Non-homologous recombination between Alu and LINE-1 repeats results in a 91 kb deletion in MERTK causing severe retinitis pigmentosa

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Purpose: Retinitis pigmentosa (RP) represents a large group of inherited retinal diseases characterized by clinical and genetic heterogeneity. Among patients with RP in northern Sweden, we identified two severely affected siblings and aimed to reveal a genetic cause underlying their disease.

Methods: Whole exome sequencing (WES) was performed on both affected individuals. Sequence variants were filtered using a custom pipeline to find a rare or novel variant predicted to affect protein function. Genome-wide genotyping was used to identify copy number variants (CNVs) and homozygous regions with potential disease causative genes.

Results: WES uncovered a novel heterozygous variant in the MER proto-oncogene, tyrosine kinase (MERTK) gene, c.2309A>G, p.Glu770Gly located in the tyrosine kinase domain and predicted to be likely pathogenic. The second variant, a large heterozygous deletion encompassing exons 1 to 7 of the MERTK gene, was revealed with genome-wide genotyping. The CNV analysis suggested breakpoints of the deletion, in the 5'-untranslated region and in intron 7. We identified genomic sequences at the site of the deletion as part of L1ME4b (LINE/L1) and AluSx3 that indicated a non-homologous recombination as a mechanism of the deletion evolution.

Conclusions: Patients with RP in this study were carriers of two novel allelic mutations in the MERTK gene, a missense variant in exon 17 and an approximate 91 kb genomic deletion. Mapping of the deletion breakpoints allowed molecular testing of a cohort of patients with RP with allele-specific PCR. These findings provide additional information about mutations in MERTK for molecular testing of unsolved recessive RP cases and highlight the necessity for analysis of large genomic deletions.

Inherited retinal diseases (IRDs) represent genetic conditions leading to visual deficiency and in some instances, to blindness [1]. The most common among IRDs is retinitis pigmentosa (RP). RP is a progressive non-syndromic rod-cone disease with clinical and genetic heterogeneity [2]. The genetic heterogeneity and founder mutations make some genes more common in isolated geographic areas. The population of northern Sweden has been previously described as ideal for genetic research because the country’s rivers and landscape features made it difficult for the inhabitants to move around hundreds of years ago, contributing to a homogenous population [3,4]. A genetic cause of recessive RP in this region is known in less than 50% of the patients; thus, the search for pathogenic disease causative variants continues.

There are at least 60 genes and three loci associated with only autosomal recessive RP (arRP) according to the Retinal Information Network. Many mutations are private or family-specific, responsible for a very small percentage of arRP cases. One of the RP genes is MER proto-oncogene, tyrosine kinase (MERTK; Gene ID ENS00000153208, OMIM 604705), which was mutated in patients with RP from consanguineous families originating in the Middle East, Saudi Arabia, Spain, and Morocco [5-8]. In general, mutations in MERTK are rare affecting less than 1% of patients with RP [6-9] although in a large French cohort the mutations account for 2% [10], and in the isolated population of the Faroe Islands, a large MERTK deletion is responsible for 30% of the RP cases [11].

In this study, we describe two siblings with a severe form of recessive RP who carried a novel missense variant in exon 17 of the MERTK gene on one allele and a novel large genomic deletion encompassing exons 1 to 7 on the second allele. We also consider a non-homologous recombination between genomic repetitive sequences such as LINE1/L1 and Alu as a plausible molecular mechanism in the appearance of large genomic deletions in the MERTK gene.
Methods

Patients and clinical examination: The now elderly siblings from a family originating from Jämtland County in northern Sweden (Figure 1) were interviewed and examined, and their blood was collected at the Östersund Eye Clinic in the early 1990s. After recent genetic findings, their medical records were reanalyzed at the Eye Clinic of the University Hospital of Umeå. The Regional Ethical Review Board in Umeå approved this research project, and the study adhering to the ARVO statement on human subjects was performed according to the Declaration of Helsinki with informed consent obtained from both patients.

DNA and RNA were available from both affected individuals, and DNA of 100 healthy individuals including blood donors and men drafted at 18 years of age from a matched geographic population was included in the study. A cohort of patients with IRDs (n=145) without known genetic cause of their visual impairment with presumed arRP was also included in this study.

High-resolution SNP array: Genomic DNA was isolated from peripheral venous blood as previously described [12]. In short, 10 ml peripheral blood was obtained by venipuncture and collected in EDTA tubes. Genomic DNA was extracted from blood leukocytes using salting out procedure [13]. Genome-wide genotyping was performed using the HumanOmnimapress-24 BeadChip with more than 715,000 single nucleotide polymorphisms (SNPs), in accordance with the manufacturer’s instructions (Illumina Inc., San Diego, CA). To identify regions of homozygosity (ROH), both siblings were genotyped. The data were analyzed using GenomeStudio software (Illumina), and cnvPartition 3.2.0 was applied for copy number variant (CNV) detection by retrieving the log R ratio (LRR, the ratio between the observed and expected probe intensities) and the B allele frequency (BAF). When a CNV is absent, the LRR is around zero, and the BAF is 0, 0.5, or 1 depending on genotypes AA, AB, and BB. Human genome GRCh38 (hg38) was used to assign all chromosome positions.

Molecular genetic analyses: As the first step, DNA from one patient (RP115) was analyzed in 2007 using the arrayed primer extension assay (APEX) available at Asper Biotech (Tartu, Estonia). The testing for arRP included 518 known variants in 12 genes (PDE6A – Gene ID ENSG00000132915, OMIM 180071; PDE6B – Gene ID ENSG00000133256, OMIM 180072; PNR/NR2E3 – Gene ID ENSG00000278570, OMIM 604485; RDH12 – Gene ID ENSG00000139988, OMIM 608830; RGR – Gene ID ENSG00000148604, OMIM 600342; RLBP1 – Gene ID ENSG00000140522, OMIM 180090; SAG – Gene ID ENSG00000130561, OMIM 181031; TULP1 – Gene ID ENSG00000134376, OMIM 602280; CRB1 – Gene ID ENSG00000134376, OMIM 604210; RPE65 – Gene ID ENSG00000100499, OMIM 180069; USH2A – Gene ID ENSG00000163646, OMIM 606397).

Whole exome sequencing (WES) was performed in both patients using the exome enrichment kit (Nimblegen SeqCap EZ Exome Library SR) and the HiSeq2000 sequencer (Illumina) using the Genomic Services at Ambry Genetics (San Diego, CA). Generated paired-end reads were aligned to the human reference genome UCSC hg19 using the Illumina CASAVA 1.8.2 software. RTA 1.12.4 (HiSeq Control Software 1.4.5) was used for the initial data processing and base calling. The sequence quality filtering script was executed in the Illumina CASAVA software (version 1.8.2; Illumina, Hayward, CA). A single BAM alignment file was used in SoftGenetics'NextGENe v2.16 for SNP and indel analysis [14]. Additionally, WES data from a control group of six individuals from the same restricted geographic area of northern Sweden (West Bothnia) were available at Computational Life Science Cluster (CLiC), Umeå University. All control individuals were clinically examined, and no signs of any retinal pathology were demonstrated.
PCR on genomic DNA was performed as described elsewhere with gene-specific primers (Table 1). The temperature profile for the PCR consisted of initial denaturing at 95 °C for 10 min, followed by seven cycles of denaturing at 95 °C for 30 s, annealing at 64 °C (−1 °C/cycle) and extension at 72 °C for 30 s; 40 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension step of 72 °C for 10 min. PCR was performed with the AmpliTaq Gold system (Applied Biosystems, Foster City, CA) with 0.2 mM dNTPs, 1X buffer, 1.5 mM MgCl₂, 0.2 mM of each primer, dimethyl sulfoxide (DMSO) 0.02%, 50 ng template, and 0.75 U polymerase in a total volume of 25 µl. The amplified PCR products were purified using Exonuclease I enzyme (Thermo Fisher scientific, Waltham, MA) before sequencing. Cycle sequencing PCR was performed using the BigDye Terminator kit, version 3.1 as recommended by the manufacturer (Applied Biosystems). The products of the sequencing reactions were analyzed on the ABI 3500xL Dx Genetic Analyzer (Applied Biosystems). Sequences were aligned and evaluated using the Sequencher software version 4.9 (Gene Codes Corporation, Ann Arbor, MI).

Allele-specific PCR followed by fragment analysis was performed as previously described with 0.4 mM of fluorescently labeled forward primer, 0.16 mM of a primer specific for wild-type allele, and 0.24 mM of primer specific for mutant alleles (Table 1). The PCR program consisted of denaturing at 94 °C for 10 min, 28 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, and final extension steps at 72 °C for 10 min and at 60 °C for 45 min. The fragment analysis based on size measurement was done with capillary electrophoresis performed in the mix of 9.5 µl Hi-Di™ Formamide 3500 Dx series, 0.5 µl of size standard 600, and 1 µl PCR product injected in the ABI 3500xL (Applied Biosystems). The results were visualized by using GeneMapper Software 5.

For reverse transcriptase PCR (RT–PCR), RNA was extracted from whole blood using TRIzol reagent (Life Technologies, Carlsbad, CA) and reverse transcribed into cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer’s recommendation. RT–PCR was performed with two different enzymes. For amplicon using primers 15F and 18R (Table 1), the conditions and program was 0.2 mM dNTPs, 1X buffer, 1.5 mM MgCl₂, 0.2 mM of each primer, 5 µl cDNA and 0.75 U AmpliTaq Gold polymerase in a total volume of 25 µl. The PCR program consisted of denaturing at 95 °C for 10 min, 39 cycles of denaturing at 95 °C for 30s, annealing at 57 °C, and extension at 72 °C for 1 min 20 s, and a final extension step at 72 °C for 7 min. Sequencing of these amplicons was performed as described previously.

In silico analyses of sequence variants were done using computational tools available via the Alamut software version 2.0 (Interactive Biosoftware, Rouen, France) that integrates Sorting Intolerant From Tolerant (SIFT), PolyPhen, Align GVGD, and MutationTaster as pathogenicity prediction tools for missense variants and allows assessment of the variants’ potential impact on splicing. All identified variants were described according to the Sequence Variant Nomenclature.

### Table 1. Primer sequences and sizes of PCR fragments for analysis of MERTK mutations.

| MERTK primer | Sequence | PCR fragment (bp) |
|--------------|----------|-------------------|
| ex17F-m13    | 5'-tgtaaaacgacggcagc CTCTGCTGTGGTCCTCACT | 419 |
| ex17R-m13    | 5'-caggaaacagctatgacc CCATACCAGCTGAGGTCATT | |
| ∆-F          | 5'-GTCATTATTGAGCTGCTAGTCAGTGC-GFAM | |
| ∆-wt-R       | 5'-GTCCTGCTTTGCTATCCTTTCCTG | 384 |
| ∆ mt-R1      | 5'-AGAAGAAGGGAGGAGGTA | 658 |
| ∆ mt-R2      | 5'-TGAGGAAAACAAGTCAGTTCAAGATCA | 354 |
| RNAex07F     | 5'-AAATAGGAGGGTGCTTCAGTGCAGTG | 1292 |
| RNAex15F     | 5'-TTTATCCCGATTGGAGACA | 372 |
| RNAex18R     | 5'-AGCTATTTCCACATGTTCA | |

M13-tag sequences and gene specific sequences are shown in lower and upper case, respectively.
recommended by the Human Genome Variation Society (HGVS) and classified according to guidelines approved by the HGVS, the Human Variome Project (HVP), and the Human Genome Organization (HUGO) [15,16]. The variants were interpreted according to standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology by Richards et al. [17].

RESULTS

Clinical findings: Both siblings presented a severe phenotype with night blindness from early childhood and affected visual acuity (VA) at an early school age, 5 and 8 years old (Table 2). The younger brother experienced severe glare problems in his teens. Both patients developed a severe visual handicap with low VA, large scotomas with only small remnants of the visual field in their teens and early adulthood leading to blindness and the need for special training for the visual handicap. Fundus changes and pigmentations were present in the posterior pole when the siblings were in their teens. Dense cataract and nystagmus were observed in RP116 in his 50s. Unfortunately, no fundus images were available from earlier medical records, and no images could be taken due to severe cataract in both patients at their most recent visit to the Eye Clinic. No other eye diseases were present. The absence of retinal disease in the siblings’ parents and other siblings strongly suggested recessive mode of inheritance.

Molecular genetic findings:

Genetic testing for known mutations—The DNA from one affected individual (RP115, Figure 1) was analyzed using the arrayed primer extension (APEX) at Asper Biotech (Tartu, Estonia) aiming to identify reported sequence variants associated with arRP. No disease-causing variants were found among the interrogated 518 variants in 12 genes.

Whole exome sequencing—DNA from both affected individuals (RP115 and RP116) was subjected to WES along with DNA from six controls from the same geographic region. Coverage for all tested samples ranged from 96.08% to 96.29% with a depth of at least 20X. The apparent autosomal recessive inheritance pattern in the family suggested two strategies for data analysis aiming at identifying either biallelic homozygous or compound heterozygous pathogenic variants. The following steps were included in the analysis of the WES data: (1) identify homozygous or heterozygous variants present in both siblings, (2) exclude variants present in at least one of the control DNA, (3) exclude all synonymous variants and variants located more than 10 bp from exon–intron junctions in all transcripts (MapView), and (4) manually analyze all variants focusing on retinal genes from the RetNet Information Network Database (the number of genes and loci was 305). The data filtering resulted in identification of shared 85 homozygous (80 genes) and 885 (724 genes) heterozygous variants out of 39,119 called variants for RP115 and 39,560 for RP116 (Appendix 1, Appendix 2, and Appendix 3). Only four homozygous variants in four genes were present in the genes associated with IRDs according to RetNet. All of them were excluded as a potential disease

| ID   | Age at examination (yo) | Visual acuity | Refraction/Right/left eye |
|------|-------------------------|---------------|---------------------------|
| RP115| 8                       | 0.4/0.6       | +1/+1                     |
|     | 13                      | 0.3/0.2       | Emmetropia                |
|     | 22                      | 0.1/0.1       | 0/+0.5                    |
|     | 39                      | HM*           | Emmetropia                |
|     | 81                      | A†            |                           |
| RP116| 5                       | 1.0/1.0       | Emmetropia                |
|     | 14                      | 0.7/0.7       | +1.5/+1.5                 |
|     | 19                      | 0.3/0.5       | +1.25/+1                  |
|     | 25                      | 0.1/0.1       | +1/+1                     |
|     | 36                      | HM            |                           |
|     | 54                      | P+L‡          |                           |
|     | 78                      | A             |                           |

Best visual acuity noted in decimal visual acuity. *HM: hand movements; †A: amaurosis fugax; ‡P+L: perception and localization.
cause because their population frequency was 10% or higher (Table 3).

Of the 885 heterozygous variants in 724 genes, 19 were found in 17 genes associated with IRDs according to RetNet (Table 4). The only gene harboring more than one variant was the LRP5 (Gene ID ENSG00000162337, OMIM 603506) associated with aberrant ocular vascularization and loss of vision in genetic disorders such as osteoporosis-pseudoglioma syndrome. In this gene, three variants were present, all with a population frequency of 11.5% or higher. Only three of the 19 variants in USH2A, WFS1 (Gene ID ENSG00000109501, OMIM 606201), and MERTK were predicted to be disease causing by all computational tools; however, USH2A and WFS1 are associated with deafness that these patients do not have. The only novel sequence variant predicted to affect protein function and cause the disease was c.2309A>G p. Glu770Gly (NM_006343.2, NP_006334) in MERTK (Table 4). Unknown by the time of detection, the allele frequency for this variant is now reported at 0.000012 in the genome Aggregation Database. Sanger sequencing of exon 17 confirmed the presence of heterozygous MERTK c.2309A>G variant in both siblings and its absence among healthy individuals (n=100) and in the IRD cohort (n=145) from the same geographic area.

**Genome-wide genotyping using the SNP array**—The presence of only one novel likely pathogenic MERTK variant in both patients motivated us to search for a second mutation, such as a large genomic deletion. High-resolution genome-wide genotyping was applied to identify CNVs and homozygosity regions shared by the two patients. The SNP array did not show any regions with neutral loss of homozygosity (regions of homozygosity, ROH) containing retinal genes or common deletions or duplications when standard cut-off values of 5 Mb for ROH and 100 kb for deletions and duplications were used. Manual analysis revealed only one common ROH on chromosome 2q13 (chr2:110,465,111–110,980,346) and one genomic deletion on the same chromosome (chr2:111,891,895–111,980,985; GRCh38). An 89,091 bp deletion present in both siblings encompassed part of the MERTK gene (Appendix 2).

**Breakpoint characterization of the MERTK deletion**—CNV analysis of the genotyping data suggested the deletion breakpoints in the upstream region and in intron 7 of the MERTK gene. Considering this information, we designed PCR primers to enable amplification of an allele containing the deletion and wild-type allele (Figure 2A). The mutant allele was amplified with primers ∆-F-∆ mt-R1 (658 bp, Figure 2B) while the wild-type allele was detected with primers ∆-F-∆ wt-R (386 bp, Figure 2B). Sanger sequencing of the 658 bp PCR fragment revealed breakpoints chr2:111,890,574–111,981,630 yielding in a 91,057 bp deletion in the MERTK gene (c.-8162_1145–1212del, p?; Figure 2C). The origin of the GCA sequence at the junction of the two breakpoints is unknown because it is present on both ends.

**Population frequency of the deletion analyzed with allele-specific PCR**—To estimate frequency of the approximate 91 kb MERTK deletion in the geographically matched control population, we performed a fragment analysis. PCR was conducted with fluorescently labeled forward primer, ∆-F (Table 1) located immediately upstream of the deletion junction, and two reverse primers, ∆ wt-R and the other reverse primer ∆ mt-R2, resulting in a deletion-specific PCR product (354 bp, Table 1, Figure 2D). The approximate 91 kb MERTK deletion was not detected in 100 control samples or in 145 samples from a cohort of patients with IRDs.

**Approximate 91 kb deletion and c.2309A>G represent allelic variants**—Two novel mutations in MERTK were considered to be likely disease causing in two affected individuals although the lack of parental and offspring samples made segregation analysis impossible. To prove that these mutations are allelic, we extracted RNA from peripheral blood of RP116 and performed RT–PCR with forward primers either in exon 7 or 15 and reverse primer in exon 18 (Table 1, Figure 3A). The PCR products of 1292 bp and 372 bp (Figure 3B) were analyzed with Sanger sequencing. In both fragments, only the G nucleotide was seen in position 2309 in exon 17 (Figure 3C). As the PCR fragments could be amplified only from the allele without the deletion, the result confirmed that the mutations are on different alleles, and both patients are compound heterozygous.

**DISCUSSION**

Mutations in the MERTK gene as a cause of IRDs are rare and explain about 1% cases of autosomal recessive RP [8]. MERTK is known as a member of the MER/AXL/TYRO3-receptor kinase family encoding a transmembrane protein with two fibronectin domains, two immunoglobulin-like domains, and one tyrosine kinase domain. The gene participates in many physiologic processes, such as cell survival, migration, differentiation, and phagocytosis of apoptotic cells. The protein has a crucial role in maintenance of retinal photoreceptors by mediating phagocytosis of shed photoreceptor outer segments [18,19]. Initially, mutations in the MerTK gene were identified in a natural animal model of recessive retinal degeneration, in the Royal College of Surgeons (RCS) rat [20], and later in human patients with IRDs [9,21]. Mutations in MERTK are described in patients with different phenotypes, such as...
Table 3. Filtering of WES data according to recessive mode of inheritance.

| Gene   | DNA    | Protein   | gnomAD* | AGVGD† | SIFT‡ | MutationTaster§ | PolyPhen2|| |
|--------|--------|-----------|---------|--------|-------|-----------------|-----------|
| BBSI2  | c.1399G>A | p.(Asp467Asn) | 0.1655  | C0     | Tolerated | Polymorphism    | Benign 0.000 |
| MERTK  | c.353G>A  | p.(Ser118Asn)  | 0.2273  | C0     | Tolerated | Polymorphism    | Benign 0.001 |
| RPIL1  | c.2375T>C | p.(Leu792Pro)  | 0.42    | C45    | Deleterious | Polymorphism    | Benign 0.001 |
| WFSI   | c.461–9A>G | p.?       | 0.6577  | NA     | NA     | NA              | NA         |

In silico analysis of non-synonymous homozygous variants in IRD genes identified by WES to be shared between 2 RP patients and absent from 6 ethnically matched controls. Prediction was performed in accordance with [14] *gnom AD [35], †AGVGD [36]; ‡SIFT – Sorting Intolerant From Tolerant [37] §MutationTaster [38]; ||PolyPhen2 [39].
Table 4. Filtering of WES data according to dominant mode of inheritance.

| Gene   | DNA        | Protein             | gnomAD* | AGVGD† | SIFT‡ | MutationTaster§ | PolyPhen2|| |
|--------|------------|---------------------|---------|--------|-------|-----------------|-------------|
| ACBD5  | c.1388C>T  | p.(Thr463Met)       | 0.0595  | C0     | Deleterious | Polymorphism    | Benign 0.333|
| AHI1   | c.2624–6A>G| p.?                | 00.163  | NA     | NA     | NA              | NA          |
| BFSP1  | c.1033G>A  | p.(Gly345Ser)       | 0.254   | C0     | Tolerated | Polymorphism    | Benign 0.001|
| C2orf71| c.1942G>A  | p.(Ala648Thr)       | 0.01    | C0     | Tolerated | Polymorphism    | Bening 0.028|
| CDHR1  | c.2434C>T  | p.(Pro812Ser)       | 0.0319  | C0     | Deleterious | Polymorphism    | Probably Damaging|
| CEP290 | c.2512A>G  | p.(Lys838Glu)       | 0.0749  | C0     | Tolerated | Polymorphism    | Benign 0.000|
| COL2A1 | c.2094+7A>G| p.?                | 0.0472  | NA     | NA     | NA              | NA          |
| GRM6   | c.733A>G   | p.(Ile245Val)       | 0.0036  | C0     | Tolerated | Disease causing | Benign 0.361|
| HSF4   | c.*8C>T    | p.?                | 0.00026 | NA     | NA     | NA              | NA          |
| LRAT   | c.403G>T   | p.(Ala135Ser)       | 0.0008  | C0     | Tolerated | Disease causing | Benign 0.094|
| LRP5   | c.884–4T>C | p.?                | 0.385   | NA     | NA     | NA              | NA          |
| LRP5   | c.1412+8G>A| p.?                | 0.1368  | NA     | NA     | NA              | NA          |
| LRP5   | c.3989C>T  | p.(Ala1330Val)      | 0.1297  | C0     | Tolerated | Polymorphism    | Benign 0.005|
| MERTK  | c.2309A>G  | p.(Glu770Gly)       | 0.00001 | C65    | Deleterious | Disease causing | Probably Damaging 1.0|
| MYO7A  | c.133–7C>T | p.?                | 0.0048  | NA     | NA     | NA              | NA          |
| NPHP3  | c.2571–7T>C| p.?                | 0.03    | NA     | NA     | NA              | NA          |
| PITPNM3| c.238G>A   | p.(Ala80Thr)        | 0.2848  | C0     | Tolerated | Polymorphism    | Benign 0.0   |
| USH2A  | c.2522C>A  | p.(Ser841Tyr)       | 0.0061  | C0     | Deleterious | Disease causing | Probably Damaging|
| WFS1   | c.2327A>T  | p.(Glu776Val)       | 0.0038  | C0     | Deleterious | Disease causing | Probably Damaging|

In silico analysis of non-synonymous heterozygous variants in IRD genes identified by WES to be shared between 2 RP patients and absent from 6 ethnically matched controls. Prediction was performed in accordance with [14]Liu et al. [14]. *gnom AD [35]; †AGVGD [36];‡SIFT – Sorting Intolerant From Tolerant [37]; §MutationTaster [38]; ||PolyPhen2 [39].
retinitis pigmentosa, rod-cone and cone-rod dystrophy, Leber congenital amaurosis, and retinal dystrophy [5,22-26].

According to the Human Gene Mutation Database (HGMD® Professional) to date, 68 mutations in MERTK have been reported, including 37 missense, 12 splice-site, ten small deletions, insertions/duplications, and small indels, and six gross deletions. In this study, we discovered one novel variant in the tyrosine kinase domain of MERTK. The variant represents a change in a highly conserved nucleotide (phyloP 4.97), and a highly conserved amino acid preserved in 11 species. Computational tools such as MutationTaster, SIFT, and PolyPhen predict c.2309A>G to be disease causing. We also assessed this variant according to standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [17]. There is a line of factors for highly likely pathogenicity of c.2309A>G such as variant location in functionally active tyrosine kinase domain (PM1); absence from controls and low frequency in the genome Aggregation Database (PM2); coexistence with the second mutation,

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**Figure 2.** Characterization of genomic deletion including exons 1 to 7 of the MERTK gene in autosomal recessive RP. **A:** Localization of allele-specific primers. Primer sequences and allele-specific PCR conditions are described in Materials and Methods and Results. **B:** PCR fragments amplified with allele-specific primers were separated with agarose gel electrophoresis. Wild-type (wt) allele (386 bp) amplified with Δ-F and Δ wt-R was detected in both affected individuals and in the unaffected control. The mutant (mt) allele (658 bp) amplified with Δ-F and Δ-mtR1 was seen only in RP115 and RP116 and not in the control due to the presence of a large genomic deletion. **C:** Sanger sequencing shows the junction of two sequences where GCA (in bold) exists on both ends of the deletion breakpoints. G in italics represents the retained nucleotide compared to the site of the deletion described in patients from the Faroe Islands with retinitis pigmentosa [11]. **D:** Identification of a mutant allele with fragment analysis. The upper panel shows the presence of wt and mt alleles in RP115. The lower panel shows only the wt allele in a control sample. Fragments migrated as 370 and 400 bp despite their actual size of 354 and 386 bp (Table 1).
a large genomic deletion in trans (PM3); and multiple lines of computational evidence support deleterious effect of the variant (PP3).

Gross deletions presented by a 1.73 kb deletion of exon 15 [27], a 9.86 kb deletion of exon 8 [7], a 25 kb deletion encompassing exons 6 to 8 [28], a 91 kb deletion encompassing exons 1 to 7 [11,29], and a large deletion encompassing exon 3 to 19 [30]. Notably, that homozygous approximate 91 kb deletion in the MERTK gene was a founder mutation in the Faroe Islands responsible for about 30% of RP in that population [11]. The breakpoints of the deletion in the present study and the deletion reported by Ostergaard et al. are very similar: They differ by only the retention of the G nucleotide in these patients at the site of the deletion [11]. In a cohort of patients with RP in northern Sweden, no MERTK deletion or the c.2309A>G mutation was detected indicating that these two MERTK variants do not represent founder mutations in this region.

A common feature of three gross deletions present in retinal dystrophy cases was the absence of exon 8 [7,11,28]. An in-frame MERTK deletion of 25 kb (c.845–1450del; p.Ala282_His483del) removes part of the protein, corresponding to two fibronectin type III domains [28]. In the case of the 91 kb deletion in the present study, both fibronectin type III domains and both immunoglobulin-like domains are deleted, but because of the deletion breakpoint is more than 8,000 bp upstream from the start codon, the gene is unlikely to be transcribed aborting protein expression. It was shown that a start codon mutation c.3G>A causing severe RP annuls protein expression that may lead to loss of MERTK function [26]. The two patients in the present study suffered a severe visual handicap during their whole life span (more than 80 years) similar to other patients with gross deletions.

In this case, at the site of the deletion, there were no sequence deviations, such as an insertion in the case of a 25 kb deletion [28] or duplication [7,27]. The mechanism of the MERTK deletion appearance seems to be complex. One hypothesis is a non-homologous recombination between two AluY elements because a complete AluY element insertion was found in the case of the exon 8 deletion [7]. The finding of homology to three intronic regions on chromosome 2, reverse complement compared to wild-type intron 5, MIR4435–2HG, and LINC00152 did not support this hypothesis [28]. As a possible explanation, the authors suggested the insertion of a transposable element located within the gene.

Figure 3. MERTK c.-8162_1145–1212del, p.? and c.2309A>G, p.Glu770Gly are allelic variants. A: Localization of MERTK-specific primers. B: Reverse transcription PCR (RT–PCR) was performed on cDNA derived from RP116 and control RNA. Using primers 7F and 18R, we could amplify only an allele without a deletion covering exon 7 (1292 bp). PCR with 15F and 18R was used as control (372 bp). Primers sequences and PCR conditions are described in Materials and Methods and Results. C: Sanger sequencing reveals the presence of the heterozygous c.2309A>G mutation in DNA of RP116 (upper panel), the presence of the mutation c.2309G in RNA of RP116 (middle panel), and the presence of a reference A in the control RNA (lower panel).
We examined the site of the deletion by performing a BLAST search of the flanking junction sequences and detected that the 5’-end sequence is part of the 286 bp repeat AluSx3 (chr2:111,890,318–111,890,603) and the 3’-end is part of the 108 bp repeat L1ME4b (chr2:111,981,585–111,981,692; Appendix 3). The AluSx3 sequence, 8,162 bp upstream from the start codon, was joined directly to a LINE L1 sequence (L1ME4b) in intron 7 6,160 bp downstream from the last nucleotide in exon 7. Notably that the 3’-breakpoint sequence of L1ME4b contained a matrix attachment region (MAR) AT-rich motif near the endpoint which was highlighted in the study of the recurrent deletion of the RB1 gene caused by a non-homologous recombination between a LINE-IHS and a MER21B element [31]. The first example of a non-homologous recombination between Alu and L1 repeats was described in a dystrophin gene causing a 430 kb deletion [32]. Sequencing of the 430 kb deletion ends revealed the presence of Alu sequence in intron 1 at the 5’-end and the L1 sequence in intron 7 at the 3’-end [32].

In summary, we highlight the possibility that MERTK deletions might be more frequent than estimated and screening for large genomic deletions should be done in IRD cases without known genetic cause. We also show that in recessive cases one should consider compound heterozygosity when one mutation is a single nucleotide change and another mutation is a large CNV. Finally, genetic diagnosis is extremely important considering the progress in the gene therapy for defects in MERTK [33,34].

APPENDIX 1. SUPPLEMENTARY FIGURE 1.

Results of variant filtering for WES data in 2 patients with retinitis pigmentosa. To access the data, click or select the words “Appendix 1.”

APPENDIX 2. SUPPLEMENTARY FIGURE 2.

CNV analysis using genome wide genotyping revealed MERTK deletion. To access the data, click or select the words “Appendix 2.”

APPENDIX 3. SUPPLEMENTARY FIGURE 3.

Deletion breakpoints in MERTK gene presented as part of AluSx3 and part of L1ME4b repeats. To access the data, click or select the words “Appendix 3.”

ACKNOWLEDGMENTS

The authors thank our study participants for providing clinical histories and DNA samples. This investigation was supported by grants from Stiftelsen Kronprinsessan Margaretas Arbetsnämnd för synskadade, Ögonfonden and Umeå University and Västerbotten County Council in cooperation in the fields of Medicine, Odontology, and Health.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 18 October 2018. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.