A Novel *NR5A1* Mutation in a Thai Boy with 46, XY DSD

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Abstract

Disorders of sex development (DSD) can be classified as 46,XX DSD, 46,XY DSD, and sex chromosome DSD. Several underlying causes including associated genes have been reported. Steroidogenic factor-1 is encoded by the *NR5A1* gene, a crucial regulator of steroidogenesis in the growth of the adrenal and gonadal tissues. It has been discovered to be responsible for 10 to 20% of 46, XY DSD cases. Here, we described a 2-month-old infant who had ambiguous genitalia and 46, XY. Using whole exome sequencing followed by polymerase chain reaction–Sanger sequencing, a novel heterozygous nonsense c.1249C > T (p.Gln417Ter) variant in the *NR5A1* gene was identified. It is present in his mother but absent in his father and maternal aunt and uncle. At the age of 7 months, the patient received a monthly intramuscular injection of low-dose testosterone for 3 months in a row. His penile length and diameter increased from 1.8 to 3 cm and from 0.8 to 1.3 cm, respectively. The patient also had normal adrenal reserve function by adrenocorticotropic hormone stimulation test. This study identified a novel causative p.Q417X (c.1249C > T) variant in *NR5A1* causing 46,XY DSD in a Thai boy which is inherited from his unaffected mother.

Keywords
- disorders of sex development
- NR5A1
- novel
- mutation

Introduction

Disorders of sex development (DSD) or differences of sexual development is a group of condition in which development of chromosomal, gonadal, or anatomical sex is atypical.[1]

This condition is an umbrella term that covers a variety of anatomical phenotypes, hormonal phenotypes, gonadal phenotypes, and chromosome complements.[2] Perceptions, approach, and care of individuals with DSD have been updated by Lee et al.[3] DSD can be classified as 46, XX DSD; 46, XY DSD; and sex chromosome DSD. The relationship between genetic factors and DSD has been extensively studied and updated.[2,4,5] However, in ~50% of 46, XY DSD children, the precise cause could be determined.[6–8]

Steroidogenic factor-1 (SF-1) is a crucial regulator of steroidogenesis in the growth of the adrenal and gonadal tissues and is encoded by the *NR5A1* gene. In males, NR5A1 also controls the expression of the steroidogenic enzymes StAR, CYP11A1, and CYP17A1 necessary for testosterone biosynthesis and the expression of insulin-like polypeptide

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3 (INSL3) required for testicular descent.[9,10] Individuals with 46, XY complete gonadal dysgenesis caused by heterozygous mutations in the *NR5A1* gene have also been reported to have adrenal insufficiency.[11,12] 46,XY DSD patients with *NR5A1* mutations may have either normal or low serum anti-Müllerian hormone (AMH) levels. Consequently, Müllerian structures may be seen in individuals with low serum AMH levels.[13,14] The genotype–phenotype correlations in reported cases of *NR5A1* mutations have not yet been established and need to be clarified. Here, we describe a case of underdeveloped genitalia in 46, XY with a novel *NR5A1* mutation in a Thai family.

Case Presentation

A 2-month-old infant was referred to King Chulalongkorn Memorial Hospital because of ambiguous genitalia. He was born at term from unrelated healthy parents with a birth weight of 4,565 g. He had both palpable gonads in labioscrotal folds with the presence of a urogenital sinus at the perineum upon physical examination. His phallus length and width were 1.5 and 0.5 cm, respectively. The external masculinization score was 3.[15]

A chromosome analysis revealed 46, XY. At the age of 2 months, hormonal testing was done, and the results showed that the serum AMH level was 12 ng/mL (40–174) and the basal serum testosterone level was 1.85 ng/mL (0.14–3.63). The intravenous 125 g adrenocorticotropic hormone stimulation test demonstrated a normal increase in serum cortisol levels from 1.76 to 32.4 µg/dL. The uterus was not demonstrated by pelvic ultrasonography. To rule out androgen insensitivity syndrome and 5α-reductase type 2 deficiency, respectively, *AR* and *SRD5A2* mutation analysis was performed. A low-dose testosterone intramuscular injection (Testoviron Depot 15 mg) was administered every month for 3 months starting at the age of 7 months. His penile length and diameter increased from 1.8 to 3 cm and from 0.8 to 1.3 cm, respectively. This suggests a positive response to the testosterone administered. At present, his parents decided to rear him as a boy. This child and his parents have received care from a multidisciplinary team that includes pediatric endocrinologists, geneticists, pediatric surgeons, and child psychiatrists.

Laboratory Investigations

Three milliliters of peripheral blood were drawn from the patient, his parents, and any other available relatives after they gave their informed consent. Using the Puregene blood kit (Qiagen, Hilden, Germany), DNA was extracted from peripheral blood leukocytes. Trio-exome sequencing was performed by Macrogen, Inc. (Seoul, Korea) as previously described.[16] Polymerase chain reaction (PCR)–Sanger sequencing of the 478-bp PCR product with primers (5′-ATGCCCATGTCTTTGATGGT-3′ and 5′-CTCGGTGGGCATCA-GAAA-3′) specific to exon 7 of the *NR5A1* gene (NM_004959.4) was subsequently performed to confirm the presence of the identified c.1249C > T variant in the patient, his parents, maternal uncle, and aunt.

A novel heterozygous nonsense c.1249C > T (p.Gln417Ter) variant (ENST00000373588.9: NM_004959.5:c.1249C > T [*https://varsome.com/gene/hg38/nr5a1*]) in exon 7 of *NR5A1* was identified in the patient by whole exome sequencing analysis. The NR5A1:c.1249C > T variant is located in the GRCh37/hg19 chr9:127245174 G > A genomic position (*https://gnomad.broadinstitute.org/gene/ENSG00000136931?dataset=gnomad_r2_1*). The c.1249C > T (p.Gln417Ter) variant showed (ref/alt; 54/46) in the patient and (ref/alt; 68/27) in his mother. In addition, no consanguinity between both parents was calculated by PI_HAT.

PCR–Sanger sequencing confirmed the presence of this mutation in the patient and mother. However, it was absent in the father, the maternal uncle, and the maternal aunt (►**Fig. 1**).

Discussion

In this study, we report on a child with ambiguous genitalia and preserved adrenal function. Genetic analysis revealed a heterozygous c.1249C > T (p.Gln417Ter) variant inherited from his mother. This causative variant has never been previously described.

The *NR5A1* gene, located on chromosome 9q33, has one untranslated exon (exon 1) with six coding exons (exons 2–7). SF-1, 461 amino acid residues, has two zinc finger DNA-binding domains, a ligand-binding domain (LBD), and two functional activation domains.[17–19] In 46, XY individuals, SF-1 induces AMH expression in Sertoli cells, which leads to the regression of Müllerian structures and induces the production of steroidogenic enzymes in Leydig cells, causing the virilization of genitalia and testes descending.[20–23] However, it promotes follicle development and maturation in 46, XX individuals.[24,25] According to some studies, mutations in *NR5A1* account for approximately 10 to 20% of 46, XY DSD cases.[26,27] Previous studies had shown phenotypic variations without any genotype–phenotype correlations.[28–30] A study of 30 Chinese children with *NR5A1* mutations also demonstrated a wide range of external genitalia and identified p.R87C and p.R313C as the most common mutations in this cohort. In addition, exon 4 is the most frequently affected exon in 40% of the patients.[27] They found de novo mutations in 80% of their patients and inherited mutations from either their mothers or fathers in the remaining 20%. According to a study by Tantawy et al, Sertoli cells in people with *NR5A1* mutations are more severely impaired than Leydig cells are, and their function declines over time.[26] Therefore, in 46, XY DSD and 46, XX DSD patients, respectively, oligozoospermia or azoospermia and premature ovarian failure may be seen.[26,31] This may be the cause of the low luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio seen in people with *NR5A1* mutations. Unfortunately, we did not measure LH or FSH to support this hypothesis in our patient. A recent analysis of 188 *NR5A1* mutations from 238 cases reported in the literature showed a wide range of different phenotypes without mutation hotspots. The phenotype variation caused by the same mutation can be used to infer the influence of additional genetic modifiers. In 17% of

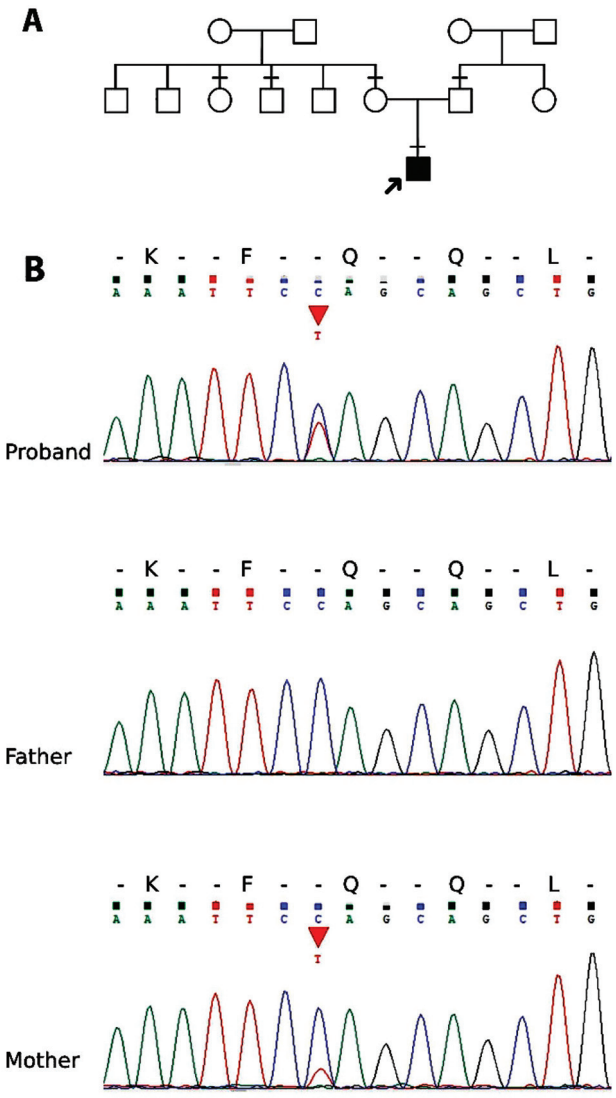

Fig. 1 The pedigree and sequencing chromatograms. (**A**) The pedigree of the patient and his family. The horizontal line mark (-) in the pedigree represented the available blood samples in this study. (**B**) The chromatograms showing the c.1249C > T (p.Gln417Ter) variant in the proband and his mother but not in his father.

46,XY cases, the uterus was found. In 25% of 46,XX, the absence of Müllerian derivative was reported. Adrenal insufficiency is undoubtedly uncommon.[32] Previous studies showed that even within the same family, the *NR5A1* p.R92W variant causes different levels of masculinization in 46,XX DSD.[33,34] They postulated that the key molecules in the female development pathway would be antagonized by the NR5A1 p.R92W variant, resulting in a decreased inhibition of the male development pathway.[33,35]

In this study, the heterozygous c.1249C > T (p.Gln417Ter) variant was identified in the patient and his mother. This variant is located next to the c.1250delA (p.Gln417Argfs*13) that was previously described by Song et al.[27] Both were in the LBD of SF-1. The c.1249C > T (p.Gln417Ter) variant in *NR5A1* is predicted to cause 45 amino acid residues missing from the mature protein starting from residue 417. MutationTaster2 predicted it to be deleterious. The VarSite predicted this nonsense variant to be fatal. ESEFinder and MMSplice showed no potential splice sites for 25 nucleotides flanking this nucleotide change.

The mother of our patient is 44 years old, has regular periods, and has no clinical evidence of ovarian failure. A previous study revealed that two from five mothers (40%) with heterozygous *NR5A1* mutations experienced premature ovarian failure, but the rest (60%) had fertility preserved.[27] However, time of follow-up is required.

Adrenal insufficiency is uncommon condition found in individuals with NR5A1 mutations.[36] According to a previous study, 10 of the 175 people whose adrenal function was assessed had some degree of adrenal dysfunction.[32] In our patient, a normal adrenal reserve function and normal testosterone level were demonstrated. When testosterone levels were measured in 46, XY DSD with *NR5A1* variants, the results were inconsistent.[37,38] Our patient's low AMH level raises the possibility that Sertoli cell function is compromised. However, the 46, XY DSD with the *NR5A1* variant cannot be ruled out by normal testosterone and AMH levels.[37]

46, XY boys with *NR5A1* mutations can have spontaneous puberty and preserve their fertility.[31,39] Our patient responded to exogenous testosterone, suggesting the possibility to achieve the male external genitalia when growing up.

Conclusion

We report a novel p.Q417X (c.1249C > T) mutation in *NR5A1* causing 46, XY DSD with normal adrenal function in a Thai boy that is inherited from his unaffected mother.

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Conflict of Interest
None declared.

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