LHX-4 Gene Mutation in a Boy with Hypopituitarism and Severe Congenital Myopathy

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INTRODUCTION AND OBJECTIVES

LIM (LIN-11, Isl1 and MEC-3) domain transcription factors (LHX4, LHX3, LHX2 or ISL1) are essential in pituitary ontogenesis (1, 2). Mutations in some of those genes result in combined pituitary hormone deficiency (CPHD) (3), and are often associated with variable pituitary and extra-pituitary anomalies on MRI.
LHX4 mutations are rare in humans. They result in large intra- and inter- familial variability of the phenotype (4, 5). The number of the deficient pituitary hormones is variable. The extra-pituitary anomalies could be absent. On the other hand, ectopic posterior pituitary, abnormal sella turcica shape or abnormal corpus callosum has been reported (6).
Only eight LHX4 mutations transmitted as an autosomal dominant trait have been reported in the literature (4, 8-11).
We present a 14 year old wheelchair bound boy, with the c.250C>T (p.Arg84Cys) LHX4 mutation, who has partial IGHD, empty sella and a congenital myopathy.

METHODS

Targeted resequencing was performed using TruSight One Sequencing Panel kit (Illumina, San Diego, CA, USA). The TruSight One Sequencing Panel contains probe sets for enrichment and analysis of >4,800 clinically relevant genes, targeting 12Mb of the human genome. Sequencing libraries were prepared according to Illumina protocol and were sequenced on an Illumina MiSeq desktop sequencer using paired-end 150bp sequencing reads. Raw sequence data were aligned against reference genome specified in the manifest file using MiSeq Reporter software. Each single variant was reported in the VCF output file which was used for the variant calling and filtering via Variant Studio software (Illumina). More comprehensive analyses were done for the variants with a global frequency under 1% according to the 1000 genomes database (http://www.1000genomes.org/) and the ExAc database (http://exac.broadinstitute.org/), particularly those in the genes implicated in growth hormone deficiency. Variants with known pathogenic clinical significance according to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and LOVD (http://www.lovd.nl/3.0/home) were also thoroughly investigated. PCR amplification and Sanger DNA sequencing of the exon 3 from LHX4 gene were performed for the confirmation of the c.250C>T (p.Arg84Cys) variant in the patient and for the analysis of the parents.

RESULTS

The first described mutation of the LHX4 gene in humans was c.607-1G>C (15)(table 1), resulting in multiple pituitary deficiencies: GH, TSH, ACTH. This affected multiple members of the family in which only one member was fertile. There were multiple MRI pituitary anomalies: hypoplasia of the pituitary, a small sella turcica, and chiari malformation.
In pituitary stalk interruption syndrome a mutation of LHX4 gene was reported (5). Early GH and TSH deficiencies, later onset ACTH deficiency were reported in the W204X mutation (16). Multiple pituitary deficiencies, small sella and chiari malformation were reported in the missense mutation (P366T) of the LHX 4 gene (9). In addition, there was severe respiratory disease and hypoglycemia was present after birth.
Pfaeffle et al. (2008)(8) reported three heterozygous missense mutations (p.R84C, p.L190R, and p.A210P) of LHX4. A proband manifested CPHD, a father and one sister harboring the identical mutation had only IGHD. Another patient with a p.L190R mutation had GH, ACTH, and TSH deficiencies. The p.R84C patient had GH, TSH and gonadotropin deficiency. This finding is in contrast to the manifestations in our patient who has only IGHD, and empty sella. In contrast to the IGHD patient, all other patients had hypoplastic anterior pituitaries, while the posterior pituitary was either in normal position or ectopic. Two siblings with p.A210P had a pituitary cyst.
Another familial mutation was a one-base insertion (c293_294insC), resulting in frame shift and producing a premature stop codon (p.Thr99fsX53) (4). Two brothers showed GH and TSH deficiency with pituitary hypoplasia and a poorly developed sella turcica. The youngest brother also had corpus callosum hypoplasia and an ectopic posterior lobe. Their father, who also harbored the identical mutation, had only GHD with pituitary hyperplasia.
Dateki et al. (2010) (17) identified the first patient with a de novo 0.5-megabase heterozygous deletion including LHX4. This patient had a small anterior pituitary, ectopic posterior lobe and underdeveloped sella turcica. Whereas GH, TSH, LH, and FSH were deficient, ACTH secretion was retained when he was evaluated at the age of 17 yr.
Filges et al. (2012) (18) reported a maternally inherited 1.5-megabase microdeletion in 1q25.2q25.3, which included the LHX4 gene. The mother was healthy. On the other hand, the patient had minor dysmorhic features (short nose, short and broad forehead, and nail hypoplasia), but as newborn a major, severe respiratory distress, heart failure hypoglycemia. Pituitary deficiencies included GH, TSH, LH, FSH and ACTH, while the anterior pituitary was small, the posterior lobe was ectopic, sella was poorly formed.
Others also reported multiple pituitary deficiencies (GH, TSH, and gonadotropin deficiency) in p. V75I mutation (11). The c.249-1G>A mutation had GHD and gradually developed hypoadrenocorticism. This mutation was also found in the healthy father and siblings.
Defect of all pituitary hormones, severe respiratory distress and hypoglycaemia was found in the P389T mutation. Pituitary anomalies were MRI evident. The p.V101A mutation had small anterior pituitary, ectopic posterior lobe. The second patient with p.V101A also had a small anterior pituitary and ectopic posterior lobe, but had a normal sized sella turcica (10).
The only homozygous missense variant (c.377C>T, p.T126M) was reported by Gregory at al. (2015)(19). All three children died in the first week of life in spite of rapid treatment with thyroxine and hydrocortisone. They all had deficiency of all pituitary hormones, anterior pituitary aplasia, posterior pituitary ectopia, mid-facial hypoplasia. In addition, a small phallus and undescended testes were also found (19).
All mutants could bind, but not activate a proximal promoter of POU1F1 (4, 7). In addition to inability to activate POU1F1, some mutants could not activate αGSU, TSHβ, FSHβ (8, 10, 16, 20).
Our patient had a severe congenital myopathy which made him wheel-chair bound. At this time there is no explanation of this association between LHX4 gene alteration and myopathy.

CONCLUSIONS

The genetic defects of LHX4 have a high variability in clinical manifestations even in the same family, no clear phenotype-genotype correlation and the phenotype may include extra-pituitary manifestations. Other genetic and/or environmental factors modify the phenotype.

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Table 1 Clinical and genetic characteristics of LHX4 genetic alterations

Author/ Year	Mutation	Age at diagnosis	Medical history of hypopituitarism in family	Pituitary deficiency	Anterior pituitary on MRI	Posterior pituitary MRI	Pituitary stalk MRI	Associated malformations
Machinis et al (2001)(15)	IVS4,G-C-1	N/A	4 family members, in 3 generations, one patient fertile. Consanguinous. Data from 2 probands siblings (quoted features),	GH, TSH, ACTH, FSH/LH not investigated	Hypoplastic, deformated into a pointed configuration in one member. In all 4 family members with short stature, pituitary and cerebellar defects, and abnormalities of the sella turcica.	Ectopic	Not specified	Chiari malformation
Tajima et al (2007)(9)	P366T	14 days	No	GH, PRL, TSH, ACTH, FSH, LH	Very small	Ectopic	Not specified	Chiari, poorly developed sella, respiratory distress sy, hypoglycaemia
Pfaffle et al (2008)(8)	A210P (sibling 1)	7 years	Father low GH and A210P	GH (persistent complete GHD at 20y), TSH, ACTH, FSH/LH	Hypoplastic, cystic lesions	Normal	Not specified	No
Pfaffle et al (2008) (8)	A210P (sibling 2)	7,8 years	Father low GH and A210P	Partial GH (partial GHD at 20y), partial TSH	Hypoplastic, cystic lesions	Normal	Not specified	No
Pfaffle et al (2008) (8)	A210P (father)	7,8 years		Low GH	Normal	Normal	Normal	No
Pfaffle et al (2008) (8)	R84C	5,75 years	No	GH, TSH, FSH/LH	Small	Ectopic	Not specified	Obesity
Pfaffle et al (2008) (8)	L190R	2,5mo	No	GH, TSH, ACTH	Small	Ectopic	Not specified	Hypoglycaemia
Castinetti et al (2008) (4)	Thr99fs	Propositus, 9 months	Father with GHD with pituitary hypoplasia	GH, TSH, FSH/LH not investigated	Hypoplastic	Not visulized	Thin	Poorly developped sella turcica, Corpus callosum hypoplasia
Castinetti et al (2008) (4)	Thr99fs	Brother, 4 years	Father with GHD with pituitary hypoplasia	GH, TSH, FSH/LH not investigated	Hypoplastic	Normal	Normal	Sella turcica poorly developed
Castinetti et al (2008) (4)	Thr99fs	Father, adult		GH FSH/LH deficiency	Hyperplasia	Normal	Normal	Sella turcica normal
Dateki et al (2010)(17)	V201I, 0.5 mb heterozygous deletion	1,6 year	No	GH, TSH FSH/LH deficiency)	Hypoplastic	Ectopic	Not specified	Underdeveloped sella turcica
Tajima et al (2010)(10)	V101A	16 months	No	GH , TSH, ACTH, FSH/LH deficiency	Hypoplastic	Ectopic	Not specified	No
Takagi et al (2012)(11)	V75I	5 years	No	GH, ACTH, FSH/LH deficiency (tentative)	Hypoplastic	Ectopic, poorly developed sella turcica	Thin	No
Takagi et al (2012)(11)	C249-1G>A	5 years	Father, brother sister with same genetic alteration	GH, TSH, ACTH (decreased with age), FSH/LH deficiency	Hypoplastic	Ectopic	Thin	No
Filges et al (2012)(18)	del 1q25.2q25.3	20 days	No; Healthy mother with same deletion	GH, TSH, ACTH, FSH/LH deficiency	Missing	Ectopic	Poorly formed sella	Short nose, short broad forehead, nail hypoplasia, heart failure, respiratory distress syndrome, hypoglycaemia
Tajima et al (2013)(20)	P389T	After birth	No	GH, TSH, ACTH, FSH/LH deficiency	Hypoplastic	Ectopic	Not specified, poorly developed sella turcica	Severe respiratory distress syndrome, hypoglycaemia
Gregory et al (2015)(19)	T126M	Newborns, 2 boys one girl deceased at 1 week with no DNA available	No	GH, TSH, ACTH, FSH/LH deficiency	Aplastic	Ectopic	Not specified	Micropenis, undescended testes,mid-facial hypoplasia.
Rochette et al (2015)(16)	W204X	2 years	No	GH (2y), TSH (2y), ACTH (9y)	Hypoplastic	Actopic	Not vizualized	No
Rochette et al (2015) (16)	DelK242	1 month	Same alternation in a healthy mother	GH (0.1y), TSH(0.1y), ACTH (0.1y)	Hypoplastic	Not specified	Not specified	No
Rochette et al (2015) (16)	N271S	2 years	Not specified	GH (2y), TSH (2y)	Normal	Ectopic	Thin	Cleft lip and palate
Rochette et al (2015) (16)	Q346R	6 years	No	GH (6y), TSH (10y)	Hypoplastic	Ectopic	Not visualized	Hypospadias, toe agenesis, short limb
Rochette et al (2015) (16)	Q346R	10 years	Not specified	GH (10y), TSH (10y), ACTH (15y), (18y), FSH/LH	Hypoplastic	Ectopic	Not visualized	No
Gucev et al. (2016)	R84C	5 years	No	GH	Hypoplastic	Normal	Normal	Severe congenital myopathy

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Growth

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