Single-Exome sequencing identified a novel *RP2* mutation in a child with X-linked retinitis pigmentosa

Hassol Lim, BS, MD, PhD,* Young-Mi Park, MS,† Jong-Keuk Lee, PhD,† Hyun Taek Lim, MD, PhD†,‡

ABSTRACT • RÉSUMÉ

Objective: To present an efficient and successful application of a single-exome sequencing study in a family clinically diagnosed with X-linked retinitis pigmentosa.
Design: Exome sequencing study based on clinical examination data.
Participants: An 8-year-old proband and his family.
Methods: The proband and his family members underwent comprehensive ophthalmologic examinations. Exome sequencing was undertaken in the proband using Agilent SureSelect Human All Exon Kit and Illumina HiSeq 2000 platform. Bioinformatic analysis used Illumina pipeline with Burrows-Wheeler Aligner-Genome Analysis Toolkit (BWA-GATK), followed by ANNOVAR to perform variant functional annotation. All variants passing filter criteria were validated by Sanger sequencing to confirm familial segregation.
Results: Analysis of exome sequence data identified a novel frameshift mutation in *RP2* gene resulting in a premature stop codon (c.665delC, p.Pro222fsTer237). Sanger sequencing revealed this mutation co-segregated with the disease phenotype in the child's family.
Conclusions: We identified a novel causative mutation in *RP2* from a single proband's exome sequence data analysis. This study highlights the effectiveness of the whole-exome sequencing in the genetic diagnosis of X-linked retinitis pigmentosa, over the conventional sequencing methods. Even using a single exome, exome sequencing technology would be able to pinpoint pathogenic variant(s) for X-linked retinitis pigmentosa, when properly applied with aid of adequate variant filtering strategy.

Objet : Présenter un cas d'application efficace et fructueuse du séquençage de l'exome à une famille avec un diagnostic de rétinite pigmentaire (RP) liée à l'X.
Nature : Étude sur le séquençage de l'exome fondée sur des données d'examen clinique.
Participants : Un proposant de 8 ans et sa famille.
Méthode : Le proposant et les membres de sa famille ont subi des examens ophtalmologiques approfondis. Nous avons réalisé le séquençage de l'exome du proposant avec la trousse SureSelect Human All Exon (Agilent) et la plateforme HiSeq 2000 (Illumina). Pour l'analyse bioinformatique, nous avons utilisé Illumina Pipeline avec BWA-GATK, puis ANNOVAR pour l'annotation fonctionnelle des variants. Nous avons validé par séquençage de Sanger tous les variants qui répondaient aux critères de filtrage afin de confirmer la ségrégation familiale.
Résultats : L'analyse des données de séquençage de l'exome a révélé une nouvelle mutation du cadre de lecture du gène RP2 qui résulte en l'apparition d'un codon d'arrêt prématuré (c.665delC, p.Pro222fsTer237). Le séquençage de Sanger a révélé une coségrégation de cette mutation avec le phénotype pathologique de la famille de l'enfant.
Conclusions : Nous avons localisé une nouvelle mutation causale du gène RP2 à partir de l'analyse des données de séquençage d'un seul exome. Cette étude montre la supériorité du séquençage de l'ensemble de l'exome dans le diagnostic génétique de la RP liée à l'X, par rapport aux méthodes de séquençage classiques. Même à partir d'un seul exome, la technologie de séquençage de l'exome, correctement appliquée et appuyée par une stratégie adéquate de filtrage des variants, pourrait permettre d'isoler des variants pathogènes pour la RP liée à l'X.

Retinitis pigmentosa (RP) is a well-known retinal dystrophy characterized by progressive degeneration of photoreceptor cells and eventual loss of vision and visual field. It is one of the most common hereditary retinal diseases with a worldwide prevalence of approximately 1 in 3000 to 5000. RP is not a single disease but rather a heterogeneous group of diseases. To date, more than 65 genes have been identified as responsible for RP, but they still explain no more than 50% of the cases. The inheritance pattern of RP is also complex, with autosomal dominant, autosomal recessive, X-linked, digenic, and even maternal or mitochondrial modes.[1,2] Among them, the X-linked pattern is a relatively severe form of RP, with an early age at onset and rapid progression, accounting for 10% to 20% of all RP cases. At least 6 different loci have been mapped for X-linked RP. However, only 2 principal genes, *RPGR* (OMIM 312610) and *RP2* (OMIM 312600), have been identified as the primary causative genes.[3–6]

Current strategy for molecular diagnosis of X-linked RP involves mutation screening of the *RPGR* and *RP2* genes by conventional Sanger sequencing or arrayed primer extension (APEX) chips.[7,8] Sanger sequencing is indeed time consuming, costly, and labour intensive, especially when screening multiple genes or regions in genetically heterogeneous diseases such as RP. However, the APEX method cannot offer a chance to identify novel genes or mutations because the array is designed to detect only known mutations in known genes. In comparison with

http://dx.doi.org/10.1016/j.jcjo.2016.03.017
ISSN 0008-4182/16

Canadian Ophthalmological Society | Société canadienne d'ophtalmologie
EYE PHYSICIANS AND SURGEONS OF CANADA | MÉDECINS ET CHIRURGIENS OPHTALMOLOGISTES DU CANADA

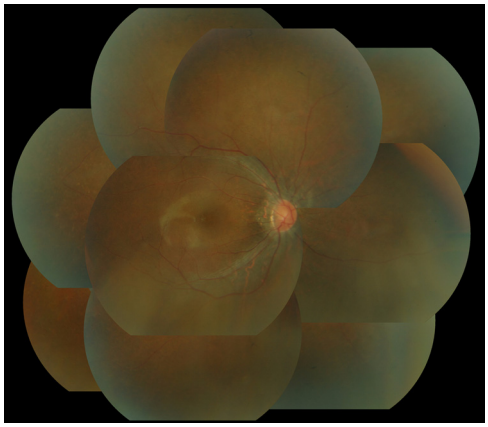

Fig. 1—Composite fundus photographs of the proband showing a mild degree of diffuse granular pigmentary changes mostly in retinal periphery.

these methods, high-throughput DNA sequencing technology has significantly lowered the hurdles for human genomic analysis. Currently, whole-exome sequencing (WES) is regarded as a powerful and effective way to unravel genetic causes of many unsolved human diseases.[9–12] It is becoming more commonly available and has also been applied for the molecular diagnosis of hereditary ocular diseases.[13–16] Several studies have recently reported the successful application of the exome sequencing technology in RP.[14,17,18] However, specifically when regarding X-linked RP, there have been only a few studies reported in the literature.[19,20]

We performed WES in a Korean family with a clinical suspicion of X-linked RP and were able to pinpoint a novel causative mutation for the disease from only a single-exome sequencing. Hence, we present our efficient application of the exome sequencing technology in X-linked RP.

Methods

Participants and clinical assessment

The present study was approved by the Institutional Review Board at the Asan Medical Center, University of Ulsan College of Medicine and was performed in accordance with the Declaration of Helsinki. Verbal and written informed consent was obtained.

The proband and his family members underwent comprehensive ophthalmologic examinations including measurement of visual acuity, slit lamp exam, ophthalmoscopy, visual field test, optical coherence tomogram, and electroretinography.

Exome sequencing and data analysis

We undertook WES in the proband to maximize our capacity to identify novel causal variants. Exome capture was carried out using the Agilent SureSelect Human All Exon 50M Kit (Agilent Inc., Santa Clara, Calif.) and DNA libraries were prepared according to the Illumina protocols. Subsequent sequencing of the exon-enriched DNA fragments was performed on Illumina HiSeq 2000 platform using standard paired-end mode sequencing protocol.

Illumina data analysis pipeline was used to perform base calling, read mapping, and variant calling. Raw reads were exported in the FASTAQ format and mapped to the human reference genome (UCSC hg19, NCBI build 37.1) with Burrows-Wheeler Aligner software (Heng Li, Broad Institute, Cambridge, MA, USA). Mapped reads were processed and sorted with Sequence Alignment/Map tools and polymerase chain reaction duplicates were removed with Picard tools. Single-nucleotide variants were called with the Genome Analysis Toolkit (version 1.4.11). For variant calling, we only used reads with a mapping quality score ≥ 20 and bases with base quality score ≥ 20. Variants were then annotated by ANNOVAR against the UCSC hg19 reference genome annotation.

Assuming an X-linked recessive mode of inheritance, we applied a series of filtering steps for the variants. First, we excluded all variants not present in the coding sequence or in intron/exon splice sites, and synonymous variants. Then, we excluded variants not present in X chromosome, and subsequently filtered against the NCBI dbSNP (build 138, http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes Project databases (http://www.1000genomes.org/),

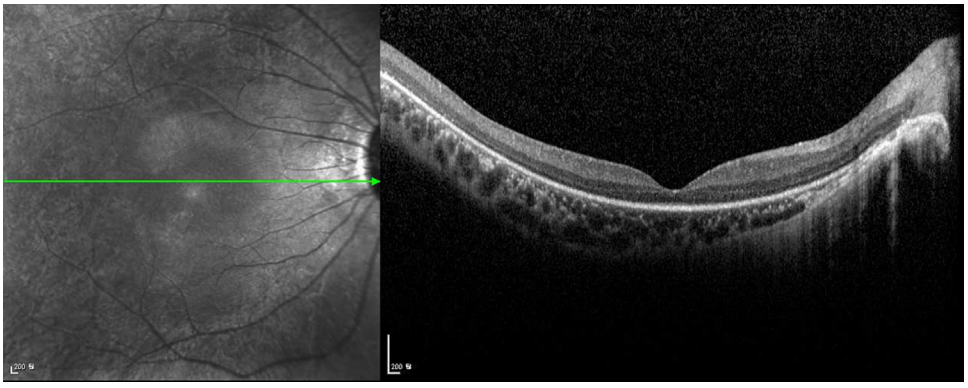

Fig. 2—Spectral domainoptical coherence tomography of the right macula shows diffuse disruption of the ellipsoid zone of the outer retina.