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A novel S269C mutation in fibroblast growth factor receptor 3 in a Japanese child with hypochondroplasia

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Abstract
Functionally activating mutations in fibroblast growth factor receptor 3 (FGFR3) can cause four types of autosomal dominant skeletal dysplasia with short-limbed dwarfism that include the mildest phenotype, hypochondroplasia (HCH). A novel mutation (c.805A>T, p.S269C) was identified in a Japanese infant with HCH through direct sequencing of all *FGFR3* exons and exon/intron boundaries. This mutation creates an additional cysteine residue in the extracellular region of FGFR3 that results in the functional activation of FGFR3.

Introduction

Functionally activating mutations of fibroblast growth factor receptor 3 (FGFR3) can cause four types of autosomal dominant skeletal dysplasia with short-limbed dwarfism: thanatophoric dysplasia (TD; OMIN187600) I and II, achondroplasia (ACH; OMIM100800), and hypochondroplasia (HCH; OMIM146000). Individuals with TD usually die after birth from respiratory distress due to pulmonary hypoplasia, and ACH and HCH are the most common genetic forms of dwarfism in children and adults[1].

The features of ACH include a short stature caused by rhizomelic shortening of the limbs, characteristic faces with frontal bossing and midface hypoplasia, exaggerated lumbar lordosis, limited elbow extension, genu varum, and trident hands. By contrast, HCH is characterized by a short-limbed short stature, lumbar lordosis, short and broad bones, and caudal narrowing of the interpediculate distance of the lumbar spine. HCH results in milder phenotypes than ACH but has a broader spectrum of phenotypes that occasionally overlap with those of patients with ACH and normal individuals of short stature.

FGFR3 encodes a member of the FGFR subfamily of tyrosine kinase (TK) receptors. The four FGFR members share a common organization that is composed of three extracellular immunoglobulin-like loops (Ig I–III), one hydrophobic transmembrane (TM) domain, and two cytoplasmic TK sub-domains (TK1 and TK2) that are related to its catalytic activity. While more than 98% of patients with ACH have a recurrent p.G380R substitution in the TM domain of the receptor[2], approximately 70% of patients with HCH have a recurrent p.N540K substitution in the cytoplasmic TK1 domain[3]. In this report, we describe a novel p.S269C mutation in *FGFR3* that was identified in an infant with HCH. This mutation creates an additional cysteine residue in the extracellular region of FGFR3 that results in its functional activation.

The patient was a boy born to nonconsanguineous Japanese parents with a birth weight and height of 2.6 kg and 45.5 cm, respectively, after 38 weeks of gestation. At birth, his father was 52 years of age and his mother was 43 years of age. He was referred to us at the age of 6 months and exhibited growth failure with a weight and height of 6.6 kg and 59.5 cm (−3.5 SD), respectively. His head circumference was 42.7 cm, which was within the normal

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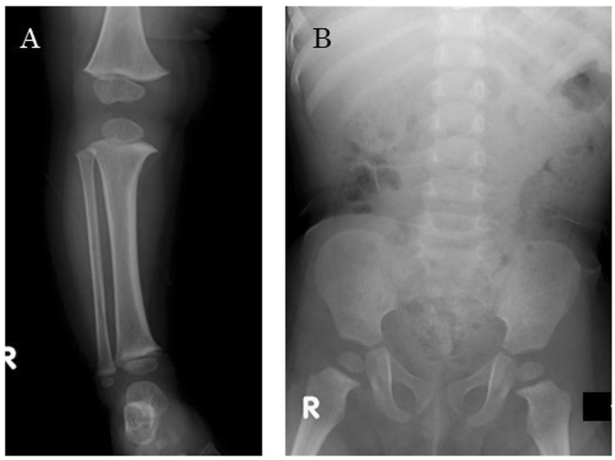

Fig. 1 Skeletal X-ray findings from a child with HCH at the age of 2 years. The skeletal X-ray findings revealed broad long bones (**a**) and hypoplastic iliac bones (**b**). Caudal narrowing of the interpediculate distance of the lumbar spine was not observed (**b**)

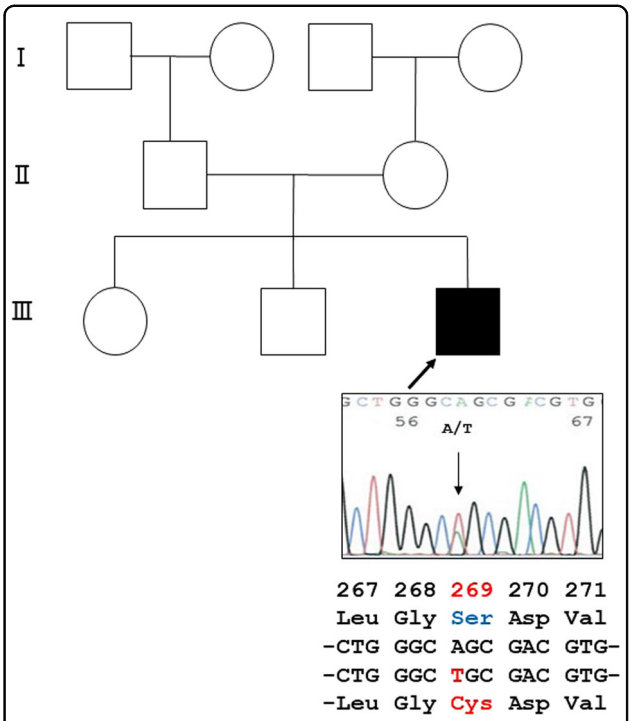

Fig. 2 A sequence chromatogram of the *FGFR3* gene from the HCH patient. A Heterozygous c.805A>T mutation was identified in the affected boy. This mutation predicts a serine-to-cysteine conversion at the 269 amino acid residue of FGFR3 that is designated as p.S269C. The parents did not have this missense mutation

range. His facial appearance was not peculiar, and he did not have triangular hands. An X-ray of the patient revealed short, broad, and hypoplastic iliac bones that were reminiscent of HCH. Following the clinical diagnosis of HCH, the patient continued to exhibit stunted growth. At the age of 2 years, skeletal X-ray findings revealed broad, long, and hypoplastic iliac bones, but caudal narrowing of the interpediculate distance of the lumbar spine was not observed (Fig. 1 a and b).

A genetic diagnosis was then performed with genomic DNA isolated from the whole blood of the patient and his parents and indicated an absence of the common recurrent mutations responsible for HCH and ACH (i.e., p.N540K and p.G380R, respectively). Subsequently, all 18 exons and exon/intron boundaries of *FGFR3* were amplified via polymerase chain reaction and then directly sequenced. The results revealed that the patient was heterozygous for the c.805A>T (p.S269C) mutation. The parents were not carriers of this missense mutation; thus, c.805A>T was a de novo mutation in *FGFR3* in the patient (Fig. 2). This study was approved by the Institutional Review Board and Ethical Committee of Akita University Graduate School of Medicine. Written informed consent was obtained from the parents of the patient.

After the initial discovery of mutations in *FGFR3* in cases of ACH, other *FGFR3* gene mutations were subsequently discovered in cases of TD and HCH. A recurrent mutation in *FGFR3*, i.e., p.G380R, was found to be responsible for more than 98% of ACH cases[2], whereas missense mutations that create cysteine residues in the extracellular domain of FGFR3 have been found to be responsible for TD[1].

Regarding HCH, a recurrent mutation, i.e., p.N540, in the intracellular TK1 domain was determined to be responsible for approximately 70% of HCH cases[3]. Subsequently, 19 missense mutations have been reported as frequent minor mutations in HCH. Of these mutations, 10 (p.S84L[4], p.R200C[4], p.N262H[4], p.G268C[4], p.Y278C[4], p.L324V[5], p.L324H[6], p.N328I[7], p.G342C[8], and p.S348C[9]) are located in the extracellular region of FGFR3, six (p.M528I[10], p.I538V[11], p.N540S[12], p.N540T[13], p.K650N[14], and p.K650Q[14]) are located in the intracellular region of FGFR3, and three (p.G380K[15], which is also responsible for AHC, p.V381E[4], and p.F384L[10]) are located in the TM region of FGFR3. In this report, we identified a novel *FGFR3* mutation that was responsible for HCH, i.e., p.S269C, which is located in the extracellular region of FGFR3 (Fig. 3). Among the mutations in *FGFR3* that have been identified thus far, those responsible for HCH are distributed along a wide genomic region of *FGFR3*. Thus, it is essential to sequence the entire *FGFR3* gene to make a genetic diagnosis of skeletal dysplasia with a short-limbed short stature, even if patients do not have the common mutations in *FGFR3*.

Notably, six of the mutations responsible for HCH are a result of an additional cysteine residue in the extracellular region of FGFR3. FGFR3 regulates a variety of biological functions, including cell proliferation, migration, and differentiation[1]. Upon ligand binding, FGFR3 dimerizes,

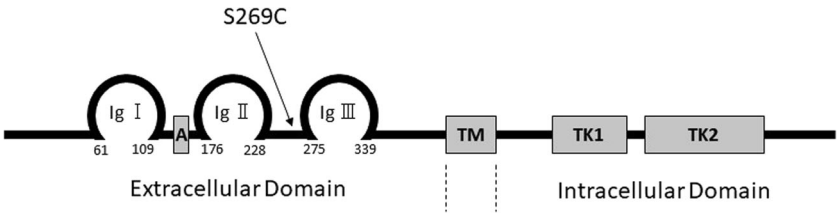

Fig. 3 Structural domains of FGFR3. The location of the novel mutation is indicated by the arrow

which results in controlled activation of a specific signal transduction pathway. Some representative mutations in FGFR3 that are responsible for TD and ACH have been biochemically studied to determine their effects on the induction and stabilization of FGFR3 dimerization[16,17]. Each of the mutations studied thus far has been demonstrated to increase the propensity for FGFR3 dimerization to some degree. The three mutations that create an additional cysteine residue in the extracellular region of FGFR3, i.e., p.G370C, p.S371C, and p.Y373C, are commonly found in patients with TD and have been biochemically analyzed and demonstrated to induce disulfide-mediated receptor dimerization and constitutive activation[18,19]. Thus, we speculate that the mutations that are responsible for HCH that create a cysteine residue may elicit constitutive activation of FGFR3 through the formation of an abnormal disulfide bond in a manner similar to the activation of FGFR3, which is associated with the common mutations responsible for TD.

HGV Database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at https://doi.org/10.6084/m9.figshare.hgv.1920.

Conflict of interest

The authors declare that they have no conflict of interest.

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