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CASE REPORT

A novel *CYP17A1* deletion causes a functional knockout of the steroid enzyme 17-hydroxylase and 17,20-lyase in a Turkish family and illustrates the precise role of the *CYP17A1* gene

Núria Camats[1], Ala Üstyol[2], Mehmet Emre Atabek[2], Bernhard Dick[3] & Christa E. Flück[1]

[1]Pediatric Endocrinology and Diabetology, Departments of Pediatrics and Clinical Research, University Children's Hospital Bern, Bern 3010, Switzerland
[2]School of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, Necmettin Erbakan University, Konya 42080, Turkey
[3]Department of Nephrology, Hypertension and Clinical Pharmacology, University Hospital Bern, Bern 3010, Switzerland

Correspondence
Núria Camats, Departments of Pediatrics and Clinical Research, University Children's Hospital Bern, Freiburgstrasse 15 C843, 3010 Bern, Switzerland. Tel: +41 31 632 0339;
Fax: +41 31 632 4792;
E-mail: nuria.camats@dkf.unibe.ch

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Key Clinical Message

A novel homozygous long-range deletion of the *CYP17A1* gene abolished protein expression and caused the severest form of 17-hydroxylase deficiency in one kindred of a Turkish family. The affected subjects presented with 46, XY sex reversal and 46,XX lack of pubertal development as well as severe hypertension.

Keywords

17α-hydroxylase/17,20-lyase deficiency, hypertension, pubertal development, sexual development, steroidogenesis.

Introduction

Childhood hypertension and disordered pubertal development are conditions with a broad range of possible underlying diseases. A common cause for both may be a disorder of steroid biosynthesis which affects both mineralocorticoid (MC) and sex steroid (SS) production [1, 2]. Patients with complete or partial, combined 17α-hydroxylase/17,20-lyase deficiency usually present in childhood to adolescence with hypertension and hypokalemia due to increased MC production, with blunted stress response due to inadequate cortisol production, and with 46,XY disordered sex development (DSD) and lack of sexual maturation due to SS deficiency [1, 2]. Most mutations of the *CYP17A1* gene cause combined 17α-hydroxylase/17,20-lyase deficiency [1, 2], but a few cases of isolated 17,20-lyase deficiency have been reported in whom only SS production is affected [3–6].

Cytochrome P450c17 (OMIM 609300) is an essential steroidogenic enzyme for glucocorticoid (GC) and SS biosynthesis. It possesses dual activity. The 17-hydroxylase activity converts pregnenolone to 17-hydroxy-pregnenolone and progesterone to 17-hydroxy-progesterone. The 17,20-lyase activity converts 17-hydroxy-pregnenolone to dehydroepiandrosterone, but has only little catalytic activity to convert 17-hydroxy-progesterone to androstenedione [1, 2, 7]. Lack of its activities results in SS and relative cortisol deficiency, and overproduction of MC precursors with some GC activity [1, 2].

We report a novel homozygous long deletion in the *CYP17A1* gene, which causes nonexpression of protein and thus the severest form of 17-hydroxylase deficiency in a Turkish family of Kurdish origin.

Case Report

The index patient (IV.2, Fig. 1A) was first seen by a general practitioner in a remote area of Turkey for epistaxis at 12 years of age. At that time she was noted to have severe hypertension and was prepubertal. Clinical and minimal biochemical work-up (data not available) indicated that the underlying cause could be 17-hydroxylase deficiency. Treatment with physiologic dose of hydrocortisone (8–10 mg/m^2/day) was recommended. Her two sisters were clinically investigated at age 10 (IV.3) and 7 (IV.4) and were suspected to suffer from the same condition (Fig. 1A). Adherence to treatment was difficult and follow-up lost. Five years after initial presentation, lack of pubertal development brought them back to medical attention. At this time, specific clinical and biochemical investigations were performed (Table 1) confirming the diagnosis of 17-hydroxylase deficiency (Fig. 1B). Additional tests revealed that the youngest sister (IV.4) was 46,XY and had no uterus and gonadal tissue on pelvic MRI (Table 1). According to her medical history, she had an operation for bilateral inguinal hernia when she was an infant, but the parents, who are first-degree cousins (III.7 and III.8; Fig. 1A), do not recall whether she was gonadectomized. The family history is remarkable for two unmarried female cousins (IV.5, IV.6; Fig. 1A) who receive hydrocortisone treatment for adrenal disease but do not wish to be further investigated.

Urine samples of patients IV.2 and IV.4 were analyzed by gas chromatography/mass spectrometry and revealed high corticosterone metabolites and low progesterone, deoxycortisol, androgen, and estrogen metabolites confirming a combined 17α-hydroxylase/17,20-lyase deficiency (Fig. 1B). Leukocyte genomic DNA was prepared from the parents and the four siblings consenting for genetic analysis. *CYP17A1* gene analysis was first performed by an exon-by-exon approach as described [8] but revealed no PCR products for exons 1–6 in the three affected subjects suggesting a big deletion. Thus, a long-frame PCR with primers located at −3 kb of the *CYP17A1* promoter (5′-AGT CCT CCT TTA TGG GAC TCT GA) and at c.2007-1198 in exon 7 (5′-CAC TCC TTC TCA TTG TGA TGC AG) was performed showing the exact architecture of the large deletion of the *CYP17A1* gene cutting out the promoter, the transcription start site, and six exons/introns (Fig. 1C). The exact boundaries of the deletion were revealed by direct sequencing (Fig. 1C; GenBank NG_007955.1). This deletion, covering more than 7 kb (7471 bp), corresponds to two long deletions with a 29-bp fragment of IVS2 in between (Fig. 1C). The first deletion c.-2011_436+119del (4238 bp) extends from −2011 to IVS2 and the second c.437-93_1140-262del (3233 bp) from IVS2 to IVS6. As this deletion cuts out the gene transcription start site, it causes a complete knockout of CYP17A1.

Discussion

This "two-in-one" deletion is the longest deletion detected in the *CYP17A1* gene [9]. Previously described deletions are 1–25 bp long or small or larger indels. However, none is located in the promoter region or involves the transcription start site; thus all of them produce some sort of a CYP17A1 protein [9]. The most prevalent *CYP17A1* deletion is D487_F489del (c.1459_1467delGACTCTTTC), which has been detected in many patients with 17α-hydroxylase/17, 20-lyase deficiency, mostly of Chinese origin [10–19]. Among people of European ancestry, the most common mutation is a 4-bp C-terminal duplication (following Ile479) among Dutch Frieslanders and their descendants [20]. The relevance of the unique large indel mutation (518-bp deletion together with a 469-bp insertion) is not clear [21]. This mutation expanding from exons 2 and 3 was identified in a 46,XY female patient with severe combined 17α-hydroxylase/17, 20-lyase deficiency, with two affected 46,XX sisters [21].

Figure 1. Steroid profiling and genetic work-up of the family with a novel *CYP17A1* deletion. (A) Family tree showing karyotype, genotype (2×, 3×, and 5× – number of healthy individuals; wt, wild type; del, deletion), and phenotype. (B) Gas chromatography/mass spectrometry profile of 24-h urine from a control and patient IV.4. Arrows indicate corticosterone metabolites THA, THB, and 5a-THB (in bold), which are elevated in CYP17 deficiency. Note also overall low androgens. Andro, androsterone; Etio, etiocholanolone; DHA, dehydroepiandrosterone; 11-oxo-etio, 11-oxo-etiocholanolone; 11β-OH-andro, 11β-hydroxyandrosterone; 17-HP, 17hydroxypregnanolone; 11β-OH-etio, 11β-hydroxyetiocholanolone; PD, pregnanediol; PT, pregnanetriol; 5-AT, 5-androstene-3β, 16α, 17β-triol; THS, tetrahydrodeoxycortisol; THDOC, tetrahydrodeoxycorticosterone; PTone, 11-oxo-pregnanetriol (or pregnanetriolone); MP(ISTD), medroxyprogesterone (recovery standard); THE, tetrahydrocortisone; THA, tetrahydro11-dehydrocorticosterone; THB, tetrahydrocorticosterone; 5a-THB, 5α-tetrahydrocorticosterone; THF, tetrahydrocortisol; 5a-THF, 5α-tetrahydrocortisol; SS(ISTD), stigmasterol (standard for derivatization and chromatography); 20β-DHF, 20β-dihydrocortisol; 20α-DHF, 20α-dihydrocortisol. (C) Genetic analysis of the identified deletion. The analytic strategy and location of the identified deletion is shown in a diagram showing the *CYP17A1* gene. PCR products of the long amplification PCR are depicted. The lower band corresponds to double deletion and the upper band to the wild-type situation. All patients are homozygous for the deletion, the parents are heterozygous whereas one sibling is genetically wild-type on both alleles (wt, wild type; del, deletion). The original sequence (reverse) of the *CYP17A1* gene with the 2-deletion breakpoints and the conserved IVS2 sequence detected in the parents and the three affected siblings are also shown.

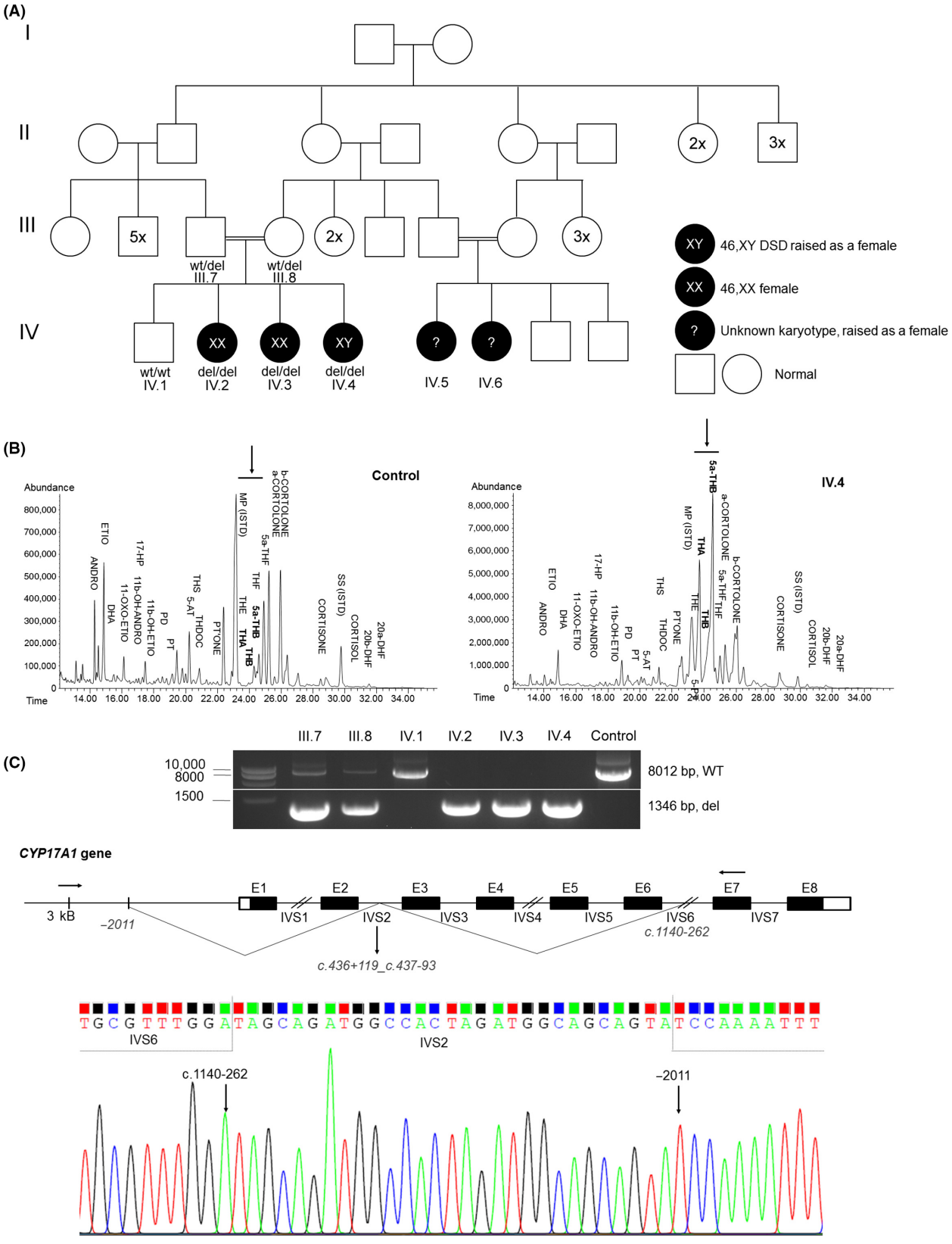

Table 1. Clinical and laboratory data of the three affected siblings.

Patient	IV.2 (index)	IV.3	IV.4
Age at diagnosis (years)	17.9	15.3	13.5
Hypertension	+	N.K.	+
Breast Tanner stage	2–3	2–3	1
Pubic hair Tanner stage	2	2	2
External genitalia	Female	Female	Female
Internal genitalia	Uterus	Uterus	Bilateral inguinal hernia repair and orchiectomy
Karyotype	46,XX	46,XX	46,XY (SRY+)
Na, mmol/L	140	141	138
K, mmol/L	3.9	3.7	3.8
ACTH, pmol/L	23.54	17.38	11.64
Cortisol, nmol/L	7.17	11.31	10.76
Progesterone, nmol/L	**27.32**	**23.69**	**24.77**
17-OH progesterone, nmol/L	**0.0063**	**0.0042**	**0.0079**
DHEA-S, μmol/L	**0.046**	**0.084**	**0.103**
Testosterone, nmol/L	**N.D.**	**N.D.**	**N.D.**
LH, IU/L	**33.7**	**33**	**30.6**
FSH, IU/L	**112.4**	**162**	**93**
Estradiol, pmol/L	**<73**	**<73**	**<73**
Inhibin B, ng/L	–	–	30
AMH, pmol/L	–	–	87.14

N.K., not known; N.D., nondetectable.
Laboratory data outside the normative range are shown in bold.

Other common mutations include point mutations W406R and R362C among Brazilians of Spanish and Portuguese ancestry, respectively [22]. However, the exact frequency of any *CYP17A1* mutation in any population has not been assessed so far.

In the reported family the index patient was 46,XX and presented with hypertension and without pubertal development manifesting at the age of puberty, which is typical for affected 46,XX individuals with CYP17 deficiency. However, bilateral inguinal hernia (likely containing male gonads) in the affected individual IV.4 in infancy should have raised suspicion of a DSD situation. Taking this into account, this patient could have been investigated in infancy for a 46,XY DSD situation with normal male internal organs (without Müllerian structures) pointing to a disorder of androgen synthesis or action. Thus her diagnosis was obviously missed at a young age. 46,XY individuals with severe 17-hydroxylase deficiency are basically not able to produce testosterone and thus present with a female phenotype of the external genitalia at birth. The gonads may be found in the genital folds, inguinal or may be located intra-abdominally [1]. Some pubertal development (breast and pubic hair) may be seen in 46, XX patients with partial activity of CYP17A1 [2, 23], and was even seen at age 15 and 18 years in our 46,XX patients with a complete loss of CYP17A1. This indicates that some androgens may be produced in the periphery from precursors and other enzymes. However, to our knowledge, the detailed metabolome of CYP17 deficiency naming those active intermediates and alternate pathways has not been described.

From this experiment of nature we learnt that CYP17A1 is not crucial for survival, even though it is essential for cortisol biosynthesis in the human adrenals. Lack of CYP17A1 for cortisol production can be compensated by enhanced production of corticosterone, which also has GC activity and is in fact the active GC in rodents, which lack CYP17A1 expression in their adrenals [24]. Late manifestation of hypertension is also known in children with 11β-hydroxylase deficiency due to elevated deoxycorticosterone [1]. Levels of MCs are physiologically higher in young children, who may also have higher blood vessel plasticity, explaining why they may not manifest with hypertension early. On the other hand, blood pressure is often not measured and hypertension therefore remains underdiagnosed in childhood.

Treatment of P450 deficiency includes physiologic replacement of GC and sex hormones (at puberty). To control blood pressure, antihypertensive drugs may be necessary. Affected 46,XY individuals with ambiguous genitalia may desire surgical repair.

In summary, we report a novel long-range *CYP17A1* deletion in several, both 46,XX and 46,XY, family members with typical clinical manifestations of severe 17-hydroxylase deficiency – a rare genetic disorder of steroid biosynthesis with manifestation in childhood.

Patient Consent

Informed consent was obtained from all the subjects of the study.

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Conflict of Interest

All authors declare no conflict of interest.

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