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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), which is characterized by the triad of holoprosencephaly, ectrodactyly and cleft lip/palate. To date, more than 200 mutations in *FGFR1* have been described; however, only 10 HS-associated mutations have been reported thus far. 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.

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Hartsfield syndrome (HS; OMIM #615465) is characterized by the triad of holoprosencephaly (HPE), ectrodactyly 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.[9–11] Here, we describe a case of typical HS (HPE, ectrodactyly 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 micropenis (2.0 cm) with undescended testes. Brain magnetic resonance imaging (MRI) at the age of 3 months revealed semilobar HPE (Figure 1c). Owing to his midline defect and micropenis, 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 micropenis (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] 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,

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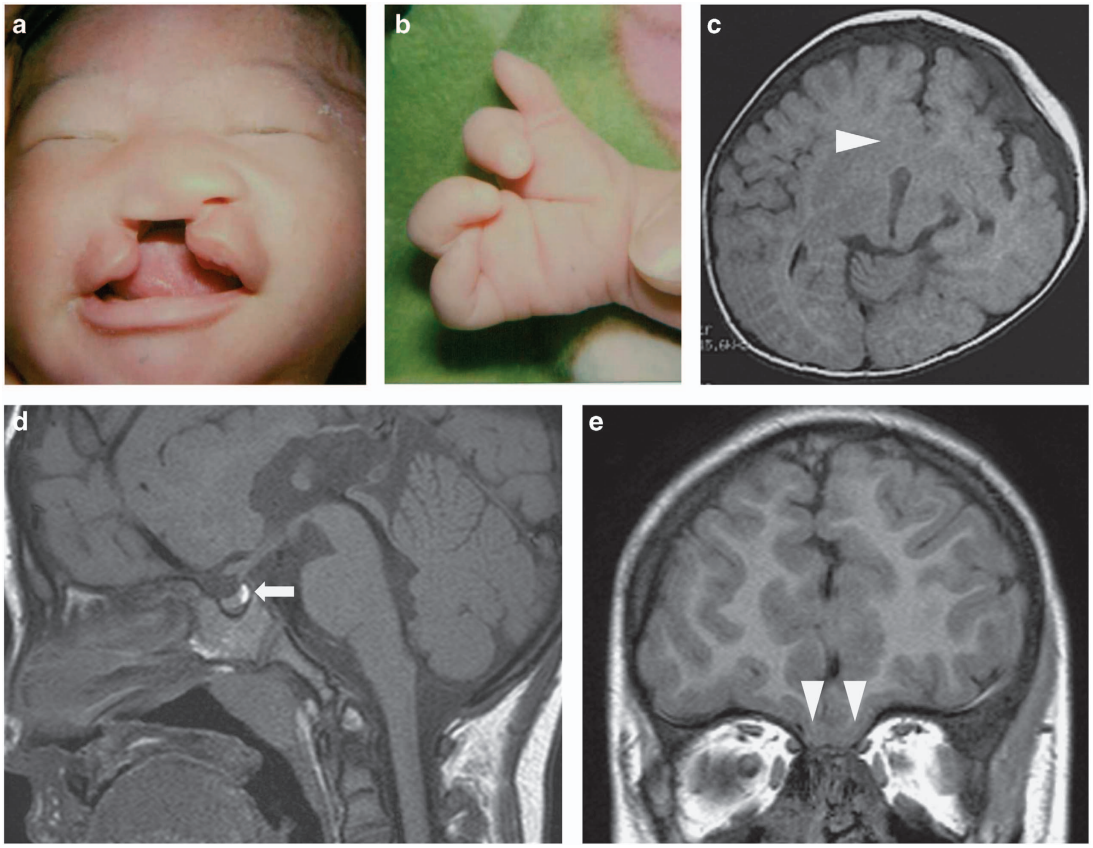

Figure 1. Clinical and radiographic features of the patient. (**a**) Photographs of the patient as a neonate. (**b**) Photographs of the patient's hands. The patient's left hand as a neonate is shown. Both hands had four fingers with deep gaps between the second and third digits. (**c**) Axial image of the brain MRI at 3 months of age. The fusion of the caudate nuclei is shown (arrowhead). (**d** and **e**) Sagittal image (**d**) and coronal image (**e**) of the brain MRI at 5 years of age showing no abnormalities in the pituitary gland (arrow) and the absence of olfactory bulbs and olfactory gyri (arrowhead). MRI, magnetic resonance image.

Table 1. Endocrinological findings in the patient

	Stimulus	*3 Months*			*11 Years*			*Reference*	
		Basal		*Peak*	*Basal*		*Peak*	*Basal*	*Peak*
GH (ng/ml)	Insulin	3.9	→	11.8					>6
LH (mIU/ml)	GnRH	<0.2	→	0.9	<0.1	→	0.43	0.17–1.63[a]	13.11–25.15[a]
FSH (mIU/ml)	GnRH	<0.2	→	0.9	1.10	→	2.24	2.12–5.24[a]	5.75–13.25[a]
ACTH (pg/ml)	Insulin	28	→	440				9.8–27.3	28–130.5
Cortisol (μg/dl)	Insulin	24.9	→	44.1				5–20	19.8[b]
TSH (mIU/ml)	TRH	2.42	→	20.04					10–35
PRL (ng/ml)	TRH	6.0	→	137.0				1.7–15.4	Increase two times
IGF-1 (ng/ml)		32.4						11–149[c]	
Free T4 (ng/dl)		1.3						1.01–1.95	
Testosterone (ng/ml)		<0.1			<0.03				

The conversion factors to the SI unit are as follows: GH 1.0 (μg/l), TSH 1.0 (mIU/l), LH 1.0 (IU/l), FSH 1.0 (IU/l), testosterone, 0.035 (nmol/l), prolactin 1.0 (μg/l), ACTH 0.22 (pmol/l), cortisol 27.59 (nmol/l), IGF-I 0.131 (nmol/l), free T4 12.87 (pmol/l) and free T3, 1.54 (pmol/l).

Abbreviations: ACTH, adrenocorticotropin; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; PRL, prolactin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

[a]Reference data of pubertal (Tanner stage II-III) Japanese boys.

[b]Reference data of UK children (younger than 10 years).

[c]Reference data of Japanese boys (0 years old).

p. 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).[9–11] 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,

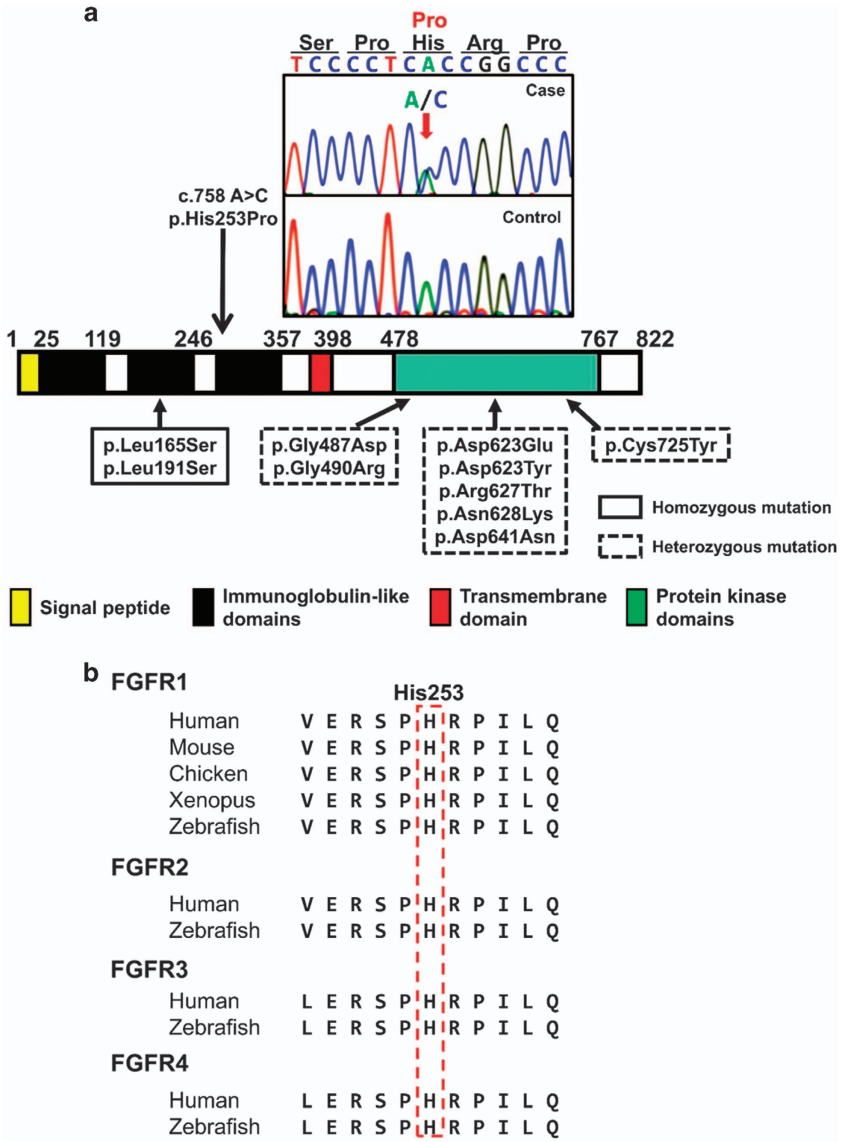

Figure 2. Identification of the sequence variation of *FGFR1*. (**a**) Partial sequence of PCR product and schematic diagrams of the FGFR1 protein. The chromatogram exhibits a heterozygous substitution of proline (CCC) in place of histidine (CAC) at codon 253, which is located at the amino terminus of the third immunoglobulin-like domain in the extracellular region of *FGFR1*. The red arrow indicates the mutated nucleotide. A summary of the reported 10 missense mutations identified in Hartsfield syndrome patients is presented. (**b**) His253 is a highly conserved amino acid in all four FGFRs. FGFR, fibroblast growth factor receptor.

p.His254Tyr and p.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,[12] indicating the pathogenesis of p.His253Pro in *FGFR1*.

Using zebrafish overexpression assays, Hong *et al.*[11] 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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