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CLINICAL CASE SEMINAR

Sporadic Heterozygous Frameshift Mutation of *HESX1* Causing Pituitary and Optic Nerve Hypoplasia and Combined Pituitary Hormone Deficiency in a Japanese Patient

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***HESX1/Hesx1* is a member of the paired-like class of homeobox genes and is essential for pituitary and forebrain development. Mice with a targeted homozygous deletion of the *Hesx1* show severe central nervous system defects, absence of optic vesicles, and a very small anterior pituitary gland. This phenotype is similar to the abnormalities observed in the human disorder called septo-optic dysplasia, a syndromic form of congenital hypopituitarism. To date, four missense mutations in the human *HESX1* have been described in individuals with phenotypes ranging from severe septo-optic dysplasia, relatively mild combined pituitary hormone deficiency (CPHD), to isolated GH deficiency. Here we report a Japanese patient with CPHD (GH, TSH, LH, FSH, and ACTH deficiency) due to a novel sporadic *HESX1* mutation. Brain magnetic resonance imaging examination revealed hypoplastic anterior pituitary, ectopic posterior lobe, and left optic nerve hypoplasia. Molecular analysis identified the insertion of a heterozygous mutation (306/307ins AG) in the exon 2 of the *HESX1*. This mutation changes a reading frame and introduces a premature stop codon soon after the mutation site. Therefore, this mutation would be predicted to generate a protein lacking the carboxyl-terminal homebox domain (DNA-binding domain) and cause the disease. Family analysis demonstrated that neither of the patient's parents harbored this mutation, indicating that the mutation had arisen *de novo*. In conclusion, a *de novo* heterozygous frameshift mutation in exon 2 of the *HESX1* causes severe CPHD with optic nerve hypoplasia in a human. (*J Clin Endocrinol Metab* 88: 45–50, 2003)**

THE ANTERIOR PITUITARY gland develops from a midline structure contiguous with the primordium of the ventral diencephalon (1). After proliferation from a well defined growth plate, different cell types arise in a distinct spatial and temporal fashion and undergo a highly selective determination and differentiation (2, 3). Thereafter, numerous cells in the pituitary gland are specialized to produce and secrete specific hormones, such as GH, PRL, TSH, LH, FSH, and ACTH (1–4). Failure of these trophic cells to differentiate and/or proliferate during embryogenesis accounts for congenital pituitary disorders that range in severity from panhypopituitarism to milder forms in which one or more of the hormone-secreting cells are absent, causing isolated or combined pituitary hormone deficiency (CPHD) (3–5).

Over the last decade, many transcription factors involved in pituitary development in humans and mice have been characterized, including POUF1/Pit1, PROP1/Prop1, SF-1/Sf-1, PITX2/Pitx2, NeuroD1, GATA-2, LHX3/Lhx3, TPIT/Tpit, and HESX1/Hesx1 (6–17). Among these factors, HESX1/Hesx1 derived from a homeobox gene expressed in embryonic stem cell is known to play an important role in the development of the optic nerve as well as the anterior pituitary gland (8, 16, 17). Hesx1 is intimately involved in orchestrating the expression of other factors involved in pituitary organogenesis (17). During mouse embryogenesis, Hesx1 expression is localized to the prospective forebrain tissue, which later develops into Rathke's pouch. Consequently, Hesx1 expression has been reported in all of the hormone-secreting cell types of the anterior pituitary. In addition, Hesx1 expression is more widespread than that of other pituitary-specific transcription factors (8, 16, 17). Homozygous *Hesx1* gene knockout mice show dramatic central nervous system defects, including absence of the optic vesicles and a small anterior pituitary gland (17). These phenotypes are very similar to the abnormalities observed in a human disorder called septo-optic dysplasia (SOD) (18–20). A number of patients with SOD and other patients with a variety of pituitary disorders were screened, and to date four different missense mutations in seven patients have been reported that are responsible for severe SOD, relatively mild CPHD, or isolated GH deficiency (17, 21, 22). Here, we report the identification of a novel heterozygous insertion mutation in the *HESX1* in a Japanese patient with sporadic pituitary and optic nerve hypoplasia.

Abbreviations: CPHD, Combined pituitary hormone deficiency; MRI, magnetic resonance imaging; SOD, septo-optic dysplasia.

Materials and Methods

Pituitary hormone assessment

Free T_4, free T_3, and TSH levels were determined by a commercially available chemiluminescent immunoassay (Ciba Corning, Inc., Medfield, MA). Serum cortisol was measured by RIA (Amerlex RIA, Ortho Clinical Diagnostics Co., Tokyo, Japan). Plasma ACTH was determined by radioimmunometric assay (ACTH IRMA Mitsubishi, Tokyo Mitsubishi Chemical Co., Tokyo, Japan). GH provocative tests were performed using arginine (0.5 g/kg, iv; Morinaga Co., Tokyo, Japan), insulin-induced hypoglycemia (0.05 U/kg, iv; Eli Lilly & Co., Indianapolis, IN), and GHRH (1 μg/kg, iv; Sumitomo Pharma Co., Osaka, Japan). GH levels were determined by RIA (Dai-Ichi Radioisotope Co., Tokyo, Japan). LH and FSH were measured in response to GnRH (2 μg/kg, iv; Tanabe Pharma Co., Tokyo, Japan). LH and FSH levels were determined by time-resolved fluoroimmunoassay (Delphia, Wallac, Inc., Turku, Finland).

DNA amplification and sequence analysis

Informed consent to participate in the study was obtained from the parents. Genomic DNA was extracted from peripheral leukocytes as described previously (23). Each exon of *HESX1* was amplified by PCR using primers previously described (17). The PCR conditions consisted of 9 min at 94 C, followed by 30 cycles of 30 sec at 94 C, 30 sec at 52 C, and 30 sec at 72 C in a Perkin-Elmer Gene Amp PCR System 2400 thermal cycler (PE Applied Biosystems, Foster City, CA). After amplification, the PCR products were purified from low melting agarose gel (23), and the purified products were sequenced directly with an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI 373A automated fluorescent sequencer (PE Applied Biosystems) from both strands (23).

To confirm the mutation, the PCR products were subcloned into pCR 2.1 vector (Invitrogen, San Diego, CA). The resulting construct was used to transform *Escherichia coli* strain JM109 (Promega Corp., Madison, WI). Both strands of positive clones were sequenced with the same primers using PCR.

Case Report

The patient is a 2-yr-old Japanese boy born at 40 wk gestation by vaginal delivery to nonconsanguineous parents. The pregnancy and delivery were without complications. His birth weight was 2764 g (−0.8 SD for the Japanese population), and length was 46.5 cm (−1.7 SD for the Japanese population). Ten hours after birth he presented with apnea and cyanosis and was found to be hypoglycemic (0.78 mmol/liter). Physical examination showed a small penis and bilateral undescended testes. Intravenous glucose infusion was started to maintain normoglycemia. As he had a small penis and hypoglycemia, he was suspected of having congenital hypopituitarism and was referred to our hospital at 14 d of age for further endocrinological evaluation. At this time his hypoglycemic episodes resolved, and he did not reveal failure to thrive. His baseline serum free T_4, free T_3, and TSH levels were low (3.2 pmol/liter, 1.2 pmol/liter, and 0.87 μU/ml, respectively). His serum cortisol level was 6.5 nmol/liter, and plasma ACTH was 2.2 pmol/liter (Table 1). Repeated measurements of serum cortisol and plasma ACTH concentrations remained low. Brain magnetic resonance imaging (MRI) examination at 20 d of age revealed a hypoplastic anterior pituitary, ectopic posterior lobe, and left optic nerve hypoplasia (Fig. 1). Hypothyroidism was evident, and replacement therapy of thyroid hormone was commenced. Hydrocortisone (5 mg/d) was also initiated to avoid the consequences of adrenal insufficiency. After initiation of treatment, he grew well, but by 5 months of age his growth gradually decelerated. At 7 months of age, arginine, insulin-induced hypoglycemia, and GHRH stimulations were performed. Arginine and insulin tolerance tests showed low stimulated GH levels; however, serum GH increased after GHRH stimulation (Table 1). It was speculated

TABLE 1. Endocrinological findings

		Normal range			Normal range (basal)
Free T_4 (pmol/liter)[a]	3.2	9.0–21.9	LH (IU/liter)[b] GnRH stimulation	0.06→0.21	0.083–1.950
Free T_3 (pmol/liter)[a]	1.2	3.4–6.3	FSH(IU/liter)[b] GnRH stimulation	0.07→0.84	0.293–1.645
TSH (μU/liter)[a]	0.87	0.3–3.50			
GH (μg/liter)[b]					
Insulin-induced hypoglycemia	0.69→3.1		ACTH (pmol/liter)[a]	2.2	3.88–7.82
Arginine stimulation	1.4→5.1		Cortisol (nmol/liter)[a]	6.5	213–672
GHRH stimulation	2.3→36.1				

[a] These values were determined at 7 d of age as described in *Materials and Methods.*
[b] These stimulation tests were performed at 7 months of age, and these values were measured as described in *Materials and Methods.*

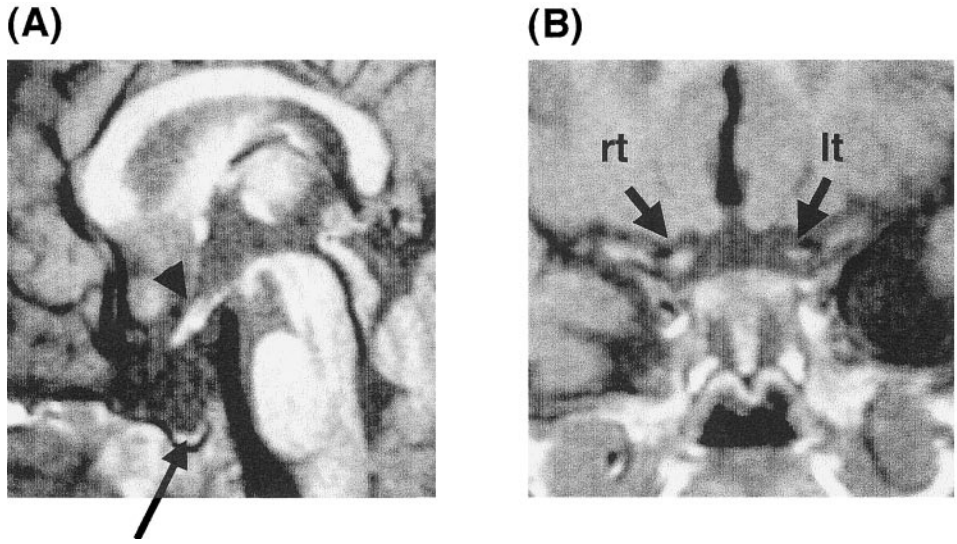

FIG. 1. A, Sagittal image showing a hypoplastic anterior pituitary (*arrow*) and an ectopic posterior lobe (*arrowhead*). B, Coronal image demonstrating left optic nerve hypoplasia (lt).

that these findings represent a combined hypothalamic-pituitary defect. LH and FSH levels after GnRH stimulation did not increase (Table 1). GH replacement therapy was commenced at this stage, and the patient responded well (Fig. 2). At 2 yr of age his body weight is 10.2 kg, and his height is 80.8 cm (−1.0 SD for normal Japanese boys).

Gene sequencing results

We identified a heterozygous two-base insertion in exon 2 of *HESX1* (306/307insAG; Fig. 3). This mutation (TTGAAAAGAGAGTTG-AGT→TTGAAAAGAGAGAGTTGAGT) may have occurred by slipped strand mispairing (24). The insertion altered an open reading frame

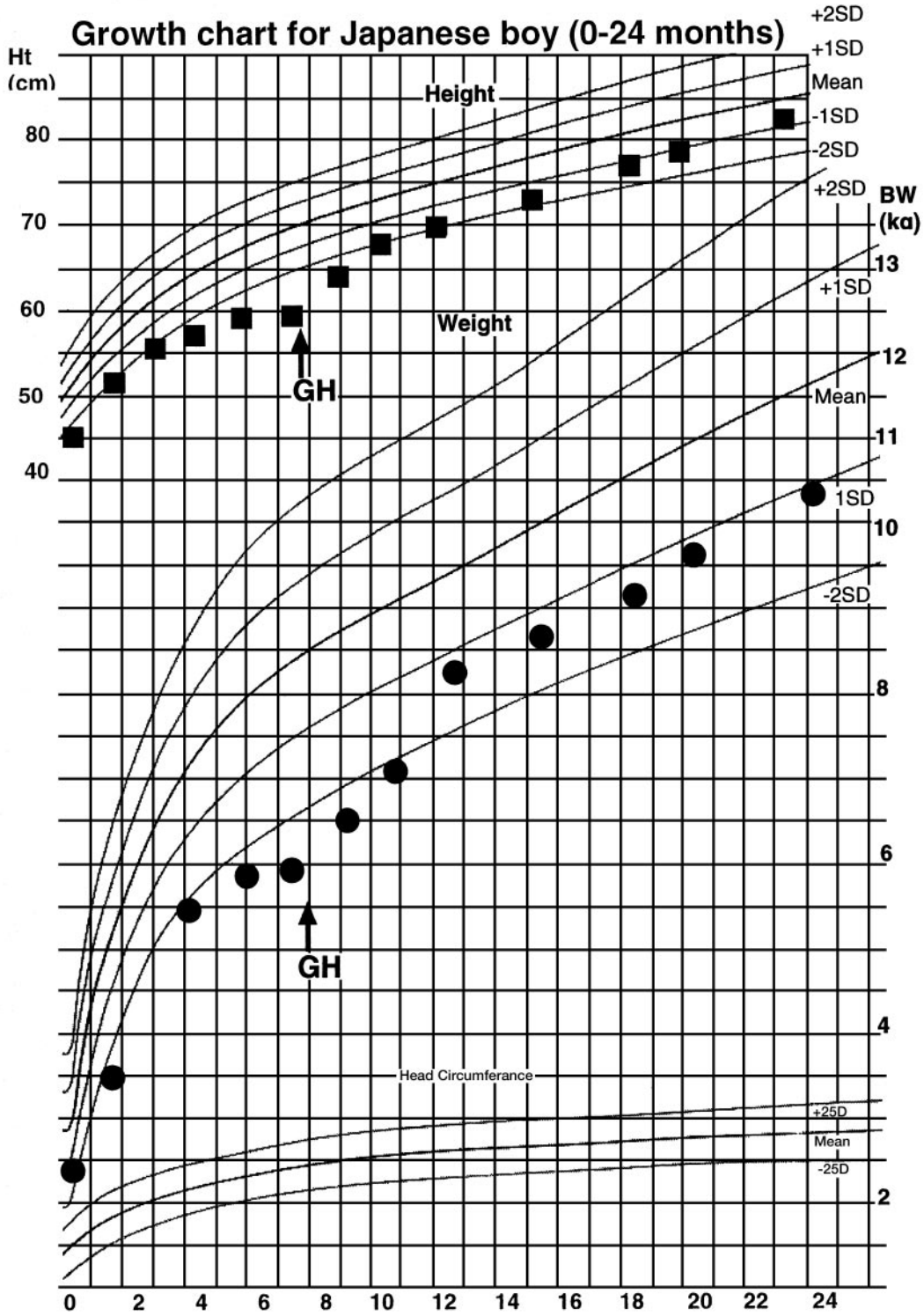

FIG. 2. Growth curve of the patient.

introducing a stop codon in the following codon (Fig. 3). DNA sequencing on the opposite strand also identified the identical mutation. We then subcloned PCR products and sequenced. Tencolonies had the mutant alleles, and seven had the wild-type alleles (data not shown). Fifty normal Japanese subjects did not harbor this mutation. Family analysis demonstrated that neither of his parents harbored the insertion, indicating that the mutation had arisen *de novo*.

Discussion

We present a sporadic case of a Japanese patient who had a heterozygous insertion mutation of *HESX1*. It is clear that the affected patient has a *de novo* insertion mutation, because neither parent exhibits mutation, and the DNA sequence shows that he does not have loss of heterozygosity at *HESX1.*

As our patient showed hypoglycemia soon after birth and had micropenis, he was suspected to have GH, LH, and FSH deficiencies, which were later confirmed by provocative tests. In addition, laboratory findings indicated hypothyroidism caused by TSH deficiency. Unlike previous reported patients with heterozygous *HESX1* mutations (21, 22), it is likely that our case also had ACTH deficiency. Hypoglycemia at birth or in the first months of life is the most common complaint leading to early diagnosis of GH deficiency (25, 26). However, it has been reported that ACTH/cortisol deficiency often accompanies congenital GH deficiency with hypoglycemia (26). In addition, the corticotrophs are the first hormone-producing cells of the pituitary to reach terminal differentiation and expression of proopiomelanocortin starts in corticotrophs on about d 12.5 of embryonic development in the anterior pituitary of the mouse (15, 27). *Hesx1* expression begins on d 8.5 of mouse embryogenesis, and its expression is later spread to all of the hormone-secreting cell types of the anterior pituitary (17). In homozygous *Hesx1* knockout mice, a striking dysmorphogenesis is evident after d 11.5 of mouse embryogenesis (17). Taken together, these data suggest that HESX1/Hesx1 may influence corticotroph differentiation, resulting in ACTH deficiency that requires further investigation in these types of patients.

We identified a frameshift mutation, resulting in a premature stop codon in exon 2 of *HESX1*. To date, *HESX1* mutations have been found in seven patients from five kindreds with various clinical presentations of pituitary hormone deficiency and MRI findings; however, all of these were missense mutations (Table 2). One mutation (R160C) was identified as homozygous in siblings with a severe form

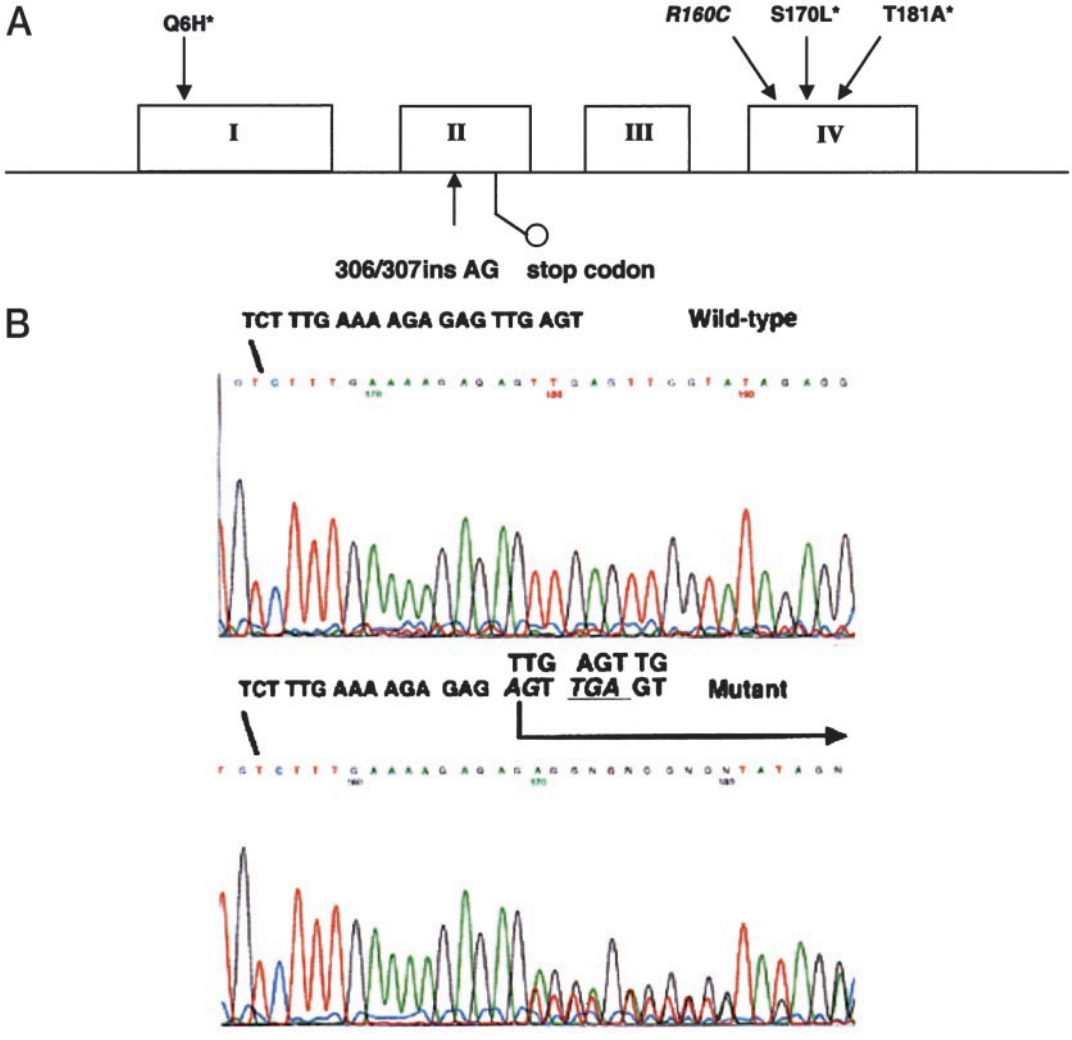

FIG. 3. A, Schematic representation of the *HESX1* gene. *Boxes* denote numbered exons. Four previously reported missense mutations and the mutation in this study (*italics*) are displayed. The insertion altered an open reading frame, introducing a stop codon in the following codon (*circle*) *Asterisks* indicate heterozygous mutations. Only the R160C mutation was identified in the homozygous state. B, A schema of the protein and functional domains of HESX1. C, Insertion of AG in exon 2 (306/307insAG). This insertion introduces a stop codon in the following codon.

TABLE 2. MRI and endocrine findings and the mutations of the *HESX 1*

Case	MRI	Deficient hormones	Mutations of the *HESX1* gene
1-1[a]	Agenesis of corpus callosum, optic nerve hypoplasia	Panhypopituitarism	R160C[b] homozygous
1-2[a]	Agenesis of corpus callosum, optic nerve hypoplasia	Panhypopituitarism	R160C[b] homozygous
2-1[c]	Bilateral optic nerve hypoplasia	GH	S170L heterozygous
2-2[c]	Normal	GH	S170L heterozygous
3[c]	Anterior pituitary hypoplasia	GH	T181A heterozygous
4[c]	Anterior pituitary hypoplasia, ectopic posterior pituitary	GH, TSH, LH/FSH	Q6H heterozygous
5[d]	Anterior pituitary hypoplasia, undescended posterior pituitary	GH	S170L heterozygous
6[e]	Left optic nerve hypoplasia, anterior pituitary hypoplasia, ectopic posterior pituitary	GH, TSH, LH/FSH ACTH(probable)	306/307Ins AG (introduce a premature stop codon) heterozygous

Patients 1-1 and 1-2 and patients 2-1 and 2-2 were siblings.
[a] Siblings were derived from Dattani *et al.* (17).
[b] This mutation was homozygous, and the other mutations were heterozygous.
[c] These patients were from Thomas *et al.* (21).
[d] This patient was derived from Brickman *et al.* (22).
[e] This is our case.

of SOD and panhypopituitarism, and *in vitro* analysis revealed that the R160C mutant may act as a null allele (17). In contrast, patients with the other three mutations of the *HESX1* gene (Q6H, S170L, and T181A) were heterozygous and had variable degrees of hypopituitarism, ranging from isolated GH deficiency to CPHD (21). Three patients with S170L showed highly variable phenotypes in terms of the presence of MRI findings and optic nerve hypoplasia (21, 22). It is speculated that milder phenotypic expression in heterozygotes compared with homozygotes is due to a gene dosage effect. This is supported by the finding that the majority of heterozygous Hesx1 knockout mice do not have a severe phenotype (17, 21). In our patient the degree of CPHD and MRI findings was milder than in the homozygous case, but was more severe than in the heterozygous patients. The exact mechanism for this is unknown. As mentioned, gene dosage-related phenotypic differences may be an explanation for this. Alternatively, as our mutation produces a premature stop codon in exon 2, the mutant protein will lack a carboxyl-terminally truncated protein of 102 amino acids; however, the amino-terminal region of the mutant may be translated. Brickman *et al.* (22) have shown that the amino-terminal region (a minimal 36 amino acids) of Hesx1 contains a repressor/dimerization domain. It is plausible that the mutant N-terminal protein generated in this patient exerts a dominant negative or modulator effect by interacting with the wild-type protein, causing the disease. This requires further study.

In conclusion, our results indicate that a *de novo* heterozygous insertion mutation introducing a premature codon in exon 2 of *HESX1* causes pituitary and optic nerve hypoplasia and severe CPHD. Further identification of the *HESX1* mutations will expand our understanding of the clinical heterogeneity and inheritance patterns of hypopituitarism.

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