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CASE REPORT

A novel *SRD5A2* mutation in a Taiwanese newborn with ambiguous genitalia

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Abstract The 5α-reductase type 2 deficiency is a rare autosomal recessive 46,XY disorder of sex development caused by the mutated *5α-reductase type 2 (SRD5A2)* gene. In this disease, defective conversion of testosterone to dihydrotestosterone leads to variable presentations of male ambiguous genitalia during fetal development. The most crucial clinical decision for the affected individual is proper gender assignment; therefore, a prompt and correct diagnosis is important. In this present study, we report a normal male karyotype manifesting microphallus, bifid scrotum/labia majora with bilateral palpable gonads, and a blind-ended pseudovagina. The mutation analysis of the *SRD5A2* gene revealed one novel C to T transition changing glutamine to a stop codon at codon 71 (p.Q71X) in exon 1 and one known G to A transition changing arginine to glutamine at codon 227 (p.R227Q) in exon 4. The p.Q71X mutation presumably results in a truncated protein, while the p.R227Q mutation is conceived to impair enzyme function and has been reported in patients of East Asian descent. This report demonstrates the essential role of hormonal and molecular studies for genetic counseling and gender assignment in males with pseudovaginal disorder of sex development, and our report helps identify a novel *SRD5A2* gene mutation in the Taiwanese population.

Introduction

The 5α-reductase type 2 deficiency (OMIM number 264600) is an autosomal recessive disorder characterized by aberrant male sex development. Affected males usually present at birth with ambiguous external genitalia, such as perineoscrotal hypospadias, pseudovagina, microphallus, and

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cryptorchidism [1]. However, a wide variety of clinical phenotypes range from complete feminization to undermasculinization or isolated infertility. Deficiency of this key enzyme fails to convert testosterone to dihydrotestosterone in a sufficient amount for normal differentiation of male genitalia during fetal development. Dihydrotestosterone also modulates psych-orientation of the prenatal human brain and mediates numerous critical steps of masculinization at puberty. Hence, residual activity of abnormal 5α-reductase type 2 enzyme leads to variable degrees of virilization at birth and has an impact on lifelong psychological functioning [2].

Several forms of 46,XY disorders of sex differentiation may share similar clinical presentations with 5α-reductase type 2 deficiency. Differential diagnosis with partial androgen insensitivity and 17β-hydroxysteroid dehydrogenase deficiency sometimes can be difficult and may be facilitated on the basis of a biochemical evaluation [3]. After human chorionic gonadotropin (hCG) stimulation, hormonal measurement of serum testosterone-to-androstenedione ratio determines a diagnosis of 17β-hydroxysteroid dehydrogenase deficiency, while serum testosterone-to-dihydrotestosterone ratio testifies a diagnosis of 5α-reductase type 2 deficiency [4]. An abnormally high baseline and hCG-stimulated testosterone-to-dihydrotestosterone ratio is the hallmark of 5α-reductase type 2 deficiency [1,5,6]. However, unremarkable elevated level of testosterone-to- dihydrotestosterone ratio after hCG stimulation does not refute this diagnosis, especially in patients with partially dysfunctional enzyme activity [7,8].

The *5α-reductase type 2 (SRD5A2)* gene, which encodes 5α-reductase type 2 enzyme, is located on chromosome 2p23. The coding region consists of 5 exons, which are translated into a 254-amino acid protein [9]. To date, more than 50 mutations have been identified in different ethnicities in previous studies. Scattered throughout the gene, missense mutations are most common, while some are nonsense or splice-junction mutations [5,8,10–14]. Nucleotide or entire deletion of the *SRD5A2* gene has also been described [15]. Accordingly, molecular analysis presently provides adjunct evidence of the definite diagnosis in the diversity of phenotypic features [16].

Previously, 5α-reductase type 2 deficiency was often suggested merely on the basis of clinical and biochemical features [17]. The characteristics of *SRD5A2* gene mutations pertaining to the Taiwanese population were scarcely known. In this article, we confirmed the diagnosis by virtue of a molecular analysis and reported a novel *SRD5A2* mutation in a newborn with ambiguous genitalia.

Case report

This patient was born at the 38th gestational week with an uneventful perinatal history. The parents were of Taiwanese origin without consanguineous history, and their previous two children did not have any specific problems. At birth, the patient manifested ambiguous external genitalia. On physical examination, bilateral gonads were palpable in bifid scrotum/labia majora. A clitoris-like phallus measured 1.3 cm, and there was a urethral orifice on the tip of underdeveloped glans. Inferior to the microphallus was a blind-pouched vagina (Fig. 1A). Pelvic ultrasonography revealed absence of Mullerian remnants. The serum hormonal profile showed an elevated total testosterone level of 2.13 ng/dL (normal range, 0.1–0.75 ng/dL), and a 17-hydroxyprogesterone level of dried blood spot on filter paper was 1.7 ng/ml (normal range, 0.4–4.3 ng/ml). The serum dihydrotestosterone level was not available. The chromosome study, performed on peripheral blood lymphocytes, showed a normal male 46,XY karyotype. Since the clinical presentations and endocrine investigations were suggestive of 5α-reductase type 2 deficiency or partial androgen insensitive syndrome, genetic analysis of these two disorders were initiated immediately under his parents' informed consent. hCG stimulation test was not performed in this case. On the basis of his final diagnosis, male gender was assigned after discussion with his family. Accordingly, he received a monthly injection of testosterone cypionate 50 mg and his penile length increased from 1.3 to 2.8 cm at 6 months of age (Fig. 1B).

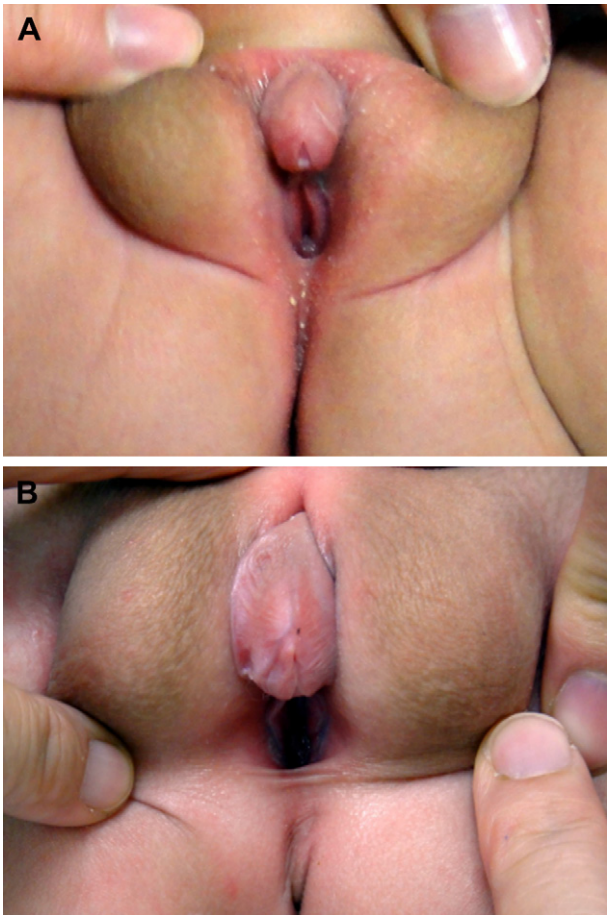

Figure 1. (A) Ambiguous genitalia with bilateral palpable gonads in bifid scrotum/labia majora, a clitoris-like phallus measuring 1.3 cm with a urethral orifice on the tip of underdeveloped glans, and a blind-pouched vagina at 1 month of age; (B) enlargement of the microphallus measuring 2.8 cm along with a pseudovagina and gonads in bifid labioscrotal folds at 6 months of age.

Table 1 Oligonucleotide primers used for polymerase chain reaction amplification of the *SRD5A2* gene (annealing temperature = 58 °C).

Exon	Forward (5′→ 3′)	Backward (5′ → 3′)	Product size (bp)
1	GCAGCGGCCACCGGCGAGG	AGCAGGGCAGTGCGCTGCACT	358
2	TGAATCCTAACCTTTCCTCCC	AGCTGGGAAGTAGGTGAGAA	235
3	TGTGAAAAAAGCACCACAATCT	CAGGGAAGAGTGAGATCTGG	208
4	TGATTGACCTTCCGATTCTT	TGGAGAAGAAGAAAGCTACGT	232
5	TCAGCCACTGCTCCATTATAT	CAGTTTTCATCAGCATTGTGG	166

Molecular diagnosis and results

Molecular analysis of the androgen receptor (AR) gene and *SRD5A2* gene was applied on peripheral blood lymphocytes collected in EDTA (Ethylenediaminetetraacetic acid)-containing tubes. Genomic DNA was extracted according to the manufacturer's instructions (Genomic DNA Extraction Kit, RBC Bioscience Corp., Taiwan). For polymerase chain reaction (PCR) amplification of *SRD5A2* gene, five sets of primers spanning all five exons and the flanking splice sites were used (Table 1). Briefly, approximately 50 ng of genomic DNA, 10 pmol of each primer, 5 μmol of each deoxyribonucleoside triphosphate, 0.2 U of pro Taq DNA polymerase (Promega, Madison, WI, USA) and

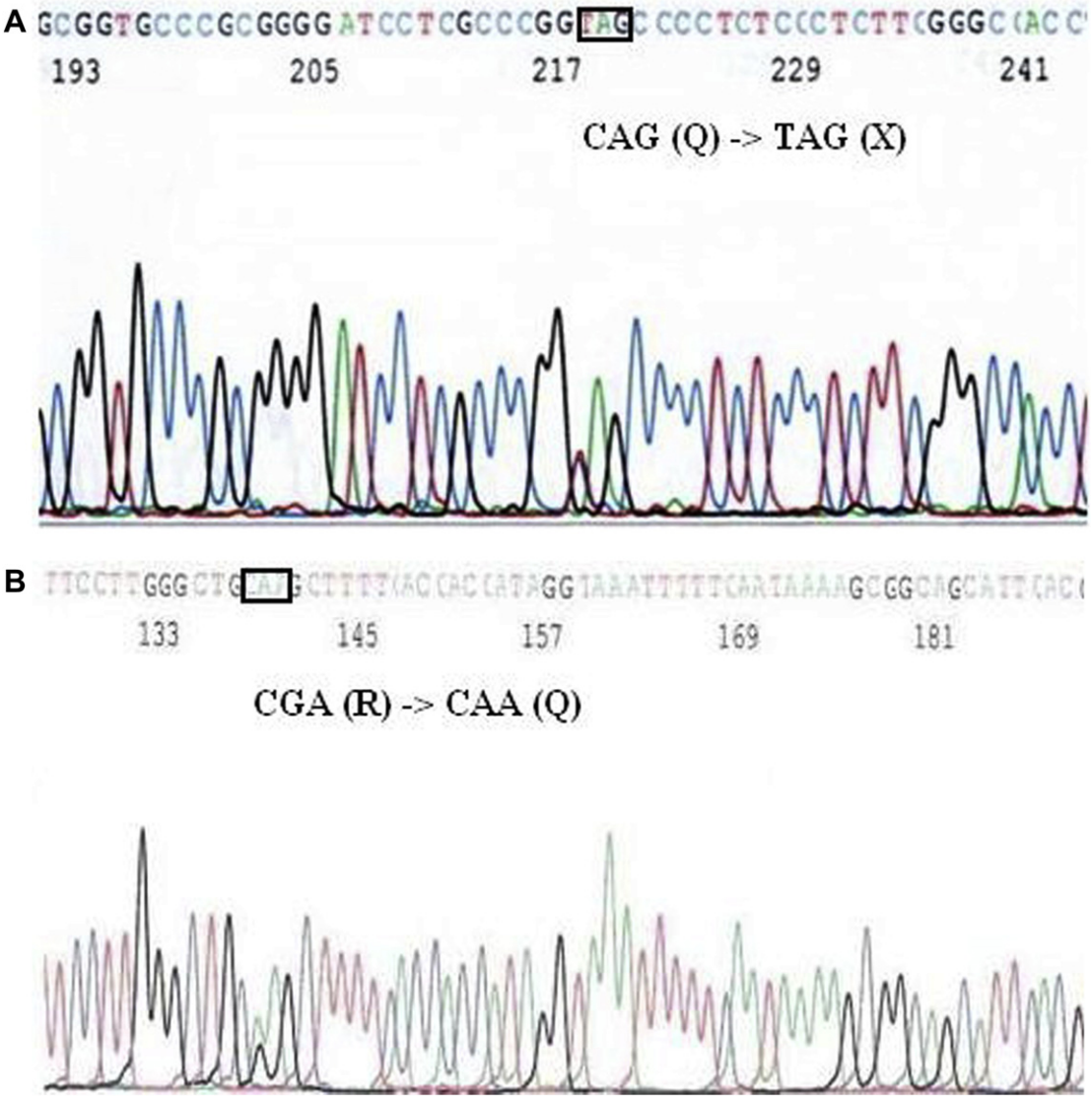

Figure 2. (A) Automated sequencing of the *SRD5A2* gene revealed one novel C to T transition resulting in a nonsense mutation at codon 71 (Q71X); and (B) one known G to A transition resulting in a missense mutation at codon 227 (R227Q).

PCR buffer were contained in a total volume of 50 μL. The amplification conditions were denaturation at 95°C for 5 minutes, followed by 35 cycles of amplification with denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds and elongation at 72°C for 40 seconds, and finally an extension at 72°C for 10 minutes. The PCR products were subsequently examined on 2% agarose and subjected to direct automated sequencing using the ABI 310 Genetic Analyzer (Applied Biosystems). The mutation analysis of the *SRD5A2* gene revealed a compound heterozygous mutation: one novel C to T transition changing glutamine to a stop codon at codon 71 (p.Q71X) in exon 1 and one known G to A transition changing arginine to glutamine at codon 227 (p.R227Q) in exon 4. (Fig. 2). No mutation was identified in the AR gene.

Discussion

Around the world, more than 50 mutations have been reported in the literature, but little was known about the molecular characterization of *SRD5A2* gene among Taiwanese patients receiving the diagnosis on the basis of biochemical results. Our patient was a compound heterozygote of one novel (p.Q71X) and one known (p.R227Q) mutations. The p.Q71X mutation has not been described yet in the literature. Harbored in exon 1 *SRD5A2* gene, the shift of glutamine to a premature stop codon is presumed to give rise to a drastically truncated mutant protein. Conceivably, the activity of 5α-reductase type 2 enzyme is abolished and results in undervirilization. Nonetheless, compound heterozygosity with a missense mutation might reduce the severity by producing a partially functional enzyme. The p.R227Q substitution has been previously reported in two brothers of Vietnamese origin [8]. The elder brother presented with scrotal hypospadia, a bifid scrotum with bilateral descended testes, and a small penis, while his younger brother merely had a small penis. Both of them were homozygotes of p.R227Q/p.R227Q mutation. Compound hetreozygotes of p.R227Q mutation have also been reported in patients of Chinese and Japanese origin [18—20]. These individuals presented diversely, with a pseudovaginal perineoscrotal hypospadia to simply a micropenis. The affected exon 4 of *SRD5A2* gene, where this mutation locates, may be responsible for the decreased affinity of this enzyme binding with nicotinamide adenine dinucleotide phosphate hydrogen and causes enzyme dysfunction [1,10]. *In vitro* reduction of the enzyme activity has been proven in some patients carrying mutation from arginine to glutamine [21]. The observation of recurrent mutations in different ethnic groups may reflect a mutational hot spot in the *SRD5A2* gene or a common founder gene from East Asian ancestry.

Although the degree of virilization was enormously variable in reported cases of *SRD5A2* mutations, our patient presented with a typical clitoris-like microphallus with bilateral palpable gonads in the bifid scrotum/labia majora and a pseudovagina. These symptoms of aberrant sex development are similar to partial androgen insensitivity and 17β-hydroxysteroid dehydrogenase deficiency. Under such circumstances, elevated testosterone/dihydrotestosterone ratio after hCG stimulation for 3 consecutive days was traditionally diagnostic of a 5α-reductase type 2 deficiency. Molecular diagnosis came recently into clinical practice with an emphasis on timely genetics and inheritance information. Our experience demonstrated that the detection of *SRD5A2* mutations provided a helpful alternative approach in patients with suggestive clinical presentations and biochemical values.

Rearing a child with intersex generally brings pressures to the parents in a Taiwanese cultural context. Previous reports have also shown the transition of masculine behavior and male psychosexual orientation in some patients with 5α-reductase type 2 deficiency assigned as females since their infancy [22]. It is postulated that prenatal androgen exposure may exert its masculinizing influence on the developmental brain with regard to sexual differentiation [23]. After a definite diagnosis, genetic counseling and discussion of gender assignment were promptly given to his parents. This patient was decided to be raised as a male under his parents' informed consent, taking into account the phenotypic features and molecular cause, before administration of testosterone injections. The importance of a timely and correct diagnosis as well as a full discussion of the bio-psycho-social factors with regard to the decision of gender assignment in patients with *SRD5A2* mutation is therefore highly advocated.

Conclusion

We report one known and one novel mutation of *SRD5A2* gene in a Taiwanese 46, XY newborn with ambiguous external genitalia. Our data delineated the genotype-phenotype relation of the *SRD5A2* gene mutation and underlined the importance of a genetic diagnosis and counseling with regard to gender assignment. More than 50 mutations have been identified among the wide-ranging coding regions of the *SRD5A2* gene. However, the genotype-phenotype correlation is not well described yet. Future investigation on a molecular basis may provide new insight and approaches to 5α-reductase type 2 deficiency. The molecular characterization of the *SRD5A2* gene among Taiwanese patients will also clarify its epidemiologic distribution and frequency.

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