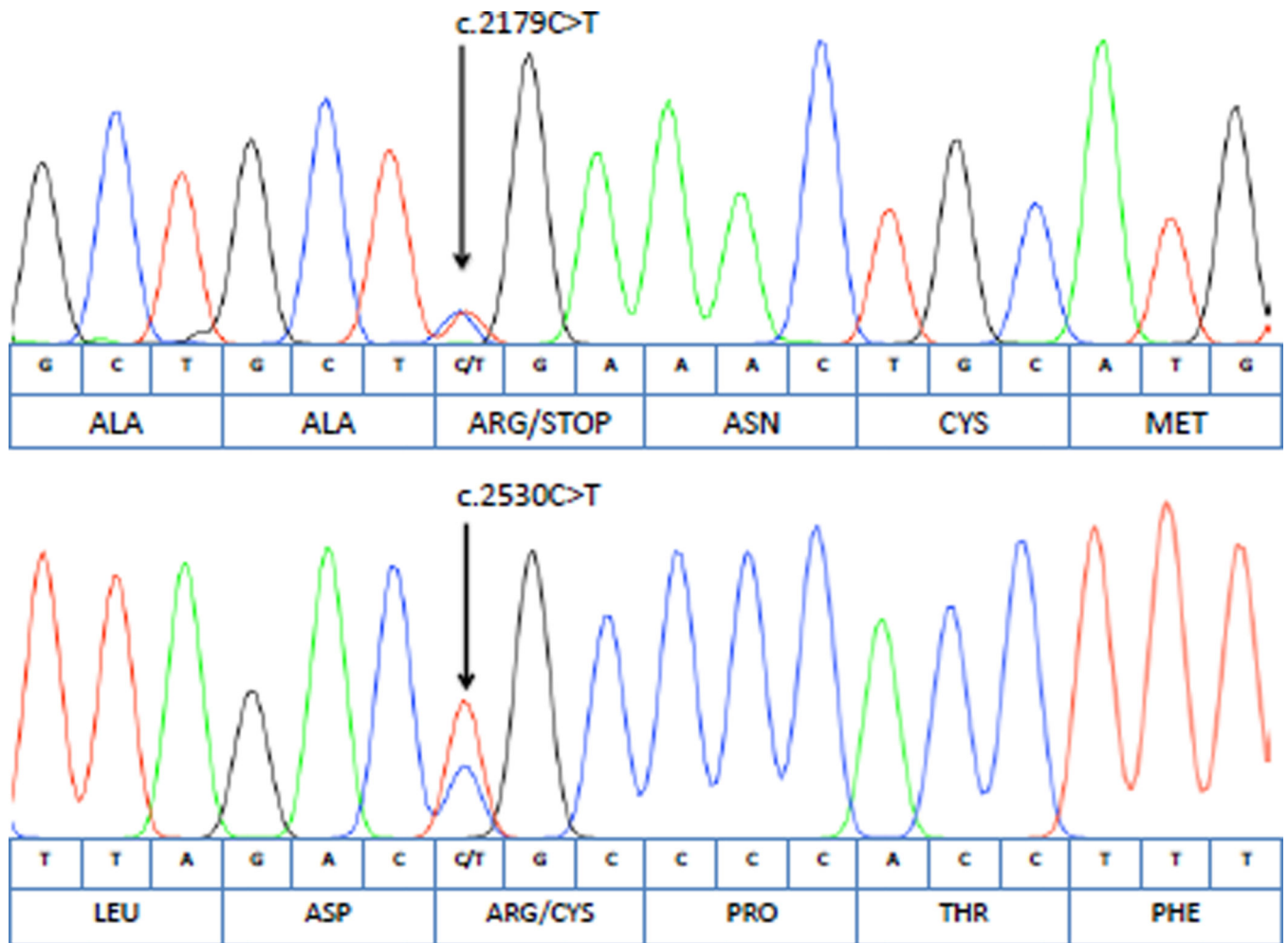


Fig 4.
Chromatograms of the Proband's Affected Brother: Sanger sequencing revealed that the proband's affected brother carried the same two heterozygous MERTK mutations as the proband. The nucleotide sequence and corresponding amino acid sequences are shown below each chromatogram.

Table 1

Eight Candidate Gene Mutations

Eight novel variants were identified in known RP-associated genes. Predicted pathogenicity was determined using four predictive tools (SIFT, PolyPhen2, MutationAssessor, and RadialSVM) for missense mutations. The nonsense mutation in *MERTK* was evaluated using snpEff.

Gene	Mutation Site	Mutation Type	SIFT Score	PolyPhen2 Score	Mutation Assessor Score	Radial SVM Score	
<i>MERTK</i>	Arg727*/2179C>T	Nonsense	Above predictive tools are not well-suited to evaluate nonsense mutations				
	Arg844Cys/2530C>T	Missense	0 (Damaging)	1 (Probably Damaging)	4.695 (High Functional Impact)	0.8992 (Damaging)	
<i>RPL11</i>	Glu1379Ala/4136A>C	Missense	0.04 (Damaging)	0.636 (Possibly Damaging)	0.345 (Neutral)	-0.9360 (Tolerated)	
	Glu1379Lys/4135G>A	Missense	0.04 (Damaging)	0.636 (Possibly Damaging)	0.345 (Neutral)	-0.9613 (Tolerated)	
	Gly1363Ala/4088G>C	Missense	0.69 (Tolerated)	0.007 (Benign)	0 (Neutral)	-0.9248 (Tolerated)	
	Gly1363Arg/4087G>A	Missense	0.46 (Tolerated)	0.075 (Benign)	0 (Neutral)	-0.9015 (Tolerated)	
	Arg266Gln/77G>A	Missense	0.54 (Tolerated)	0.0 (Benign)	0 (Neutral)	-0.9924 (Tolerated)	
<i>PRPF31</i>	Gly436Asp/1307G>A	Missense	0 (Damaging)	1.0 (Probably Damaging)	2.85 (Medium Functional Impact)	0.0704 (Damaging)	