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Novel Missense Mutation in the P-Box of Androgen Receptor in a Patient with Androgen Insensitivity Syndrome

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Abstract. Mutations in the X-linked *AR* gene cause androgen insensitivity syndrome (AIS) by impairing androgen-dependent male sex differentiation to various degree. Here we describe a partial AIS patient with confliction with the assigned female sex. Although the patient was noticed to have ambiguous genitalia at birth, the patient was reared as a female with no medical intervention. At the age of 31 years, the patient visited us because the patient was dissatisfied with the assigned female sex. The patient was treated with systemic testosterone and topical dihydrotestosterone, but the external genitalia responded only minimally to the treatment. The genetic analysis revealed a novel missense K580R mutation in the P-box of the DNA-binding domain of androgen receptor, which was the first missense mutation shared by AIS and prostate cancer. Although the best predictor of the adult gender identity is documented to be the initial gender assignment in patients with partial AIS as well as those with complete AIS, deciding gender assignment for infants with partial AIS is still challenging.

Key words: Androgen insensitivity syndrome, Androgen receptor, Gene, Mutation, Gender identity
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ANDROGEN insensitivity syndrome (AIS) is an X-linked disorder caused by mutations in the *AR* gene. This leads to deficient masculinization in 46,XY individuals despite normal or increased androgen production by the testes. There is a wide variation in the degree of undermasculinization, ranging from infertility in an otherwise normal male, to ambiguous genitalia (partial AIS), to a complete female external phenotype (complete AIS) [1].

Androgen receptor (AR) is a member of the steroid receptor family, which in turn belongs to a large nuclear receptor superfamily [2, 3]. AR is encoded by the *AR* gene of 8 exons, located at Xq11-12. AR consists of four main domains: an N-terminal, constitutive activation domain, a DNA-binding domain, a hinge region containing the nuclear targeting signal, and a C-terminal ligand-binding domain. AR binds androgens, and the ligand-receptor complex interacts directly with its target genes and regulates their transcription to mediate the effects of androgens [1].

To date, more than 300 mutations in the *AR* gene have been identified in patients with AIS, and the majority of them have been detected in the ligand-binding domain of AR (http://www.mcgill.ca/androgendb/) [4]. In the present study we describe a novel missense mutation in the DNA-binding domain of AR in a patient with partial AIS who had confliction with the assigned female sex.

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Patient and Methods

Patient

The patient was the first daughter of unrelated

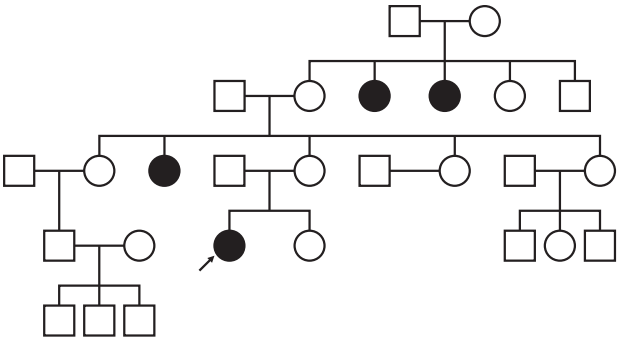

Fig. 1. Family pedigree. The arrow indicates an index case. Black circles indicate individuals with primary amenorrhea.

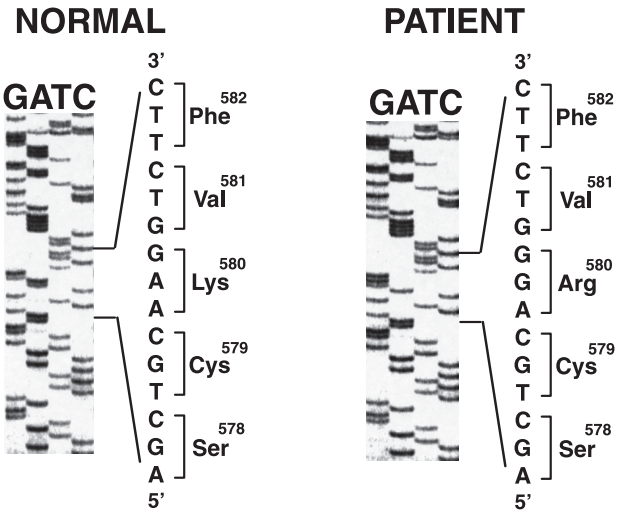

Fig. 2. Direct sequencing of exon 2 of the *AR* gene. The patient was hemizygous for a K580R mutation, converting codon 580 (AAG) encoding Lys to AGG encoding Arg.

healthy Japanese parents. The family tree is given in Fig. 1. Allegedly, one maternal aunt and two maternal grandaunts of the patient had primary amenorrhea, suggesting they suffered from AIS, but no further detailed information was available. The patient was born in 1953 after a term uncomplicated pregnancy and delivery. Her birth weight was 3.0 kg. Although atypical genitalia were noticed at birth, the patient was reared as a female with no medical intervention. At 3 years of age, she was seen at another hospital because of ambiguous genitalia, and was diagnosed as hermaphroditism. Subsequently, the patient and her parents did not ask for a medical examination or intervention until adulthood.

At 31 years of age, the patient came to our hospital because of dissatisfaction with the assigned female sex; the patient wanted to be masculinized. Although the patient socially lived as a female, she had not experienced menarche, and had a female sex partner. On examination, her height was 170 cm. Breast development was Tanner stage 1. Pubic hair was absent, so was axillary hair. The patient had ambiguous external genitalia characterized by an enlarged clitoris (5.0 cm), a single perineal opening, and bilateral inguinal masses. The karyotype was 46,XY. Genitourography failed to visualize a vagina. At 32 years of age, the gonads were removed to eliminate the risk of gonadal malignancy at another hospital. Histopathological examination revealed hypoplastic testes (right 5.8 × 2.5 × 1 cm; left 4.5 × 4.5 × 0.7 cm) accompanied by epididymides. Since the patient refused to be feminized by taking estrogen and wanted to be masculinized, the patient was treated with testosterone enanthate (125 mg, im, every 2 weeks) without reconstructive surgery after gonadectomy. The clitoral length increased to 6.5 cm after 6 months of treatment, so the dose was doubled to 250 mg, im, every 2 weeks, during the following 6 months without further improvement in clitoral length or pubic hair growth. Because the patient was unsatisfied, the therapy was changed into topical testosterone treatment for 2 years, and then topical dihydrotestosterone treatment in addition to intramuscular testosterone injection (250 mg, every 2 weeks) for another 10 years, but the patient failed to show further increment in clitoral length or pubic hair growth. The patient is now in her fifties, and she is still on intramuscular testosterone treatment.

Based on these clinical findings, the patient was diagnosed as having partial AIS, although hormonal examinations were not performed.

Analysis of the AR gene

Only the patient was studied genetically in the family. The genetic study was reviewed and approved by the Institutional Review Board at the National Center for Child Health and Development. Genomic DNA was extracted from peripheral white blood cells by proteinase K digestion and phenol/chloroform extraction after informed consent for genetic analyses was obtained from the patient. Exons of the *AR* gene were amplified as reported previously [5], and directly sequenced.

The K580R mutation, creating a new recognition site for a restriction enzyme *Pst* I, was confirmed by digestion of PCR products with the restriction enzyme *Pst* I (New England Biolabs, Inc., Beverly, MA, USA), followed by electrophoresis on a 2% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME, USA).

Results

In order to elucidate the molecular basis of undermasculinization of the patient, we analyzed the *AR* gene in the patient. As shown in Fig. 2, a hemizygous K580R mutation, changing codon 580 (AAG) encoding Lys to AGG encoding Arg, was identified in exon 2 of the *AR* gene in the patient. The CAG repeat number in exon 1 was 27, which was within normal range [6, 7].

Since the K580R mutation creates a new recognition site for a restriction enzyme *Pst* I (CTGCAG; the mutated nucleotide is underlined), the PCR product from the patient was digested with the enzyme, followed by electrophoresis and the patient was confirmed to be hemizygous for the K580R mutation (data not shown). We analyzed 50 normal males with the same method, and none was found to have the K580R mutation.

Discussion

Although we were unable to perform endocrinological examinations in our patient, it was highly likely that the patient suffered from partial AIS due to defective AR because of the following reasons. First of all, the family history suggested X-linked transmission of the disease. Secondly, the patient exhibited highly undermasculinized features in the presence of testes, although some signs of masculinization such as clitromegaly, a single perineal opening, and the development of epididymides were observed. Thirdly, the patient responded only minimally to systemic and topical testosterone administration. Finally, the patient showed no responses to topical dihydrotestosterone treatment. Thus, our patient had severe androgen resistance, and 5α-reductase 2 deficiency was very unlikely as a cause of undermasculinization of the patient. Therefore, we analyzed the *AR* gene, and found the K580R mutation.

The K580R mutation has not previously been described in patients with AIS (http://www.mcgill.ca/androgendb/) [4]. The K580R mutation resides in the P-box of the DNA-binding domain of AR. The DNA-binding domain of AR, like that of other members of the steroid receptor family, comprises a pair of zinc-binding motifs, commonly referred to as zinc fingers, and is essential for receptor-DNA interaction. The first zinc finger determines the recognition specificity of the hormone responsive element (HRE), whereas the second finger stabilizes receptor-DNA interaction by contact with the DNA phosphate backbone and mediates receptor dimerization [2]. The five amino acids, termed 'P-box' in the base of the first zinc finger are especially crucial for cognition of the HRE sequence [2, 8]. The lysine residue at position 580, adjacent to an invariable cysteine residue in the P box is highly conserved among the members of the steroid receptor family [2, 3]. Thus, the substitution of the lysine residue with the arginine residue is expected to impair the AR function, although it is a conservative substitution.

Indeed, the K580R mutation was identified in a lymph node metastasis of a prostate cancer [9], and the mutant AR was expressed and functionally characterized in yeast [10]. Surprisingly, in the yeast system, the K580R mutant AR exhibited weak constitutive activity, and was transcriptionally activated not only by androgens such as dihydrotestosterone and dehydroepiandrosterone, but also by estradiol, progesterone, hydrocortisone, flutamide and bicalutamide. Recently, the K580R mutant AR was stably expressed in the SV-40-immortalized human prostate epithelial cell line pRNS-1-1, which does not express either AR or prostate-specific antigen (PSA), and was further characterized [11]. The luciferase assay with the PSA promoter (–630/+12) demonstrated that treatment with a synthetic androgen R1881 resulted in a 15-fold increase of luciferase activity in the pRNS-1-1 cells expressing the wild-type AR compared to the untreated cells, and a 5.5-fold increase in the cells expressing the K580R mutant, which exhibited the constitutive activity. The growth of the pRNS-1-1 cells expressing the wild-type AR was markedly inhibited in the presence of R1881, while those expressing the K580R mutant exhibited dose-dependently enhanced growth in response to the R1881 treatment. The pRNS-1-1 cells expressing the K580R mutant AR were able to form tumors in some of the xenografted male nude mice, whereas those expressing the wild-type AR did not form tumors in any of the mice. The microarray profiling followed by the RT-PCR analysis revealed that at least 29 genes were differentially expressed in the RNS-1-1 cells express-

ing the K580R mutant AR compared with those expressing the wild-type AR. Taken together, the K580R mutant AR regulates the gene expressions quite differently from the wild-type AR, thus the K580R mutant is speculated to behave not only as a loss-of-function mutation in the process of male sex differentiation, but also as a gain-of-function mutation in that of tumorigenesis.

The best predictor of the adult gender identity is documented to be the initial gender assignment in patients with partial AIS as well as those with complete AIS [12]. Thus, it is noteworthy that our patient failed to accept the assigned female sex. This might have been caused by the residual activity of the K580R mutant AR, which could have masculinized the developing central nervous system (CNS) of the patient during fetal life. It is also possible that the sustained exposure to testosterone in the presence of the residual mutant AR activity could have had some effect on the CNS involved in the development of gender identity, because the patient underwent gonadectomy in her thirties.

In conclusion, we have identified a novel missense mutation K580R, which is the first mutation shared by AIS and prostate cancer, in the P box of the DNA-binding domain of AR in a patient with partial AIS, who was dissatisfied with the assigned female sex, and deciding gender assignment for infants with partial AIS is still challenging.

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