Identification of a Novel Nonsense Mutation in the ARSE Gene of a Patient with X-Linked Recessive Chondrodysplasia Punctata

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ABSTRACT

X-linked recessive chondrodysplasia punctata (CDPX1) is caused by a hemizygous mutation in the arylsulfatase E (ARSE) gene located on chromosome Xp22.3. It is a rare congenital disorder of punctate calcifications in cartilages, leading to short stature and facial and limb anomalies. These clinical features are frequently observed in all types of chondrodysplasia punctata and have also been seen in other cartilage developmental disorders. Because of the phenotypical similarities, specific testing for only one gene is inefficient and time consuming. The advent of next-generation sequencing has provided an opportunity to improve diagnostic accuracy as well as save on time and cost. Here, we report on a patient diagnosed with CDPX1, who was identified via diagnostic exome sequencing to have a novel nonsense mutation in the ARSE gene, that was inherited from the mother.

Key Words: Arylsulfatase E, X-linked recessive chondrodysplasia punctata, Next-generation sequencing, Stippled epiphyses

INTRODUCTION

Chondrodysplasia punctata (CDP) is a clinically and genetically diverse group of rare disease that share the features of stippled epiphyses and skeletal changes. This disorder is divided into 3 types according to inheritance pattern: an autosomal recessive type, named rhizomelic chondrodysplasia punctata (RCDP), that has 3 associated genes (RCDP1 caused by the peroxisomal biogenesis factor 7 [PEX7] gene, RCDP2 caused by the glyceronephosphate O-acyltransferase [GNPAT] gene, and RCDP3 caused by the alkylglycerone phosphate synthase [AGPS] gene)¹ ²; an X-linked recessive type [CDPX1 caused by the arylsulfatase E (ARSE;NM_000047.2) gene]³; and an X-linked dominant form (CDPX2 caused by the emopamil binding protein [EBP] gene)² ⁴. When case of CDP

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is suspected, genes such as PEX7, GNPAT, AGPS, ARSE, and EBP need to be analyzed. However the Sanger sequencing method can analyze only one gene at a time; therefore, failure to identify the variant of the suspected gene necessitates repeated sequencing analysis for identifying the other genes, which is very time-consuming and costly. In contrast, next-generation sequencing (NGS) provides a unique glimpse into the complexity of genetic disorders and has been applied to many areas of study, including medical genetics. Here, we report on a patient with CDPX1, who was confirmed via diagnostic exome sequencing (DES) (an NGS technique that uses targeted exome sequencing analysis and Sanger sequencing) to have a novel nonsense mutation in the ARSE gene and whose mother was confirmed to be an asymptomatic carrier of the mutation by Sanger sequencing.

CASE REPORT

The male infant was born at 38 weeks of gestation from the first pregnancy of a healthy 27-year-old mother and a 31-year-old father. His mother was under routine prenatal follow-up during pregnancy and showed no specific abnormalities. She did not have any chronic diseases, and there was no history of exposure to any known embryopathic agent such as warfarin. Her family history was negative for malformations or other genetic conditions.

The infant was transferred to our neonatal intensive care unit because of desaturation and dysmorphism. Chest X-ray showed pneumomediastinum; however, desaturation was recovered after oxygen supplementation. Physical examination revealed macrocephaly with a flat occiput and large fontanel, frontal bossing, short neck, and low nasal bridge, as well as brachytelephalangy (Figure 1A) and rhizomelic shortening of the arms. The patient had a body weight of 3,610 g (50-75th percentile).

Figure 1. (A) The patient shows frontal bossing, a low nasal bridge, a short neck, and a short stature. (B) Both the feet and humerus show punctate calcification on epiphyses. (C) Infantogram showing shortening of both humerus bones (humerus < radius, ulna; normal humerus:radius ratio is about 1.7).
percentile), height of 46 cm (5-10<sup>th</sup> percentile), and head circumference of 37.5 cm (>95<sup>th</sup> percentile). Radiographs showed incomplete ossification with epiphyseal punctate calcifications of the distal humerus, femur, and tarsal bones (Figure 1B), along with nasomaxillary hypoplasia and symmetric shortening of the humerus (rhizomelic shortening) (Figure 1C). The neonatal screening test and tandem mass screening test were normal. Chromosomal analysis showed a 46, XY karyotype without any structural abnormalities. Further evaluation for other associated dysfunctions revealed that the infant did not have cardiac anomalies, renal anomalies, or any optic problems such as cataract or retinopathy. Under suspicion of cartilage developmental disorder, we performed DES to identify possible variants of genes associated with this disorder. After obtaining informed consents from the parents, blood samples were collected from the patient, and genomic DNA was extracted from peripheral whole blood. We prepared indexed, paired-end libraries, followed by enrichment using the TruSight One Sequencing Panel (Illumina, San Diego, CA, USA), which covers 12 Mb of genomic content, including 4813 genes associated with a clinical phenotype (Table 1).

The results revealed a C-to-T transition at the 109<sup>th</sup> nucleotide

Table 1. Summary of Exome Sequencing Data

<table>
<thead>
<tr>
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<th>Proband</th>
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<tbody>
<tr>
<td>Total captured region size</td>
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<tr>
<td>Number of gene</td>
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<tr>
<td>% of captured regions with ≥10X</td>
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<tr>
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<tr>
<td>Non-synonymous SNV</td>
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</tr>
<tr>
<td>Rare variants</td>
<td>240</td>
</tr>
</tbody>
</table>

Abbreviation: SNV, single nucleotide variant.

Figure 2. (A) Sequencing analysis of the Arylsulfatase E (ARSE) gene in the patient. Exome sequencing of the ARSE gene revealed that the patient was hemizygous for a C-to-T transition in exon 3 (c.109C>T). (B) Sanger sequencing analysis of the ARSE gene showed that the c.109C>T variation was inherited from the patient’s mother. The patient was hemizygous and the mother was heterozygous for a C-to-T transition in exon 3 (ARSE: NM_000047.2:c.109C>T:p. R37X).
on exon 3 in the \textit{ARSE} gene, which changes an arginine into a termination codon at amino acid position 37 (c.109C>T; p.Arg37*, Figure 2A). The variant was presumed to be pathogenic based on the following 3 criteria in the guidelines for the interpretation of sequence variants of the American College of Medical Genetics and the Association for Molecular Pathology$^5$: 1) it is a truncating variant (PVS1), 2) the variant is absent from controls (PM2), and 3) the patient’s phenotype is highly specific for a disease with a single genetic etiology (PP4). To identify the origin of the variant, we collected blood from the parents and performed Sanger sequencing analysis after obtaining informed consents from both parents for genetic analysis (Figure 2B). As a result, we identified that it was a sequence variant of maternal origin.

The patient was discharged without any respiratory or feeding problems on hospital day 21. On last follow-up at age of 11 months, his height was still below 3%. He also had developmental delay, especially in motor and speech development. On a previous follow-up at age of 8 months, he had no optic problems such as cataract or retinopathy.

\section*{DISCUSSION}

\textit{CDPX1} is one of the types of chondrodysplasia punctata that share the features of stippled epiphyses and skeletal changes. This X-linked recessive variant is caused by a mutation in the \textit{ARSE} gene located on chromosome Xp22.3$^3$. Patients with mild phenotypes of \textit{CDPX1} were first reported by Sheffield et al.$^6$. The clinical characteristics of \textit{CDPX1} are short stature, brachytelephalangy, dysmorphic face, nasomaxillary hypoplasia, ichthyosis, cervical myelopathy, and hearing loss. Although most affected males have minimal morbidity and skeletal findings that improve by adulthood, some have significant medical problems including respiratory compromise, cervical spine stenosis and instability, mixed conductive and sensorineural hearing loss, and intellectual disability. Stippled epiphyses on X-ray are the most important radiological feature of \textit{CDPX1}$^4,5$. In approximately 25% of patients with features of \textit{CDPX1}, karyotype analysis identified gene rearrangements (such as deletions, insertions, or splicing) of the short arm of the X chromosome (Xp) that includes the \textit{ARSE} gene, and 60% to 75% of patients with clinical features of \textit{CDPX1} were diagnosed by identifying mutations in the \textit{ARSE} gene through sequencing analysis$^4$. This shows that we need to perform not only karyotyping and microarray assays but also sequencing analysis to diagnose \textit{CDPX1}.

According to the Leiden Open Variation Database, 55 mutations have been reported for the \textit{ARSE} gene, and there are extra reported cases of Korean and Slovenian patients$^8,10$. Thirteen cases were caused by partial or whole gene deletions, whereas all other cases involved point mutations. In the past, statistical studies on \textit{CDPX1} were not feasible because of the scarcity of cases. However, the accumulation of cases to date has led to a few such studies being reported. The genotype-phenotype correlation for \textit{CDPX1} has been controversial. A recent prospective study of \textit{CDPX1} revealed no obvious genotype-phenotype correlations$^{11}$. However, this study was limited to missense mutations of \textit{ARSE}. In the case report of Vrecar et al.$^{10}$, mutations in exon11 presented with more specific and severe clinical symptoms. For this reason, a confirmation of diagnosis by genetic analysis is necessary because it can be helpful for predicting the patient’s prognosis as well as for genetic counseling to the patient’s parents who are preparing for pregnancy.

Pediatricians usually base their decision of the method of genetic analysis for a disease under suspicion on the clinical characteristics and family history. In the case of a well-known chromosome disorder or single gene disorder, the choice of the genetic analysis method is relatively easy and leads to a highly accurate diagnosis. In the case of CDP, however, the differential diagnosis is complex since it could include diastrophic dysplasia, spondyloepiphyseal dysplasia congenital, Binder syndrome, and other forms of chondrodystrophy. Furthermore, CDP has many subtypes with clinical features that range from a mild disease to an extremely severe condition. The clinical suspicion and the genetically confirmed diagnosis would be different, especially in diseases that are rare and have few references. Therefore, NGS can be applied effectively in cases that have many possible candidate genes for a specific phenotype. The advantages of NGS its wide genome coverage and the ability to obtain from a single DNA strand. In a study on the clinical analytical sensitivity and specificity of NGS, the sensitivity was found to be 92.7%$^{12}$. Therefore, in the cases of many other Mendelian genetic disorders that have many possible candidate genes, we recommend NGS.

However, DES does have some limitations. Sanger sequencing is more cost-effective than DES when there is a specific candidate gene involved. In addition, large rearrangements, copy
number variation mutations, and mitochondrial mutations are only detectable by microarray assay not DES. For these reasons, DES is not always applicable for every case. Nonetheless, our results highlight the appropriate use of this NGS technique and its effectiveness in the diagnostic field.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**


