DATA REPORT

Novel heterozygous mutation in the extracellular domain of FGFR1 associated with Hartsfield syndrome

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Heterozygous kinase domain mutations or homozygous extracellular domain mutations in FGFR1 have been reported to cause Hartsfield syndrome (HS; OMIM #615465) is characterized by the triad of holoprosencephaly (HPE), ectodactyly and cleft lip/palate, and more than 15 cases have been reported to date.1–11 However, the causative gene responsible for HS remained unknown until recently, when Simonis et al.9 identified homozygous or heterozygous mutations in the fibroblast growth factor receptor 1 (FGFR1) in several cases of HS. Since this discovery, 10 mutations in FGFR1 have been reported in HS patients.3–11 Here, we describe a case of typical HS with hypogonadotropic hypogonadism (HH) harboring a novel heterozygous mutation, p.His253Pro, in the extracellular domain of FGFR1. This is the first report of an HS-associated heterozygous mutation located in the extracellular domain of FGFR1, thus expanding our understanding of the phenotypic features and further developmental course associated with FGFR1 mutations.

Hartfield syndrome (HS; OMIM #615465) is characterized by the triad of holoprosencephaly (HPE), ectodactyly and cleft lip/palate, and more than 15 cases have been reported to date.1–11 However, the causative gene responsible for HS remained unknown until recently, when Simonis et al.9 identified homozygous or heterozygous mutations in the fibroblast growth factor receptor 1 (FGFR1) in several cases of HS. Since this discovery, 10 mutations in FGFR1 have been reported in HS patients.3–11 Here, we describe a case of typical HS (HPE, ectodactyly and cleft lip/palate) harboring a novel heterozygous p.His253Pro mutation in the extracellular domain of FGFR1.

The details of the clinical course in this patient’s early childhood were described by Takenouchi et al.8 Previously, in brief, he was born at 42 weeks of gestation with a constellation of malformations, including a cleft lip and palate (Figure 1a), a depressed nasal bridge, bilateral ectrodactyly of the hands (Figure 1b) and a microopenis (2.0 cm) with undescended testes. Brain magnetic resonance imaging (MRI) at the age of 3 years showed semilobar HPE (Figure 1c). Owing to his midline defect and microopenis, his pituitary gland function was evaluated at the age of 3 months. The secretions of luteinizing hormone and follicle-stimulating hormone (FSH) in response to gonadotropin-releasing hormone (GnRH) were decreased (Table 1) in the period of ‘mini-puberty,’ indicating hypogonadotropic hypogonadism (HH). Brain MRI at the age of 5 years showed no abnormalities in the pituitary gland (Figure 1d), whereas the absence of the olfactory bulb and fusion of the olfactory gyri were shown (Figure 1e).

At the age of 11 years, he showed bilateral undescended testes, no pubic hair (P1) and a microopenis (3 cm). His height was 136.9 cm (−1.1 s.d.) and his weight was 33.6 kg (−0.6 s.d.). Hormonal assays revealed very low plasma testosterone levels. The HH diagnosis was confirmed again by a GnRH-stimulating test (Table 1). Ultrasonography and MRI revealed that bilateral testes were undetectable. After recombinant FSH pretreatment, gonadotropin replacement therapy was started at the age of 13 years and 9 months. Bilateral testes became detectable (right: 1.8×0.8 cm, left: 1.4×0.6 cm) in inguinal canals, and bilateral orchiopexy was performed at the age of 14 years. During his last examination at the age of 14 years and 2 months, his height was 149.3 cm (−2.2 s.d.) and his weight was 45.8 kg (−0.8 s.d.).

This study was approved by the Institutional Review Board of Tokyo Metropolitan Children’s Medical Center (H25–73). We checked all coding exons and flanking introns of FGFR1, and found a novel de novo heterozygous c.758A>C transition (p.His253Pro) in the patient (Figure 2a). This sequence variation was absent from all selected databases, including the dbSNP, 1000 Genomes Project, Exome Variant Server, NHLBI Exome Sequencing Project and Human Genetic Variation Database for the Japanese population databases. His253 is located at the third immunoglobulin-like domain in the extracellular region of FGFR1 (Figure 2a) and is an evolutionarily conserved residue in all FGFR families (Figure 2b).

Because both of the two reported HS-associated mutations in the extracellular domain of FGFR1 are homozygous,9,11 we performed multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA KIT P133; MRC-Holland, Amsterdam, the Netherlands) to determine whether the patient had exon-level deletion or duplication of FGFR1 in another allele, with negative results.

We also analyzed all coding exons and flanking introns of SHH, GLI2, SIX3, TGIF1 and FGF8, which are the genes responsible for HPE, using PCR and direct sequencing. No mutation was found in these genes.

To date, more than 200 mutations in FGFR1 have been described; however, only 10 HS-associated mutations have been reported thus far. Among these 10 mutations, 8 were heterozygous within the intracellular protein kinase domain (p.Gly487Asp, p.Gly490Arg,
Asp623Glu, p.Asp623Tyr, p.Arg627Thr, p.Asn628Lys, p.Asp641Asn and p.Cys725Tyr), whereas the remaining 2 were homozygous and were located in the second immunoglobulin-like domain in the extracellular region (p.Leu165Ser and p.Leu191Ser). Therefore, the possible mutation discovered in the present case, p.His253Pro, could be the first reported heterozygous HS-associated mutation located in the extracellular domain of FGFR1.

Most of the mutations at the third immunoglobulin-like domain in the extracellular domain of FGFR2 cause craniosynostosis syndromes, such as Crouzon syndrome (e.g., p.Ser252Leu, p.Asp623Glu, p.Asp623Tyr, p.Arg627Thr, p.Asn628Lys, p.Asp641Asn, p.Cys725Tyr), whereas the remaining 2 were homozygous and were located in the second immunoglobulin-like domain in the extracellular region (p.Leu165Ser and p.Leu191Ser). Therefore, the possible mutation discovered in the present case, p.His253Pro, could be the first reported heterozygous HS-associated mutation located in the extracellular domain of FGFR1.
His254Tyr and Pro263Leu) or Apert syndrome (p.Ser252Trp and p.Pro253Arg). Notably, the mutation p.His254Tyr in FGFR2, which affects the 254th histidine residue (aligned with the 253rd histidine residue in FGFR1 (Figure 2b)), causes Crouzon syndrome, indicating the pathogenesis of p.His253Pro in FGFR1.

Using zebrafish overexpression assays, Hong et al. recently demonstrated that kinase domain mutations in FGFR1, resulting in HS/HPE phenotypes, showed dominant-negative effects, which were associated with the clinical severity of HS. Although the functional significance of our heterozygous mutation remains unknown, a de novo substitution in an evolutionarily highly conserved amino acid is likely to be pathogenic. However, further studies are necessary to clarify the contribution of heterozygous extracellular domain mutations in FGFR1 to the development of HS.

In summary, we describe a patient with typical HS harboring a novel de novo heterozygous sequence variation in FGFR1. This is the first report of an HS-associated heterozygous mutation located in the extracellular domain of FGFR1, thus expanding our understanding of the phenotypic features and further developmental course associated with FGFR1 mutations.

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HGV DATABASE
The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.873 (2016).
COMPETING INTERESTS
The authors declare no conflict of interest.

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