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46 XY undervirulized male DSD: Reporting a patient with prenatally diagnosed disorder/difference of sex development (DSD) with heterozygous *LHCGR* mutations

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ABSTRACT

Leydig cell hypoplasia is a rare autosomal recessive condition caused by mutations in luteinizing hormone/chorionic gonadotropin receptor (LHCGR) genes in which 46, XY patients demonstrate a wide spectrum of disorders/differences of sex development (DSD) phenotypes ranging from normal female external genitalia in severe subtypes to micropenis or hypospadias in patients with less severe presentations. Although most patients with LHCGR defects are diagnosed at puberty, here we describe the prenatal diagnosis of 46, XY DSD due to two likely pathogenic variants in *LHCGR*, one of which has never been reported.

1. Introduction

Disorders/differences of sexual development (DSD) comprise congenital abnormalities of chromosomal, gonadal, or anatomical sexual characteristics with a prevalence of 1 in 4500 live births.[1] Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition caused by mutations in luteinizing hormone/chorionic gonadotropin receptor gene (*LHCGR*) in which 46, XY patients demonstrate a wide spectrum of DSD phenotypes.[2] The degree of genital atypia is dependent on the remaining activity of LHCG receptor. Significant involvements in sex development arise if *LHCGR* mutations lead to complete loss of function of LHCG receptor. These patients, categorized as type I, present like individuals with complete androgen insensitivity syndrome in that they have external genitalia resembling a typical female, dissolution of Müllerian duct structures, and testes located in the labia, inguinal, or intra-abdominal regions.[3] With less profound loss of function mutations, the LHCGR is able to partially function, which causes hypogonadism and undervirulized male external genitalia ranging from micropenis to proximal hypospadias. In this group of patients, categorized as type II, testes may be intrascrotal.[3] Here we report a patient with DSD wherein a complete prenatal genetic workup yielded compound heterozygous mutations in *LHCGR*, including a novel variant that had an unknown significance prenatally.

2. Case presentation

The patient was born from Caucasian parents with no prior history of familial genitourinary disorders or consanguinity. The mother was a 32-year-old G1P0 with a normal, uncomplicated pregnancy, and no history of prenatal alcohol, tobacco, or recreational drug exposures. Cell free DNA testing at her 9th week of pregnancy demonstrated a 46, XY fetal karyotype; however, 20-week ultrasonographic (U/S) assessment showed external genitalia consistent with a female fetus. A second U/S study at 21 weeks confirmed the absence of any elongation to the clitorophallic structure. This discordant information prompted a complete prenatal genetic workup which included amniocentesis (46, XY

Abbreviations: LHCGR, luteinizing hormone/chorionic gonadotropin receptor; DSD, disorder/difference in sex development; LCH, Leydig cell hypoplasia; LH, luteinizing hormone.

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Table 1
Endocrinology investigations of the newborn on the third day and 6th week of life. Testosterone blood level was evaluated by tandem mass spectrophotometry. Normal ranges are expressed for full term infants at Tanner stage 1. Testosterone blood level was very low and below the normal ranges for both male and female neonates in the same age group. Dihydrotestosterone concentration is lower than male newborns and is close to the normal range of female children in this age group. Anti-Müllerian Hormone (AMH) blood level was within the normal range of male newborns. Luteinizing Hormone (LH) was within the normal range.

Parameters	Testosterone (ng/dL)	Dihydrotestosterone (pg/mL)	LH (mU/mL)	AMH (ng/mL)
Blood Level Day 3	2	21.3	<0.1	86.47
Blood Level 6th Week	5	–	0.8	–
Normal Range	Male: 14-363 Female: 20-64	Male: 50 - 600 Female: <20	Male: 0-1 Female: 0-9	Male: 57-495 Female: 0.256–6.345

karyotype), a cytogenomic microarray (46, XY) and a comprehensive DSD gene panel assessment. The gene panel demonstrated two abnormal copies of *LHCGR*. Based on the 30-week U/S showing no changes from the initial U/S, the family was counseled to expect either very severe hypospadias or a phenotypic female. The child was delivered at 39 weeks, had normal APGARs, and a typical female phenotype confirmed by a pediatric urologist (AJS). Specifically, the child had a clearly visualized vaginal opening, a urethra in the typical position, and a typical clitoral hood; there was no evidence of any virulization. Gonads were palpated in each inguinal canal. Endocrine evaluation began on the third day of life (DOL) (Table 1). DOL3 pelvic U/S showed no evidence of Müllerian structures in the pelvis, but inguinal gonads with the sonographic appearance of testicles. A selected endocrine evaluation continued at 6th week and showed levels consistent with testosterone suppression but did not demonstrate a hormone profile completely consistent with LHCGR mutation (Table 1). An incidental inguinal herniorrhaphy was carried out at five months of age; parents desired a gonadal biopsy during herniorrhaphy to further understand and confirm the diagnosis.

3. Genetic analysis and histopathologic evaluations

The Prevention Genetics (Marshfield, WI) Ambiguous Genitalia Panel was used to screen for known genes associated with DSD. This panel covers the full coding regions of over 100 known genes associated with genital development plus ~10 bases of non-coding DNA flanking each exon. Genetic studies revealed 2 heterozygous variants in *LHCGR*. The c.1868A > C variant results in an amino acid substitution p. Tyr623Ser, a known inactivating mutation in the LHR gene. The second variant, previously unidentified and designated c.29_55del, results in an in-frame deletion of nine amino acids p.Leu10_Gln18del, producing a presumed loss of function change. *LHCGR* mutations only cause phenotypic abnormalities if one abnormal copy is inherited from each parent. Subsequent parental testing showed that one parent carried the c.1868A > C and one carried the c.29_55 deletion.

Histological assessment of gonadal biopsies showed normal seminiferous tubules containing Sertoli and spermatogonial cells but an interstitium absent Leydig cells. Calretinin immunohistochemistry is routinely used to identify Leydig cells. It was used on patient's testicular biopsy, and showed no immunoreactivity, while a control sample from same age group showed positive reaction for this marker (Fig. 1). This confirmed the diagnosis of LCH as the cause of 46, XY undervirulized male DSD.

4. Discussion

In this study, we report a patient with 46, XY DSD, female external genitalia, and Leydig cell hypoplasia that had a complete genetic assessment prenatally which yielded a known inactivating mutation and a previously undescribed *LHCGR* gene variant. This prenatal diagnosis and postnatal confirmation are rare and unique in that most patients with *LHCGR* mutations present at or after puberty due to absent secondary sexual characteristics, amenorrhea, or infertility.[4] Here we

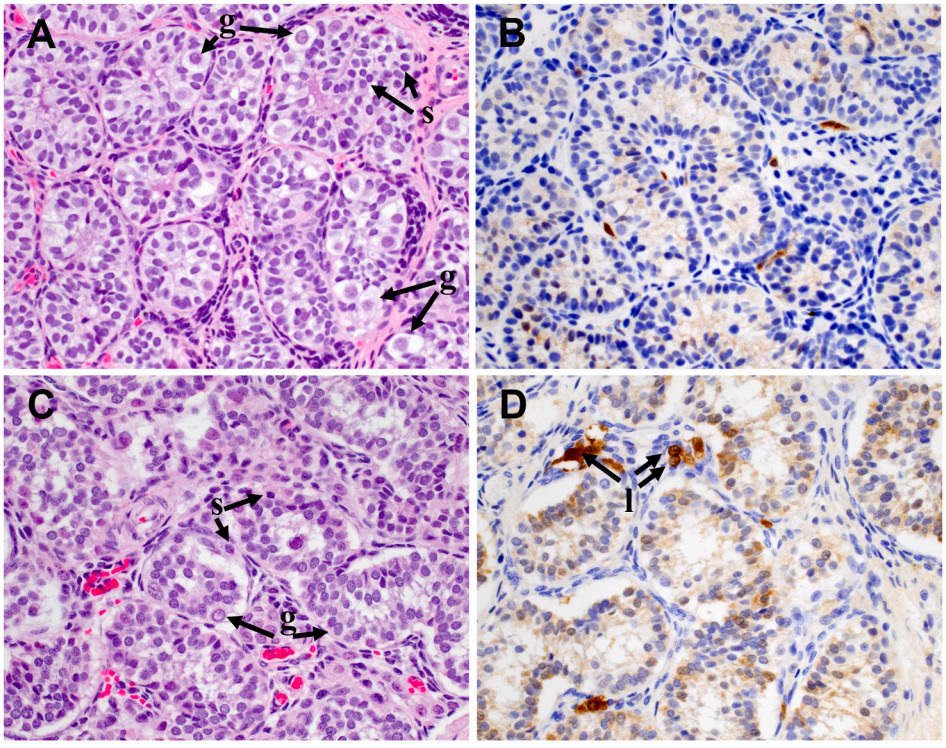

Fig. 1. Histopathologic and immunohistochemical evaluation of biopsied testis (A&B) and comparison with control samples (C&D).
Histological evaluation of the biopsied samples from the patient using hematoxylin and eosin (H&E) staining (A: 40X) demonstrated testicular seminiferous tubules showing gonocytes with large nuclei and clear cytoplasm (g) and Sertoli cells with spheric hyperchromatic nuclei (s). No apparent Leydig cells were seen in the testicular interstitial connection tissue. Calretinin immunohistochemistry can be used to identify Leydig cells. Calretinin (B: 40X) did not stain any Leydig cells in the patient's specimen (the brown areas in C are extracellular artifact), which is consistent with Leydig cell hypoplasia. For comparison, evaluation of the samples from normal testis stained by H&E (C: 40X) also showed no apparent Leydig cells (polyhedral cells with eosinophilic cytoplasm) stained sections, but Calretinin stained (D: 40X) scattered Leydig cells (l) within interstitial connective tissue. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

showed that discordance between the outcomes of prenatal cell free DNA test and U/S imaging led to a complete prenatal DSD genetic evaluation. We suppose that with increased utilization of cell free DNA testing, prenatal diagnosis of DSD may become more common.

There are more than 77 different inactivating mutations of *LHCGR* gene which result in hypoplasia of the Leydig cells and decreased or absent production of testosterone.[5] To the best of our knowledge, the c.29_55 deletion has not been previously reported in the literature, and its clinical significance was unknown. While further molecular investigation into the structural and functional ramifications of the c.29_55 mutation could be accomplished in a research setting, the observation here of an infant with a typical female phenotype and absent Leydig cells suggests that when combined with the known inactivating variant, c1868A > C, profound loss of function results. Thus, the findings suggest that the c.29_55del change is likely an inactivating mutation. Furthermore, this alteration could be associated with blocked downstream expression, potentially allowing the other inactivating mutation to manifest itself. Histological and immunohistochemical evaluations of the patient's gonadal biopsy were consistent with LCH in this patient.

5. Conclusion

Here, we describe a prenatally diagnosed 46, XY DSD undervirulized male with a typical female phenotype caused by two heterozygous variants in *LHCG*R including a novel c.29_55 alteration. The phenotype of the child suggests that the c.29_55 variant is an inactivating mutation.

Declaration of competing interest

None.

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References

1. Hughes IA. Disorders of sex development: a new definition and classification. *Best Pract Res Clin Endocrinol Metabol*. 2008;22(1):119–134.
2. Inactivating mutations of the human luteinizing hormone receptor in both sexes. In: Latronico AC, Arnhold IJ, eds. *Seminars in Reproductive Medicine*. Thieme Medical Publishers; 2012.
3. Wu S-M, Chan W-Y. Male pseudohermaphroditism due to inactivating luteinizing hormone receptor mutations. *Arch Med Res*. 1999;30(6):495–500.
4. Hassan HA, Essawi M, Mekkawy M, Mazen I. Novel mutations of the LHCGR gene in two families with 46, XY DSD causing Leydig cell hypoplasia I. *Hormones (Basel)*. 2020;19(4):573–579.
5. Yan M, Dilihuma J, Luo Y, Reyilanmu B, Shen Y, Mireguli M. Novel compound heterozygous variants in the LHCGR gene in a genetically male patient with female external genitalia. *J. Clin. Res. Pediatr. Endocrinol.* 2019;11(2):211.