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LETTER TO THE EDITOR

Male Reproduction

A missense mutation in the androgen receptor gene causing androgen insensitivity syndrome in a Chinese family

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Dear Editor,

Androgen Insensitivity Syndrome (AIS) is an X-linked recessive genetic disorder presenting as male pseudohermaphroditism with gender ambiguity or reversal.¹ According to the reaction to androgens, subjects with AIS can exhibit varying degrees of feminization, mainly in two forms. One is complete androgen insensitivity syndrome (CAIS), in which all patients are phenotypically female but with the karyotype 46, XY; the other is partial androgen insensitivity syndrome (PAIS) with a few male characteristics.² As a result, they often come to hospital with primary amenorrhea or infertility. The pathogenesis of AIS is unclear, so it is crucial to explore its mechanism. *AR* (GenBank accession no. NC_000023.11), located on chromosome Xq11–12, contains a 2763-bp coding sequence divided into eight exons separated by introns varying in size up to 90 kb, and codes for an 110 kD protein composed of 919 amino acids.^{3,4} Highly expressed in the epididymis (which is dependent on it for structure and function), the *AR* plays an important role in binding to androgen in the cytosol to exert its normal effect. *AR* mutations may interfere with the binding of androgen to the *AR*, leading to loss-of-function or gain-of-function alterations.⁵ Because of the very complex pathogenesis of *AR* mutations AIS, different mutations may lead to different clinical phenotypes. In this research, we focused on an *AR* mutation and described the gene mutation associated with the genotype-phenotype of a Chinese family with AIS.

The proband had female characteristics with normal development of the external female genitalia. She came to hospital with the bilateral inguinal masses. Ultrasonography and pathology showed that the essence of masses is testicular tissue, and no uterus or accessories were found. However, the levels of androgens were consisted with a male phenotype. She had a normal male karyotype of 46, XY without apparent numerical or structural chromosomal

abnormalities. Therefore, the proband was confirmed to have AIS by clinical presentations, biochemistry, ultrasonography, and pathology. Genomic DNA was extracted from peripheral blood leukocytes using standard methods. All eight exons of *AR* were amplified by polymerase chain reaction (PCR) using appropriate primers designed by Primer Premier and Oligo (Premier Biosoft Co. Ltd., Palo Alto, California, USA). Amplified PCR products were purified and sequenced using the appropriate PCR primers and run on an ABI3500 automated sequencer (Applied Biosystems, Carlsbad, California, USA) to perform the mutational analysis. The study protocol was approved by the Human Ethics Committee of the Linyi People's Hospital and The Affiliated Hospital of Qingdao University and informed written consent was obtained from every participant.

Sequencing of the entire coding region of the proband's *AR* revealed a homozygous missense mutation 2599G>A (mRNA sequence reference; **Figure 1a**) that was predicted to result in a valine-to-methionine substitution at codon 867 in exon 7 (c. 2599G>A; p.V867M). DNA sequencing was carried out on the proband's mother and sister, showing a heterozygous mutation at the same site (**Figure 1b and 1c**). Analysis of 200 normal chromosomes did not identify the same change in healthy control subjects. We obtained the *AR* sequence from the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>) and used DNAMAN software (Lynnon Biosoft, Quebec, Canada) to obtain multiple sequence alignments in various species, including humans (*Homo sapiens*), *Bos taurus*, *Danio rerio*, *Mus musculus*, *Oryctolagus cuniculus*, *Rattus norvegicus*, and *Sus scrofa*. We found that codon 867, where the compound homozygous mutation occurred in this family, was located in a highly conserved region of the *AR*.

AIS is a type of male pseudohermaphroditism and its pathogenesis is associated with functional defects in the *AR*. Mutation in *AR* will result in the inadequate response to the androgen and then lead to disorders in sexual development. According to the *AR* mutation database (<http://androgendb.mcgill.ca/>), at least, 800 different mutations have been found in most exons in the human *AR* throughout its coding region.⁶ It is crucial to analyze these mutations, which can lead to the dysfunction and then result in the changes in clinical phenotypes that can clarify the pathogenesis and be helpful for clinical treatment and in designing therapies for AIS.⁷ Here, we identified a homozygous mutation in the *AR* of the proband and a heterozygous mutation in her mother and sister at the same site in exon 7 (p.V867M). This encodes

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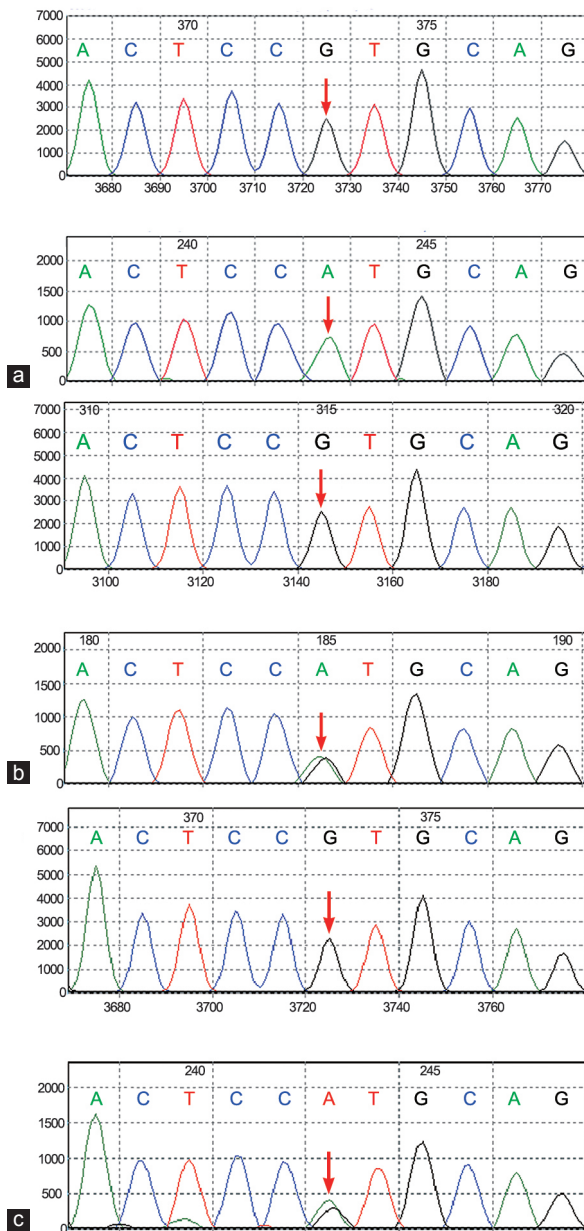


Figure 1: Partial sequence of the *AR* from affected individuals. (a) Arrowhead indicates the homozygous G and A at nucleotide 2599 in the proband; (b and c) Arrowhead indicates the heterozygous G and A at nucleotide 2599 in the proband's mother and sister.

the carboxy-1 ligand-binding domain (LBD) that can determine the specificity of hormone binding.⁸ Because the AR is activated by binding with androgens, which is referred to ligands-dependent activation, mutations in the LBD may lead to decreased binding specificity of the AR to androgens, and impair its activity.

According to the *AR* mutation database, this is a known mutation which has been proved to be associated with AIS. Different mutations in the *AR* can lead to different clinical phenotypes. Saunders *et al.*⁹ have found this particular mutation to be associated with PAIS while Kazemi-Esfarjani *et al.*¹⁰ have found that it was associated with CAIS, consistent with our result. According to the co-segregation analysis, the proband's mother and sister carried heterozygous *AR* mutations,

indicating that the proband was likely to be derived from the mother because AIS was transmitted in an X-linked recessive fashion. In addition, analysis of 200 control subjects did not identify the same mutation. Therefore, we think it must be a pathogenic mutation for the proband. However, the definite conclusion needs to be validated in the future study of the functional experiment.

In conclusion, we describe a case of AIS in a Chinese family caused by a homozygous mutation of the *AR*. To our knowledge, this is the first report of this missense mutation (p.V867M) in the Chinese Han population. Our study will be helpful in understanding the molecular genetics of AIS. Furthermore, these findings also provide an important basis for prenatal diagnosis to guide the fertility. At the same time, functional assays on mutations in the *AR* will be helpful for understanding structural-functional relationships in the *AR*.

AUTHOR CONTRIBUTIONS

SGL and FYC conceived the study, participated in its design and guided drafting of the manuscript. LL and WML participated in statistical analysis and drafted the manuscript. MXL, JXZ, and SQZ were responsible for the clinical diagnosis and clinical phenotype analysis. All authors have read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing financial interests.

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