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DATA REPORT

A novel *KAL1* mutation is associated with combined pituitary hormone deficiency

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Using a next-generation sequencing strategy, we identified a novel *KAL1* missense mutation (p.His568Gln) in a patient with combined pituitary hormone deficiency, right microphthalmia, right renal aplasia and severe developmental delay. Our findings will provide additional evidence that *KAL1* mutations are associated with hypopituitarism, in addition to luteinizing hormone, and follicle-stimulating hormone deficiencies, and improve our understanding of the phenotypic features and developmental course associated with *KAL1* mutations.

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Kallmann syndrome (KS) is a genetically heterogeneous condition, defined by hypogonadotropic hypogonadism (HH) and anosmia/hyposmia. Several genes have been linked to KS pathogenesis, including *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *CHD7* and *KAL1*.[1–6] Increasing evidence shows that overlapping genotypes/phenotypes exist between KS, combined pituitary hormone deficiency (CPHD) and septo-optic dysplasia (SOD), a condition characterized by pituitary hormone deficiencies, optic nerve hypoplasia and midline defects, including agenesis of the septum pellucidum and/or corpus callosum. Mutations in *FGFR1*, *FGF8* and *PROKR2*, the genes responsible KS, have been identified in a small number of CPHD/SOD, suggesting that the genetic overlap between KS, CPHD and SOD is significant.[7–10] Unlike *FGFR1*, *FGF8* and *PROKR2*, the contribution of *KAL1*, which is mutated in 5% of KS cases,[11] to CPHD/SOD development has not been clearly established.

Here, we report a case of CPHD patient with extra-pituitary phenotypes, including right microphthalmia, right renal aplasia, mild hearing impairment in both ears and severe developmental delay. Using a next-generation sequencing strategy, we identified a novel missense mutation in *KAL1* (p.His568Gln). Our findings provide additional evidence that *KAL1* mutations are associated with hypopituitarism, in addition to luteinizing hormone (LH), and follicle-stimulating hormone (FSH) deficiencies, and will further our understanding of the phenotypic features, and developmental course associated with *KAL1* mutations.

The propositus was a 13-year-old Japanese boy born at 40 weeks of gestation after an uncomplicated pregnancy and delivery. He had no family history of pituitary dysfunction. His parents were nonconsanguineous and phenotypically normal. The patient's birth weight, length and head circumference were 2638 g (below the 3rd percentile), 46.0 cm (3rd–10th percentile) and 31.8 cm (3rd–10th percentile), respectively. A constellation of malformations was noticed, including right microphthalmia and small and uplifted earlobes with very-small external auditory canals. The testes were undescended, the scrotum small and the foreskin hypoplastic. Echography revealed right renal aplasia. An auditory brainstem response examination revealed mild hearing impairment in both ears. Owing to severe psychomotor retardation, he remains wheelchair-bound and nonverbal at 13 years of age.

Frequent episodes of hypoglycemia were noted at the age of 3 months. He was diagnosed with central adrenal insufficiency based on low cortisol (2.2 μg/dl) and adrenocorticotropin (ACTH) (32 pg/ml) at a time of severe hypoglycemia (glucose 1.3 mmol/l). Further endocrine studies indicated that the patient also had central hypothyroidism on the basis of a low free T4 (0.50 ng/dl: Ref. 0.99–1.91) with an inadequately increased thyroid-stimulating hormone (TSH) level of 3.98 mU/l (Ref. 0.77–7.3), and growth hormone (GH) deficiency (Supplementary Table 1). The brain MRI exhibited anterior pituitary hypoplasia, visible but thin stalk, cerebellar hypoplasia and eutopic posterior pituitary. The olfactory bulb was difficult to identify. Replacement therapy with L-thyroxine, hydrocortisone and recombinant human GH was started at 3 months of age. At 13 years of age, he showed typical signs of hypogonadism, with small intrascrotal testes (1 ml), no pubic hair (Tanner stage 1) and a micropenis (stretched penile length 2.0 cm). Hormone assays revealed very-low plasma testosterone levels. The HH diagnosis was confirmed by LH-releasing hormone stimulating test (Supplementary Table 1).

After obtaining informed consent, and with the approval of the Institutional Review Board of Keio University School of Medicine, and the Institutional Review Board of Tokyo Metropolitan Children's Medical Center, genomic DNA was extracted from peripheral blood leucocytes of the propositus. We sequenced 9 genes implicated in CPHD, including *POU1F1*, *PROP1*, *LHX3*, *LHX4*, *HESX1*, *OTX2*, *SOX3*, *SOX2*, *GLI2*, and 12 genes implicated in KS/HH, including *CHD7*, *FGFR1*, *FGF8*, *GNRH1*, *GNRHR*, *KISS1*, *KISS1R*, *PROK2*, *PROKR2*, *TAC3*, *TACR3* and *KAL1* using the MiSeq instrument (Illumina, San Diego, CA, USA) according to the SureSelect protocol (Agilent Technologies, Santa Clara, CA, USA). In brief, 3 μg of genomic DNA was used for the SureSelect capture methods. Exons of 122 genes known to be associated with congenital endocrine disorders (including 9 CPHD and 12 KS/HH-related genes) were identified in the University of California Santa Cruz table browser (http://genome.ucsc.edu/). In total, we targeted 1321 regions comprising 2 46 158 bp using SureSelect. DNA

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obtained from the SureSelect solution-based sequence capture was subjected to MiSeq sequencing. Base calling, read filtering and demultiplexing were performed with the standard Illumina processing pipeline. We used BWA 0.6.1 and SAMtools 0.1.18 for alignment and variant detection against the human reference genome (NCBI build 37; hg19) with the default settings. Local realignment, quality score recalibration and variant calling were performed by GATK 2.3-9 with the default settings. We used ANNOVAR for annotation of called variants. As for *LHX3* and *SOX3*, we screened by PCR and direct sequencing.

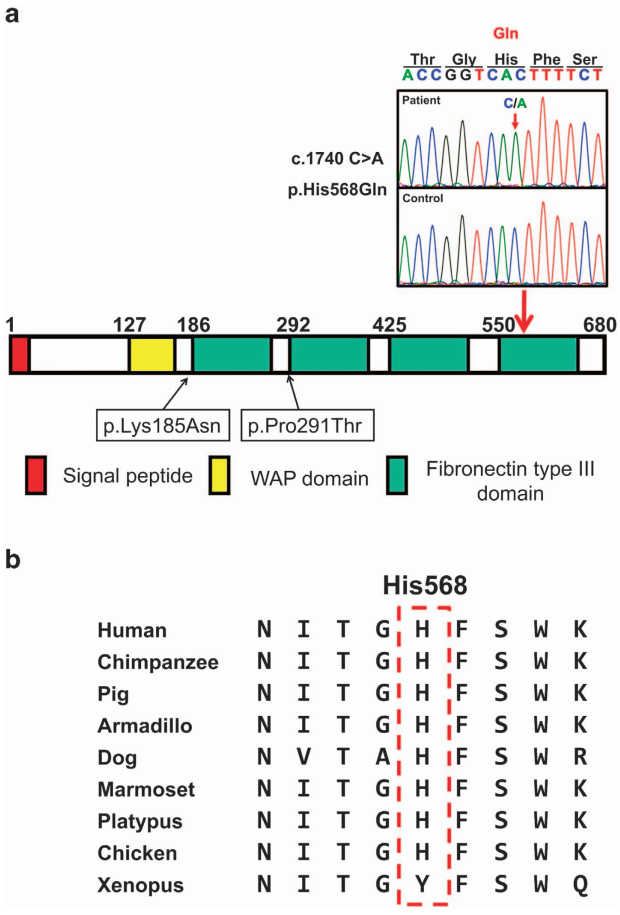

Figure 1. Identification of sequence variation of *KAL1*. (**a**) Partial sequence of PCR product and schematic diagrams of the anosmin-1 (coded by *KAL1* gene) protein. The chromatogram represents a hemizygous substitution of glutamine (CAA) in place of histidine (CAC) at codon 568, located in the fourth FnIII domain. The arrow indicates the mutated nucleotide. The reported 3 missense mutations identified in CPHD/SOD patients are summarized. (**b**) His568 is a highly evolutionarily conserved amino acid across mammals.

We identified a novel hemizygous *KAL1* mutation, c.1704C > A (p.His568Gln), the only gene among 9 CPHD and 12 KS/HH-related genes for which unknown variants were identified. We performed Sanger sequencing on PCR products from genomic DNA to confirm the *KAL1* variant (Figure 1a). The p.His568Gln was not detected in 150 healthy Japanese controls and was absent from database, including dbSNP, the 1000 Genomes Project, Exome Variant Server, NHLBI Exome Sequencing Project and the Human Genetic Variation Database in Japanese. The p.His568Gln mutation in *KAL1* was submitted to *in silico* analysis. The results exhibited that this mutation was predicted to cause functional damage by PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/ (damage score 0.928, sensitivity 0.81 and specificity 0.94). Parental analysis was refused. *KAL1* encodes anosmin-1 (680 amino acids), a protein that consists of a whey acidic protein-like domain followed by four fibronectin type III (FnIII) domains. His568 is a highly evolutionarily conserved amino acid across mammals, located in the fourth FnIII domain (Figures 1a and b).

Genomic DNAs were also subjected to array comparative genomic hybridization with the Agilent 4 × 180 K SurePrint G3 Human CGH Microarray (catalog no. G4449A; Agilent Technologies). No significant copy-number changes were identified.

Anosmin-1 has a key role in the migration of gonadotropin-releasing hormone neurons and olfactory nerves to the hypothalamus and olfactory bulbs, and is involved in FGF signaling, which has a positive role in pituitary cell proliferation.[12,13] To date, only four CPHD/SOD patients harboring *KAL1* mutations have been reported.[7,14] Raivio *et al.*[7] reported one male CPHD patient (GH, TSH, ACTH and LH/FSH deficiencies) carrying a *KAL1* mutation (p.His459Tyr). However, this patient also carried a heterozygous p.Arg85His mutation in *PROKR2*, which had been reported to be causative for KS. Therefore, the pure contribution of the *KAL1* mutation to this CPHD phenotype is not clear. McCabe *et al.*[14] reported three female patients with SOD harboring heterozygous *KAL1* mutations among 421 CPHD/SOD patients examined. Although these 421 patients were only screened for *KAL1*, this result implies that *KAL1* mutations only contribute to CPHD/SOD etiology to a minor extent, if any. In Table 1, we summarize the clinical phenotypes and MRI findings of *KAL1* mutations within CPHD/SOD patients reported to date. We believe that our findings contribute additional evidence that *KAL1* mutations are associated with hypopituitarism, and provide clinical information to expand the phenotypic spectrum of patients harboring *KAL1* mutations.

We used a next-generation sequencing strategy to analyze 122 genes associated with congenital endocrine disorders. The genetic

Table 1. Summary of the clinical phenotypes and MRI findings of CPHD/SOD patients with *KAL1* mutations

Case	*Sex*	*Clinical findings*	*Affected pituitary hormones*	*MRI findings*	*KAL1 mutation*	*Ref*
I-1[a]	Male	Micropenis, no ocular defect, no midline defects	GH, TSH, LH/FSH, ACTH	NA	p.H459Y	7
II-1	Female	SOD	GH, TSH	EPP, ONH	p.K185N	14
III-1	Female	SOD	GH	ONH	p.P291T	14
III-2	Female	SOD	GH	ONH		
Our case	Male	Right microphthalmia, right renal aplasia, bilateral hearing impairment, severe developmental delay, micropenis, cryptorchidism	GH, TSH, LH/FSH, ACTH	APH, CH, NPP	p.H568Q	

Abbreviations: ACTH, adrenocorticotropin; APH, anterior pituitary hypoplasia; CH, cerebellar hypoplasia; CPHD, combined pituitary hormone deficiency; EPP, ectopic posterior pituitary; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; NPP, normal posterior pituitary; NA, not available; ONH, optic nerve hypoplasia; SOD, septo-optic dysplasia; TSH, thyroid-stimulating hormone. [a]This patient also carried a heterozygous p.R85H mutation in *PROKR2*, which had been reported to be causative for Kallmann syndrome.

etiologies of CPHD and KS/HH are quite heterogeneous, and recent studies have showed that KS/HH is not strictly a monogenic Mendelian disease as previously thought; instead, it is emerging as a digenic or potentially oligogenic disease.[15,16] When multiple genes need to be analyzed simultaneously for mutations, targeted sequence analysis of interesting genomic regions is an attractive approach.

Relative to the four previously described CPHD/SOD patients carrying *KAL1* mutations, our patient had severe pituitary (GH, TSH and ACTH deficiencies in addition to LH/FSH deficiencies) and extra-pituitary phenotypes, including ocular malformation as well as severe growth and psychomotor retardation. Severe HH and predominantly right-sided renal aplasia are consistent with a previously reported phenotype of a male patient harboring *KAL1* mutations;[17] however, the phenotypical variation could be partly due to the impact of other genes that are important, but have not yet been recognized as genes involved in pituitary development. Therefore, further studies are necessary to clarify the independent contribution of *KAL1* mutations to the development of CPHD.

In conclusion, we identified a novel *KAL1* mutation in a CPHD patient with extra-pituitary phenotypes, including a right microphthalmia, right renal aplasia, mild hearing impairment in both ears and severe developmental delay. This study expands our understanding of the phenotypic features and developmental course associated with *KAL1* mutations.

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.505.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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