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Whole exome sequencing reveals a mutation in an osteogenesis imperfecta patient

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ABSTRACT

Osteogenesis imperfecta (OI) is an autosomal dominant disorder characterized mainly by bone fragility and blue sclerae. OI is caused by mutations in type I collagen genes, *COL1A1* and *COL1A2*. Dentinogenesis imperfecta is a common disorder for osteogenesis imperfecta patients. More than half of the OI patients have also dentinogenesis imperfecta. Whole exome sequencing (WES), involves exome capture, which limits sequencing of the protein coding regions of the genome, composed of about 20,000 genes, 180,000 exons, and constituting approximately 1% of the whole genome. A major indication for use is molecular diagnosis of patients with suspected genetic disorders or of patients with known genetic disorders with substantial genetic heterogeneity involving substantial gene complexity. In this study, we performed WES for a patient prediagnosed as Osteogenesis imperfecta. He had also dentinogenesis imperfecta. The WES results confirmed with Sanger sequencing revealed as a missense mutation at codon 560 of *COL1A1* gene: c.1678G>A p.(Gly560Cys). The mutation was in exon 25 and according to the dbSNP database this mutation corresponded to rs67507747. As a conclusion, it is very important to perform WES after an algorithm. This algorithm has to include, a suspect of a mendelian disorder, multiple genetic conditions in the differential diagnosis, and even if it is available the conventional diagnosis is prohibitively expensive. Finally, Sanger sequencing in order to confirm the results is also advised.

1. Introduction

Osteogenesis imperfecta (OI) is an autosomal dominant disorder characterized mainly by bone fragility and blue sclerae. OI is caused by mutations in type I collagen genes, *COL1A1* and *COL1A2*, which are responsible for the synthesis of this main protein of bones, skin, ligaments, tendons and most other connective tissues (Gajko-Galicka, 2012). In OI, multiple bone fractures generally result from minimal trauma. Also affected individuals have blue sclerae, normal teeth, and normal or near-normal stature (Byers, 1993). Although individuals with OI type I have distinctly blue sclerae which remain intensely blue throughout life, in OI types III and IV sclera may also be blue at birth and during infancy (Sillence et al., 1993).

Sillence et al. (1979) classified OI into types I to IV using clinical, radiographic, and genetic criteria. Type I: a dominant form with blue sclerae (166,200), type II (166,210): a perinatal lethal OI syndrome, also known as congenital OI, type III (259,420) a progressively deforming form with normal sclerae, and type IV (166,220) a dominant form with normal sclerae (Sillence et al., 1979). Levin et al. (1980) suggested that OI subtypes could be further divided into types A and B based on the absence or presence of dentinogenesis imperfecta (Levin et al., 1980).

Dentinogenesis imperfecta is a common disorder for osteogenesis imperfecta patients. More than half of the OI patients have also dentinogenesis imperfecta. There are three types of this disorder and Type I is mainly associated with osteogenesis imperfecta. The inheritance of type I dentinogenesis imperfecta usually is autosomal dominant but can be recessive if the associated osteogenesis imperfecta is of recessive type. Deficient collagen formation is the cause of Type I of DI. Types II and III are related to dentin sialophosphoprotein (DSPP) gene mutation. In Type I of dentinogenesis imperfecta both dentition was affected, although primary dentition was more severely affected. Teeth discoloration is a main clinical manifestation of dentinogenesis imperfecta, which can be generally in yellow/brown and gray. Enamel fractures also can be seen because of weak dentin. In radiographic examination bulbous crowns and short roots are common signs of DI. Histological examinations show similar changes in dentin such as normal mantle dentin, irregularity in circumpulpal dentin, abnormal dentin tubules and some atubular areas can be seen (Teixeira et al., 2008).

The collagen, type I, alpha 1 (*COL1A1*) gene is localized on 17q21.33 and encodes the pro-alpha1 chains of type I collagen whose triple helix

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comprises two alpha1 chains and one alpha2 chain. Type I is a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon. The mutations in this gene have been reported to be associated with osteogenesis imperfecta types I–IV, Ehlers-Danlos syndrome type VIIA, Ehlers-Danlos syndrome Classical type, Caffey Disease and idiopathic osteoporosis (Dalgleish, 1997). *COL1A1* gene has been registered in Osteogenesis imperfecta variant database; http://www.le.ac.uk/genetics/collagen/.

Whole exome sequencing (WES), involves exome capture, which limits sequencing of the protein coding regions of the genome, composed of about 20,000 genes, 180,000 exons, and constituting approximately 1% of the whole genome. It has been reported that the exome contains about 85% of heritable disease-causing mutations (Choi et al., 2009).

WES has the advantages of speed and efficiency relative to Sanger sequencing, delivering results in weeks to months. If candidate variants can be sufficiently reduced in number, targeted Sanger sequencing can be used to confirm the presence or absence of the potential variant(s) in the individual being diagnosed. It is very important to perform exome sequencing as a clinical service for patients to determine a molecular diagnosis by identifying causal variants in known genes (Lyon and Wang, 2010).

However, there are also some limitations of WES similar to Sanger sequencing, such as; causative variants that occur in non-coding regulatory regions or large deletions, duplications, rearrangements, nucleotide repeats, and epigenetic changes cannot be detected (Lyon and Wang, 2010; McDonald et al., 2012). Due to these and other detection limitations, the total proportion of gene variants that will be missed by current methods may be as high as 15–20% (Fuchs et al., 2012). Parla et al. (2011) determined that the genotypic information resulting from exome capture and sequencing provides sensitivities up to 97%, false discovery rates up to 0.67% for all variants and up to approximately 1.5% for heterozygous variants (Parla et al., 2011).

Additionally, public databases containing information on putative disease-causing mutations are incomplete and may have high error rates requiring manual curation; associations for some mutations in the database may not be causal (Bell et al., 2011).

The preparation for ordering WES has been reported to include four key elements: gathering information on family history, systematically evaluating the patient's phenotype, searching the medical literature and databases, and obtaining informed consent (Biesecker and Green, 2014).

A major indication for use is molecular diagnosis of patients with suspected genetic disorders or of patients with known genetic disorders with substantial genetic heterogeneity involving substantial gene complexity. The laboratory indications for WES testing includes; when a patient with a diagnosis that suggests the involvement of one or more of than many different genes and if available sequencing is expensive (Blue Cross and Blue Shield Association, 2013).

In this study, we performed WES for a patient prediagnosed as Osteogenesis Imperfecta. First of all, our aim was to find the causative mutation for OI and then to delineate the subtypes of OI and lastly to give a better counseling for the family for their future pregnancies. According to the Levin's classification, the diagnosis of our case was finally suggested as OI type IVB.

2. Materials and methods

2.1. Clinical report

A 32 year old male patient admitted to our Medical Genetics Department because of his recurrent femur fractures. In his history, he indicated that the fractures had been started since he was 7 years. Also, dentinogenesis imperfecta was also diagnosed.

He has been married for 5 years. Recently a D&C had been performed because the fetus had short femur. A previous sanger sequencing for *COL1A1* had been performed revealing no disease causing mutation for Osteogenesis Imperfecta. So, in order to rule out genetic heterogeneity, whole exome sequencing was planned for our patient in order to identify the causative mutation for OI.

2.2. DNA isolation

A 5 ml of peripheral blood sample of the patient was collected with consent from in EDTA tube. The isolation of the DNA was performed with the NucleoSpin® Blood kit (Macherey-Nagel, Düren, Germany) according to the protocol. The concentration and quality of eluted DNA sample was analyzed by a Spectrophotometer (NanoDrop ND 1000, USA).

2.3. Whole exome sequencing

Regarding the WES analysis the enrichment was performed by Nextera Rapid Capture Expanded Exome Kit with the Illumina HiSeq platform with a coverage of $\times 70$. The analysis was performed by the Arpeggi Engine. This pipeline has been used for alignment, variant calling, and variant annotation. The raw data had been provided in VCF format.

2.4. Analysis of sequence variations

The VCF data had been analyzed by a pipeline for ranking nonsynonymous single nucleotide variants given a specific phenotype, eXtasy (Sifrim et al., 2013). Also, online tools including Sorting Intolerant From Tolerant (SIFT) (Ng and Henikoff, 2001) and Polymorphism Phenotyping v2 (PolyPhen-2) were used to confirm the effect of the detected *COL1A1* c.1678G>A missense mutation (Adzhubei et al., 2010).

In our case, the raw data revealed 56.386 variants. We used the online eXtasy program (Sifrim et al., 2013). This program had 2 scores: "complete" and "imputed", and these scores ranged from 0 to 1, representing the score of 1 to be "disease-causing". Also, a score above 0.5 was set to be the threshold. We chose the "complete" score in order to reduce the variants from 56.386 to 7.167. Also, filtered the "complete" scores ranging between 0.5 and 1 in order to reduce the variants from 7.167 to 36 genes. Among 36 genes, only *COL1A1* gene was selected as no other genes were reported to be in the etiology of OI. Then, we performed Sanger sequencing in order to confirm this causative mutation found in *COL1A1* gene.

2.5. Sanger sequencing

The result of WES was confirmed by Sanger sequencing. We designed primers with an online tool, Primer3 (Untergasser et al., 2012). The sequencing results were then aligned with the reference gene sequences available in the NCBI database. Sequencing reactions were conducted by using Genetic Analyzer 310 (ABI/Life Technologies). The sequencing results also confirmed the WES results (Fig. 1).

3. Results and discussion

The WES results confirmed with Sanger sequencing revealed as a missense mutation at codon 560 of *COL1A1* gene: c.1678G>A p.(Gly560Cys). The mutation was in exon 25 and according to the dbSNP database this mutation corresponded to rs67507747 (Fig. 1).

Regarding the WES results preimplantation genetic diagnosis was performed and the family had a healthy baby.

WES is currently indicated for the detection of rare variants in patients with a phenotype suspected to be due to a mendelian genetic disorder, after known single-gene candidates have been eliminated from consideration or when a multigene testing approach is prohibitively expensive (Biesecker and Green, 2014). In this study, our patient was