

CLINICAL STUDY

Novel insertion frameshift mutation of the LH receptor gene: problematic clinical distinction of Leydig cell hypoplasia from enzyme defects primarily affecting testosterone biosynthesis

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Abstract

Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals and is caused by inactivating mutations of the LH receptor gene. The clinical and biochemical diagnostic parameters of LCH are not always specific and may therefore show significant overlap with other causes of insufficient testicular steroid biosynthesis. We have studied a 46,XY newborn with completely female external genitalia and palpable testes. Due to an increased basal serum ratio of androstenedione/testosterone, 17 β -hydroxysteroid dehydrogenase type 3 (17 β -HSD 3) deficiency was initially suspected. DNA analysis of the corresponding HSD17B3 gene, however, showed no abnormalities in the entire coding region. In contrast, direct sequencing of the LH receptor gene revealed a novel homozygous single nucleotide insertion in exon 11 (codon A589fs) producing a frame shift in the open reading frame predicting for premature termination of translation 17 amino acids downstream. From the genetic perspective, this mutation represents the first frame shift mutation in the LH receptor gene ever reported to date. From the clinical standpoint, LCH should always be considered in the differential diagnosis as steroid profiles may not be informative. Therefore, molecular genetic analysis should be warranted for androgen biosynthesis defects in all cases.

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Introduction

Failure of the human luteinizing hormone (LH) receptor of Leydig cells to respond to LH and the placental LH homologue human chorionic gonadotropin (hCG) leads to a decrease or absent testosterone secretion from the testes and results in a variable clinical presentation of Leydig cell hypoplasia (LCH). The spectrum ranges from 46,XY women with a normal-appearing female phenotype (1, 2) to patients with hypoplastic male external genitalia or hypospadias (3, 4).

Clinical diagnosis of these patients is often difficult due to a similar phenotype found in patients with enzyme defects primarily affecting testicular testosterone biosynthesis. This includes the differential diagnosis of isolated 17,20 lyase and 17 β -hydroxysteroid dehydrogenase type 3 (17 β -HSD 3) deficiency.

The inactivation of the LH receptor can be caused by different mutations. Nonsense mutations resulting in truncation of the protein (5–7), deletions (8, 9) and even missense mutations (1, 2, 4, 10, 11) resulting in an amino acid change are ways in which the receptor

protein can be non-functional. Additionally, an insertion in exon 1 has been described which causes profound impairment of signal transduction mediated by the mutated LH receptor (12). Although deletions or insertions of single nucleotides resulting in reading frame shift and translational stop are known in other genes (13, 14), these mutations have not been reported for the LH receptor gene so far. We have studied a 46,XY phenotypic girl who was suspected to have a 17 β -HSD3 deficiency from the serum steroid profile, but turned out to have the first reported frame shift mutation of the LH receptor gene.

Patient and methods

Patient

The unambiguously phenotypically girl was born as the first child from healthy, consanguineous parents from Turkey. At birth, she presented with a ventricular septum defect and an ear appendage. Lumps were palpable in the labio maiora and lead to further diagnostic

procedures. By ultrasound, testicular tissue was visualized (Fig. 1). Müllerian structures were not detected. The karyotype was 46,XY; serum testosterone levels were very low (<0.01 nmol/l) and could not be stimulated by hCG treatment at the age of 5 weeks. When the child was 8 months old the hCG stimulation test was repeated with 5000 IU/m^2 body surface and an adrenocorticotropin (ACTH) test was performed. The results are shown in Table 1. Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) gave no evidence for 21-hydroxylase, 3β -HSD, 11β -hydroxylase and 17-hydroxylase/17-lyase deficiency. The androstenedione/testosterone ratio in serum, determined by stable isotope dilution GC-MS (15), was initially

interpreted as pathological and an analysis of the HSD17B3 gene was performed. No mutation was found. Due to absent stimulation of testicular androgens after hCG, analysis of the LH receptor gene was initiated. Informed consent for this study was obtained from the patient's parents.

DNA analysis

Genomic DNA was extracted from peripheral blood using QIAamp DNA mini kit (Qiagen, Hilden, Germany). Exon 1–10 and two overlapping fragments of exon 11 of the LH receptor gene as well as the adjacent exon-intron boundaries partly up to 70 intron

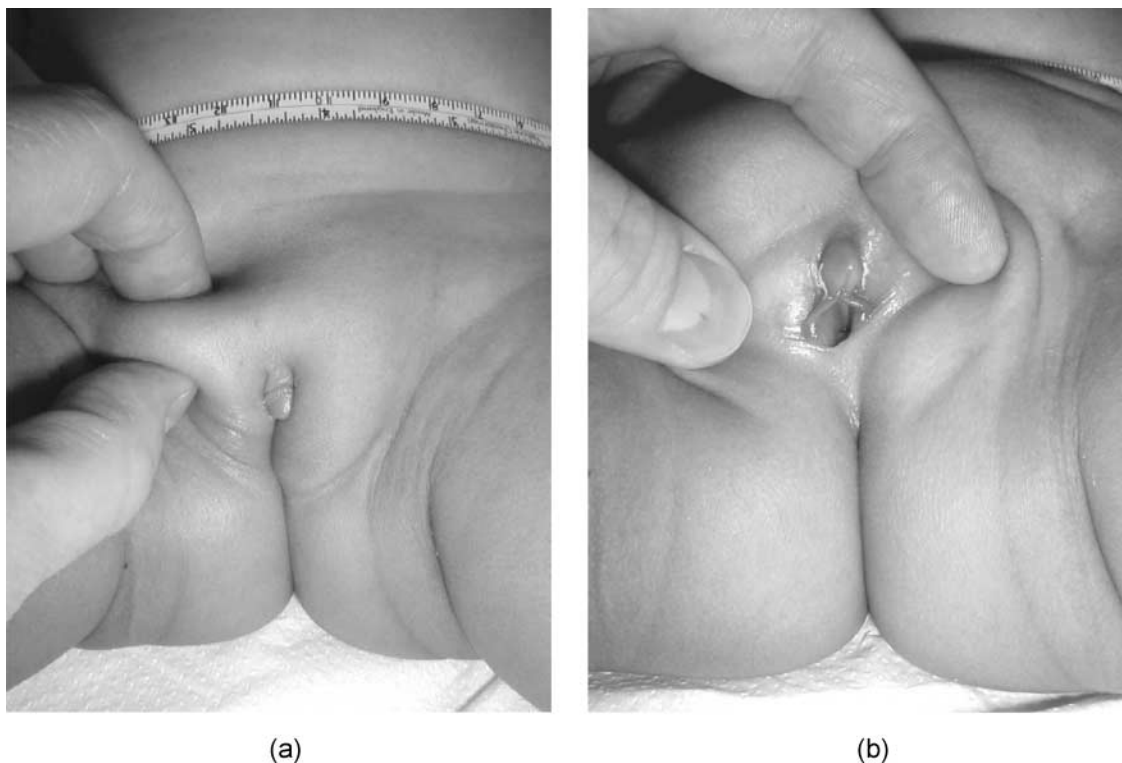


Figure 1 External female genitalia of the 46,XY girl are shown suggesting that no androgen actions during male sex differentiation were present. Gonads are palpable in the inguinal regions.

Table 1 Results of the hCG stimulation test and the ACTH test at the age of 8 months are shown. All steroid serum levels were analyzed by GC-MS.

	Basal	After ACTH stimulation*	After hCG stimulation**
Testosterone	n.d.	n.d.	n.d.
4-androstenedione	0.18 ng/ml	0.32 ng/ml	0.16 ng/ml
Dehydroepiandrosterone	1.22 ng/ml	2.17 ng/ml	1.40 ng/ml
Androstenediol	0.06 ng/ml	0.06 ng/ml	0.19 ng/ml
5 α -dihydrotestosterone	n.d.	n.d.	n.d.
17 α -hydroxyprogesterone	0.22 ng/ml	0.87 ng/ml	0.27 ng/ml
17 α -hydroxypregnenolone	1.81 ng/ml	13.08 ng/ml	3.41 ng/ml
11-desoxycortisol	n.d.	2.06 ng/ml	0.79 ng/ml
Cortisol	10.33 $\mu\text{g/ml}$	27.49 $\mu\text{g/ml}$	16.33 $\mu\text{g/ml}$

*Maximum serum level after 250 μg ACTH; **maximum serum level after 5000 IU/m^2 body surface; n.d., not detectable.

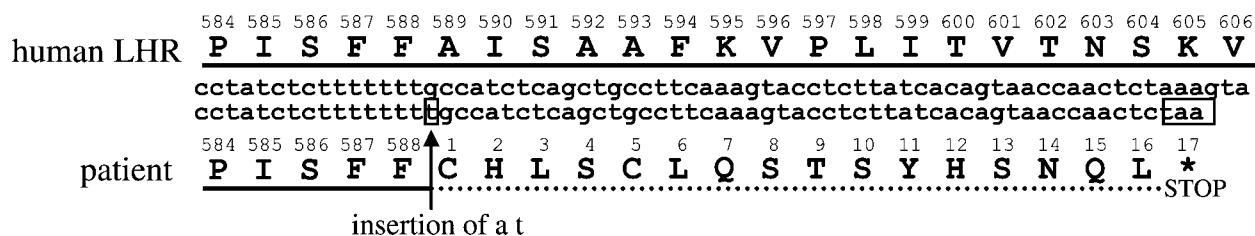


Figure 2 Sequences of wild-type (human LH receptor (LHR)) and patient encoding amino acid residues 584–606 are presented. The altered sequence of 17 amino acids is not underlined. Insertion of a single thymine residue at position 1765 and the stop codon (taa) are indicated.

nucleotides (intron 1) were amplified by PCR using primers and conditions previously described (16, 17). The PCR fragments were checked on agarose gels, purified with the High Pure PCR Purification Kit (Boehringer Mannheim, Mannheim, Germany) and sequenced using the Thermo Sequenase Dye Terminator Cycle sequencing kit for PCR fragments (Amersham Pharmacia Biotech, Amersham, Bucks, UK).

Results and discussion

Sequencing of the complete coding sequence of the LH receptor gene of our 46,XY girl revealed a homozygous single nucleotide insertion at position 1741. This frame shift mutation resulted in an altered amino acid sequence from codon 589 to 604 followed by a STOP (TCT AAA to CTC TAA) (Figs 2 and 3).

Androgenic steroids are essential for male sexual differentiation. Disorders of male development in 46,XY individuals who have no abnormalities in adrenal steroid biosynthesis, absent Müllerian ducts and normally structured testis in the inguinal regions can be due to a defective LH receptor, impaired testosterone biosynthesis or androgen insensitivity. Enzyme defects that are known to primarily affect testicular testosterone biosynthesis are isolated 17,20 lyase and 17 β -HSD 3 deficiency (18, 19).

In prepubertal children, basal androgen levels are generally low, therefore stimulation of the Leydig cells has to be performed. However, a missing or reduced rise of testosterone after the hCG stimulation test may demonstrate a defective LH receptor or a defect in testosterone biosynthesis itself. A 17 β -HSD 3 defect may be suspected if a disproportionate rise in plasma androstendione and estrone levels, compared with testosterone and estradiol concentrations, is found. A plasma testosterone to androstendione ratio of less than 1.0 is typically found in 17 β -HSD 3 patients (20) whereas an abnormal increase in plasma 17-hydroxyprogesterone and 17-hydroxypregnenolone and an increased ratio of 17-hydroxy C21-deoxysteroids to C19-steroids (dehydroepiandrosterone, androstendione) following hCG may account for deficiency of the 17,20-lyase activity of the P450c17 complex (21).

In our patient, GC-MS profiling of steroids in urine gave no evidence of a form of congenital adrenal

hyperplasia, in particular of 17,20 lyase deficiency. A 17-ketoreductase deficiency cannot be excluded by this analysis. While the ratio of testosterone/androstendione was less than 1.0 after hCG stimulation, neither steroids rose, and thus the diagnosis of 17 β -HSD 3 deficiency could be excluded both by the laboratory data as well as the molecular genetic analysis of the HSD17B3 gene (22, 23). Remarkably, there was an increase of 17 β -hydroxypregnenolone after ACTH and hCG in this patient (Table 1). A possible explanation for this finding may be an adrenal origin of the detected testosterone precursors whereas no gonadal androgens could be produced. Comparison of androgens levels (basal and in response to hCG) before and after gonadectomy may help to differentiate the origin of the androgen precursors in the patient.

The poor or totally lacking responsiveness to LH/hCG has led to the hypothesis that loss-of-function mutations in the LH receptor gene may be the underlying cause of LCH. Many different types of mutations may cause full inactivation of function of

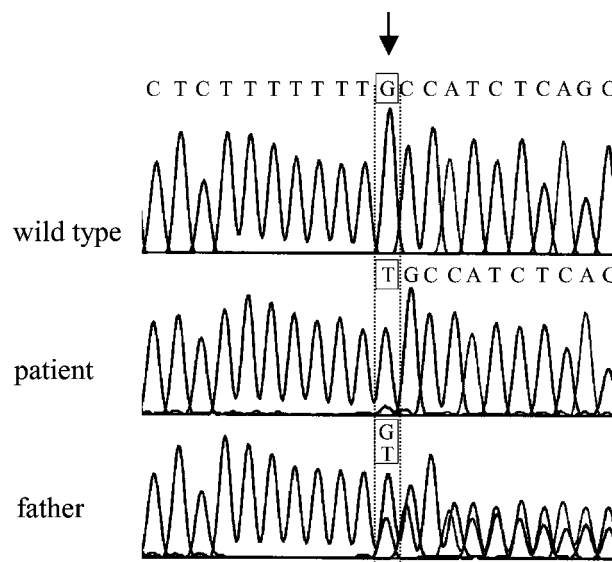


Figure 3 Sequences of a normal control (wild type), the patient and the father are shown. The nucleotide at position 1765 is indicated. Analysis of the patient's DNA revealed a homozygous insertion of a single thymine residue. Father and mother (not shown) are both heterozygous for this mutation.

the LH receptor gene product. Three nonsense mutations have been found in different regions of the transmembrane domain: helix 4 (W491*) (5), helix 5 (C545*) (6) and intracellular loop 3 (R554*) (7). These mutations cause truncation of the LH receptor protein and corresponding absence of at least part of the intracellular loop 3 and transmembrane domains 6 and 7 that are important regions for G protein coupling. We identified in our patient a frameshift-inducing base insertion in the LH receptor gene, which causes a premature truncation of the LH receptor protein and loss of its function. The single nucleotide insertion is located in a highly conserved region in the 6th α helix. The result of the reading frame shift and the translational stop codon 605 may be caused by a duplication of one nucleotide t in a t-rich region (tct¹t²t³t⁴t⁵t⁶t⁷ gcc to tct¹t²t³t⁴t⁵t⁶t⁷t⁸gc) (Fig. 3). We did not test this mutation *in vitro* because the importance of the interaction of the 6th transmembrane domain in LH receptor-mediated G protein activation has been shown using site-directed mutagenesis of these regions (24). Additionally a truncation of the C-terminal tail of the rLH receptor at residue 628 enhances complete hCG-induced cAMP response (25).

Our patient may be compared with a 'human knockout mouse' or 'knockout LH receptor human'. Zhang *et al.* (26) generated mice with LH receptor knockout by inactivating, through homologous recombination, exon 11 of the LH receptor gene. The knockout model produced total inactivation of LH receptor function. The male 'knockout LH receptor mouse' should represent the male 'knockout LH receptor human'. In humans, a clear correlation exists between receptor activity and the resulting phenotype. Our patient underlines that a total inactivation of the LH receptor protein leads to external female genitalia without a significant sign of the impact of androgens (Fig. 1). However, male knockout LH receptor mice are born phenotypically normal. They can be distinguished from wild-type mice by external examination only after day 30–35 by their small penis, short anogenital distance and underdeveloped scrotum. Although the receptor inactivation in LH receptor knockout mice is total, the male phenotype is less dramatic than in connection with similar human mutations. This indicates that a gonadotropin-independent component of fetal Leydig cell androgen production is more prominent in the mouse than in the human. However, shared similarities like infertility, phenotype of internal genitalia and histology of the testis are predominant. The different phenotypes in the males could reflect developmental differences between mice and humans and emphasizes the need to be careful in transferring models to the human conditions.

In conclusion, the diagnosis of severe male under-virilization due to isolated 17,20 lyase or 17 β -HSD 3 deficiency gene must be distinguished from a loss-of-function mutation of the LH receptor gene and it is

difficult to detect solely by steroid analysis. This warrants detailed molecular genetic analysis of the underlying genes in order to make the correct diagnosis.

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