A diagnosis of hypochondroplasia by next generation sequencing

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Achondroplasia and hypochondroplasia are the two most common forms of short-limb dwarfism. They are autosomal dominant diseases that are characterized by a rhizomelic shortening of the limbs, large head with frontal bossing, hypoplasia of the mid-face, *genu varum* and trident hands. Mutations in the fibroblast growth factor receptor-3 (*FGFR3*) gene, which is located on chromosome 4p16.3, have been reported to cause achondroplasia and hypochondroplasia. More than 98% of achondroplasia cases are caused by the G380R mutation (c.1138G>A or c.1138G>C). In contrast, the N540K mutation (c.1620C>A) is detected in 60-65% of hypochondroplasia cases. Tests for common mutations are often unable to detect the mutation in patients with a clinical diagnosis of hypochondroplasia. In this study, we presented a case of familial hypochondroplasia with a rare mutation in *FGFR3* identified by next generation sequencing.

Key words: Achondroplasia, Hypochondroplasia, Dwarfism, Receptor, fibroblast growth factor, type 3, High-throughput nucleotide sequencing.

Introduction

Achondroplasia (ACH; OMIM #100800) and hypochondroplasia (HCH; OMIM #146000) are the two most common autosomal dominant skeletal dysplasias characterized by short-limb dwarfism [1]. Patients with ACH typically show characteristic phenotypes at birth including relative macrocephaly, midface hypoplasia, rhizomelic shortening of the limbs, brachydactyly, narrowing of the spinal canal, lumbar lordosis, and *genu varum*. HCH, a milder form of ACH, causes a less severe phenotype, which is typically not evident before 2 years of age [2,3].

Both ACH and HCH are caused by missense mutation in the fibroblast growth factor receptor-3 gene (*FGFR3*) located on chromosome 4p16.3 [4]. Recent studies reported that more than 98% of patients with ACH have a glycine-to-arginine

substitution at residue 380 (G380R) of *FGFR3*, located within the *FGFR3* transmembrane domain, as a result of guanine (G) to adenine (A) or cytosine (C) mutation at nucleotide 1,138 of the *FGFR3* gene [5]. In the case of HCH, only 65% of patients have the common asparagine-to-lysine substitution (N540K), located in the *FGFR3* tyrosine kinase domain, caused by a C to A mutation at position 1,620 [5]. It is thought that the unaccounted HCH cases may be caused by a yet-to-be identified mutation in *FGFR3* or a different gene.

The proband patient in this study was a 28-month-old female child who presented the typical HCH phenotype, with no common ACH or HCH mutation detected. After a verbal informed consent was received from parents, the genomic DNA was isolated from the peripheral blood of the patient using the SureSelect Human All Exon Kit (Agilent Technologies, Santa

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Conflict of interest: The authors declare that they do not have any conflicts of interest.

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Clara, CA, USA). A sequence analysis was carried out using a HiSeq 2000 sequencing system (Illumina, San Diego, CA, USA). An S84L amino acid substitution of *FGFR3* was identified in the patient. In this study, we reported the first HCH case with an S84L mutation in *FGFR3* in Korea, identified using next generation sequencing.

Case

A 28-month-old female child was referred to the Department of Pediatrics of the Hallym University Chuncheon Sacred Heart Hospital (Chuncheon, Korea) for an evaluation of short stature and *genu varum*. She was born to non-consanguineous parents after 40 weeks of gestation by spontaneous vaginal delivery without perinatal complications. The patient's birth weight was 3.2 kg (25-50th percentile), and there was no developmental delay or intellectual disability reported. The patient is the second of four children of a Korean father and a Filipino mother. Her height and weight on referral were 87 cm (25th percentile) and 16 kg (90-97th percentile), respectively. Her father has a short stature of approximately 155 cm (<3rd percentile) and a mild rhizomelic shortening of the limbs without any other orthopedic deformities. The height of the patient's mother, brother, and twin younger sisters' was within the normal range. The height of the mother and twin sisters' was 155 cm (10-25th percentile), 82.4 cm (75-90th percentile), and 76.4 cm (10-25th percentile),

respectively with no morphological abnormalities. The height of the older brother was not evaluated.

During the physical examination, a mild mid-facial hypoplasia, rhizomelic shortening of extremities, *genu varum*, and lumbar lordosis were observed; however, the result of a neurologic examination was normal (Fig. 1). Laboratory examinations showed normal blood cell count and thyroid function. Other endocrinology laboratory findings were within the normal range, including an insulin-like growth factor 1 level of 129.2 ng/mL, a cortisol level of 3.3 µg/dL, an ACTH level of 8.5 pg/mL, and a 25-hydroxyvitamin D level of 14.6 ng/mL. A toluidine blue urine spot test for mucopolysaccharidosis was also performed and the result showed no abnormality relative to those observed in the same age group. Skeletal radiographs, including those of the paranasal sinus (PNS), chest, whole spine, both hands, forearms, and knees, were obtained. Radiographs of the PNS and both hands showed normal findings (Figs. 2A-C). The spinal anteroposterior (AP) X-ray showed normal interpedicular distances; however, a lumbar lordosis with horizontally tilted sacrum was apparent in the lateral view (Figs. 2D and E). Radiographs of forearms and knees showed normal findings.

Typically, when ACH or HCH is suspected based on clinical symptoms of the patient, we first assess the presence of the G380R mutation (G1138A or G1138C) in a genetic analysis. However, neither of the two major mutations was detected in the proband patient. We subsequently sequenced six exons

Fig. 1. Photographs of a 28-month-old female child with hypochondroplasia. (A, B) Photographs showing mild mid-facial hypoplasia, which is especially apparent in the lateral view. (C) All extremities show relatively short proximal limbs (when compared to the distal parts) and *genu varum*. (D) A lateral photograph of the patient shows excessive lumbar curvature and a sacral lesion of the spine.

Fig. 2. Radiographs of the patient. (A, B) Anteroposterior (AP) and lateral views of the paranasal sinus show no abnormal findings. (C) An AP view of both hands shows no trident fingers. (D) A lumbarosacral spine radiograph shows normal interpedicular distances. (E) A lateral view lumbarosacral spine radiograph shows lumbar lordosis.

(exon 6, 7, 8, 11, 13, and 17) of *FGFR3*, which include sites often mutated in ACH and HCH. However, none of the common mutations, including Y373C, G380R, N540S, and K650N, was found in the patient. Therefore, a whole genome analysis was performed by next generation sequencing to identify a mutation in other gene or elsewhere in *FGFR3*. In the analysis, for the first time in Korea, we found a c.251C>T mutation in *FGFR3* resulting in a S84L amino acid substitution associated with HCH. The patient's father, but not her mother or three siblings, was also found to carry the same *FGFR3* mutation, as assessed by a genetic analysis using next generation sequencing.

Discussion

The *FGFR3* gene is located on chromosome 4p16.3 and consists of 18 coding exons [6]. The *FGFR3* gene encodes for a transmembrane tyrosine kinase receptor that binds to fibroblast growth factors. The protein has three extracellular

immunoglobulin-like domains (Ig-like domains I-III), a transmembrane domain, and an intracellular tyrosine kinase domain [6]. *FGFR3* has essential roles in the regulation of chondrocyte differentiation, proliferation, and apoptosis, and has been reported to regulate endochondral ossification during a normal skeletal development through a negative feedback mechanism [7,8]. An *FGFR3* gain-of-function mutation was reported to negatively affect both the proliferation and differentiation of the growth plate chondrocytes, causing various forms of skeletal dysplasia, including HCH, ACH, and lethal thanatophoric dysplasia (OMIM #187600), depending on the severity of symptoms [9,10].

ACH has an estimated prevalence of one in 16,000-26,000 live births [6]. The clinical manifestations of ACH include bulging forehead and a rhizomelic shortening of the limbs with a subsequent development of lumbar lordosis and *genu varum* [11]. Radiographic findings include a shortening of the base of the skull with a small *foramen magnum*, a reduced spinal

canal size throughout its length, a flared metaphysis of the long bones, a decrease in interpedicular distances from the first to fifth lumbar vertebral bodies, and an AP shortening of the lumbar pedicles [11,12]. A narrowing of the spinal canal in the thoracolumbar and lumbar regions often causes neurological issues, resulting in spinal stenosis, which can progress paralysis [11].

Symptoms of HCH, which is also caused by *FGFR3* mutation, are similar to those of ACH with the exception of less severe changes in the skull and phenotype when compared to those observed in ACH [11]. Lamy and Maroteaux [13] suggested the use of term HCH for this syndrome. Beals [14] reported five kindred cases, which were classified as HCH. The study reported limb shortening without rhizomelia, mesomelia, or acromelia, and a mild metaphyseal flaring in the five cases. Brachydactyly and mild limitation of elbow extension were apparent in these patients. The typical manifestations observed included an AP shortening of the lumbar pedicles, unchanged or caudal narrowing of the spinal canal, and a noticeable lumbar lordosis. Specht and Daentl [15] subsequently reported six additional cases of HCH displaying rhizomelic shortening of the limbs with a normal craniofacial and hand appearance.

Both ACH and HCH are allelic disorders caused by mutation in *FGFR3*. Bellus et al. [16] identified recurrent mutations associated with HCH. In the study, a specific mutation in the tyrosine kinase domain of *FGFR3* was identified in eight of 14 unrelated patients with HCH [16]. The nucleotide at position 1,620 of *FGFR3* was mutated from C to A resulting in an N540K substitution. Because six of the 14 patients did not carry the N540K substitution, it was determined that HCH can be caused by an unidentified mutation in other gene or elsewhere in *FGFR3*. To identify novel *FGFR3* mutations, Heuertz et al. [17] conducted a study on a cohort of 25 HCH and one ACH patients in whom the common *FGFR3* mutations were ruled out. Seven novel mutations were found, including the Y278C, G268C, R200C, N262H, V381E, S84L substitutions for HCH, and S279C for ACH [17]. Mutations that resulted in an amino acid substitution to cysteine were found to cause more severe phenotypes. The S84L mutation was located in the extracellular Ig-like I domain of *FGFR3*. In the particular case reported by Heuertz et al. [17], the mother and grandfather of the patient also had the S84L mutation.

In this study, we identified the first case of S84L mutation in Korea. Only one case with this mutation was reported outside Korea [17]. ACH and HCH are typically diagnosed based on clinical symptoms and radiographic findings; however, there are no definite diagnostic biomarkers available. Thus, it is essential

to sequence the mutation hotspots in *FGFR3* first, and in cases where the common mutations are not found, a whole exome analysis can be subsequently performed in order to identify other mutation(s) in *FGFR3* for both diagnosis and genetic counseling purposes.

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