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Aberrant transcription of the *LHCGR* gene caused by a mutation in exon 6A leads to Leydig cell hypoplasia type II

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ABSTRACT

The luteinizing hormone/chorionic gonadotropin receptor (LHCGR) is essential for normal male sex differentiation. Recently, the additional primate-specific exon 6A of the *LHCGR* was discovered and it was shown to act as regulatory element at the transcriptional level.

Compound heterozygous mutations in exon 6A (c.580 A > G) and exon 11 (c.1244T > C) were identified in the *LHCGR* of a male 46,XY patient with genital malformation. Analysis revealed that mutation c.580A > G in exon 6A affects the splicing pattern resulting in an increase of transcripts containing the internal variants of exon 6A prone to nonsense-mediated decay. In contrast, mutation c.1244T > C results in an amino acid substitution (Ile415Thr), which abolishes signal transduction due to structural changes. When inherited in a compound heterozygous fashion these mutations result in Leydig cell hypoplasia (LCH) type II. Thus this study provides proof that mutations causing aberrant transcription can impair receptor function and thereby be causative of LCH.

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1. Introduction

Male fetal sex differentiation is dependent on the androgen production of fetal testicular Leydig cells (Diez d'Aux and Pearson Murphy, 1974; Sharpe, 2006), which is induced by placental human chorionic gonadotropin (hCG). Later during gestation it is maintained by luteinizing hormone (LH), secreted by the fetal pituitary gland (Fowler et al., 2009). As the action of both glycoprotein hormones is mediated by the luteinizing hormone/chorionic gonadotropin receptor (LHCGR), this receptor is essential for male sex differentiation as well as for normal reproductive function in the adult. The human *LHCGR* gene (NCBI GeneID 3973; http://www.ncbi.nlm.nih.gov/) is located on chromosome 2p21 and consists of 12 exons. Exons 1–10 and a part of exon 11 encode for the extracellular domain, which is responsible for ligand binding. The remaining exon 11 encodes the transmembrane domain consisting of seven helices (TMH) and the intracellular C-terminal tail, which are involved in signal transduction (Ascoli et al., 2002). A distinctive feature of the *LHCGR* gene is the presence of the primate specific exon 6A, which is located in the intronic region between exons 6 and 7. Exon 6A displays composite characteristics of an internal/terminal exon and gives rise to three splice variants through alternative splicing (Kossack et al., 2008). All three variants are expressed in fetal and adult human testes (Fowler et al., 2009; Kossack et al., 2008). Transcripts with the internal exon 6A are characterized by the presence of premature termination codons and are therefore targets for nonsense-mediated mRNA decay (NMD) resulting in low mRNA expression levels. In contrast to that, transcripts bearing the terminal *LHCGR* exon 6A variant are expressed at higher levels. These transcripts give rise to a putatively truncated LHCGR protein of 209 amino acids, consisting only of a part of the extracellular domain and lacking the transmembrane domain. As solely transcripts without exon 6A can give rise to functional receptor proteins, this exon acts as a novel regulatory element at the transcriptional level within the *LHCGR* gene (Kossack et al., 2008).

The essential regulatory function of exon 6A for LHCGR signaling was shown by the identification of mutations in exon 6A in patients suffering from Leydig cell hypoplasia (LCH) (Kossack et al., 2008). These mutations led to an increased amount of transcripts containing exon 6A and a reduced amount of functional full-length transcripts. Therefore these mutations were causative of the severe form of LCH (type I). LCH is a rare 46,XY disorder of sex development caused by inactivating mutations in the *LHCGR* gene predominantly affecting the amino acid sequence. Depending on the severity of the LHCGR inactivation, a spectrum of phenotypes has been described ranging from a predominantly female phenotype to incomplete male sex differentiation (Martens et al., 1998). The severe form of LCH (type I) is caused by mutations, deletions or insertions, which result in a complete inactivation of LHCGR

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function. Affected 46,XY patients have a predominantly female phenotype with abdominal testicular structures, a blind-ending vagina, absence of breast development and primary amenorrhea (Themmen and Huhtaniemi, 2000). Apart from this severe form, a milder form of LCH (type II) has been described, which is caused by *LHCGR* mutations that solely impair function and therefore cause undervirilization of affected individuals (Martens et al., 1998; Themmen and Huhtaniemi, 2000; Toledo et al., 1985).

In this study we describe a novel mutation in exon 6A of the *LHCGR*, which does not affect the amino acid sequence but rather causes transcriptional changes resulting in altered ratios of *LHCGR* transcripts with an increased amount of *LHCGR* variants including the internal exon 6A. In contrast to the previously known mutations in exon 6A, which result in the severe form of LCH (type I), this mutation is causative of the mild form of LCH (type II). We provide novel information on the effects of mutations affecting transcriptional ratios of *LHCGR* mRNA variants, the essential role of exon 6A for receptor functioning and delineate a concept in which significantly reduced LH/hCG responsiveness by an altered receptor is sufficient for initial but insufficient for complete male sexual development.

2. Patient and methods

2.1. Patient

The patient's parents gave written informed consent for this study and the publication of case details. The patient was born to unrelated parents and was evaluated because of genital malformation with micropenis (Fig. 1A). He had an increased birth weight (height: 55 cm; weight: 5.35 kg; head circumference: 39.5 cm) and remained slightly overweight at 2.5 months (height: 61 cm; weight: 7.0 kg), the time of his first visit. While the left testis was located in the upper scrotal compartment, the right gonad was not palpable initially but could be located in the inguinal region at 7 months of age. The patient's hormone levels were analyzed at three consecutive time points (2.5, 7 and 8 months; Supplementary Table 1). At 2.5 months of age the patient revealed normal gonadotropin, slightly elevated 17α-hydroxyprogesterone and undetectable testosterone levels. Low testosterone levels (<0.1 ng/l) persisted at 7 months and therefore a standard hCG-treatment with 2× 1000–1500 IU hCG was performed. At the age of 8 months, 3 days after the second hCG injection, the testosterone levels of the patient remained low.

Further treatment included application of a dihydrotestosterone salve, which induced penile growth in length and width.

No *family history* of LCH or related features is known in either parent. The patient has a maternal half-brother, who is of normal height and has normally developed genitalia.

2.2. DNA isolation and sequencing analysis

Genomic DNA was isolated from EDTA-treated blood samples using the FlexiGene DNA Kit (Qiagen, Hilden, Germany). Primers for the detection of mutations in exons 1–11 were as published (Gromoll et al., 2000) and are listed in Supplementary Table 2 for exon 6A.

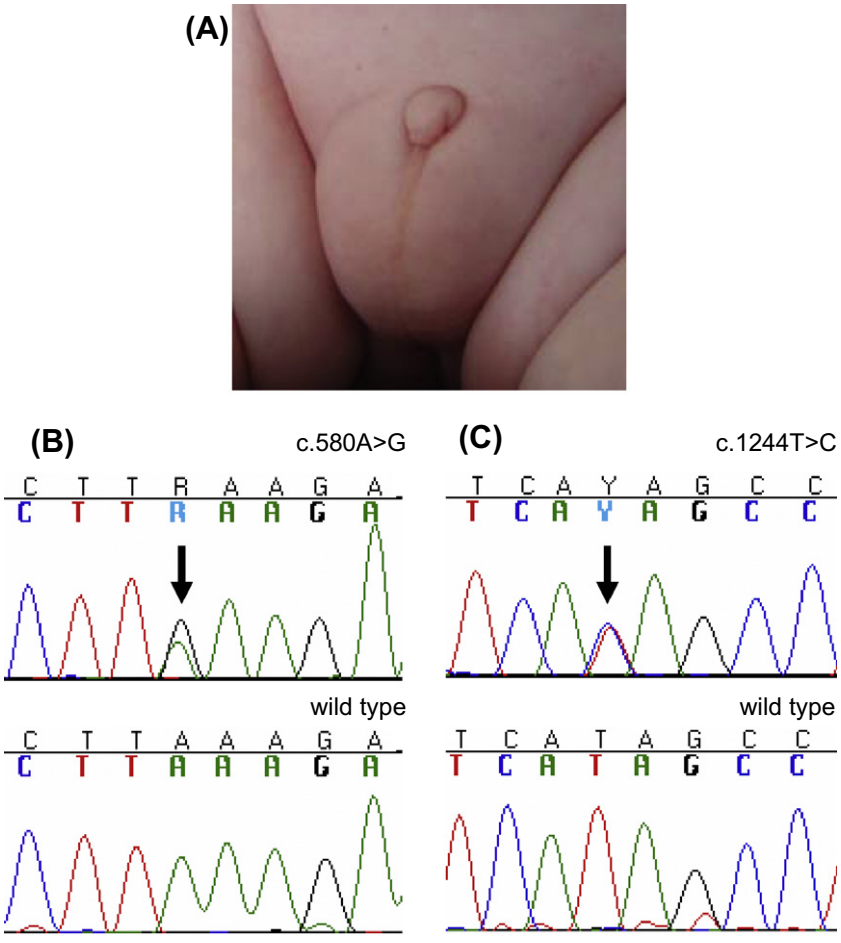

Fig. 1. Patient phenotype and genomic analysis of the *LHCGR* gene. (A) Photograph of the patient's genitalia at the age of 2.5 months. The patient showed malformation of the genitalia with micropenis. (B and C) DNA sequence analysis of the *LHCGR* gene. The upper images show the results for the patient and the lower images the results for normal controls (wild type sequence). The position of the heterozygous mutations c.580A > G in exon 6A (B) and c.1244T > C in exon 11 (C) are indicated by arrows.

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