Asian Journal of Andrology (2018) 20, 308–310
www.asiaandro.com; www.ajandrology.com

Open Access

LETTER TO THE EDITOR

Male Reproduction

# A rare polypyrimidine tract mutation in the androgen receptor gene results in complete androgen insensitivity syndrome

**Shi-Min Yuan[1,2,*], Huan Huang[3,*], Chao-Feng Tu[1], Juan Du[1,2], Da-Bao Xu[3], Ge Lin[1,2], Guang-Xiu Lu[1,2], Yue-Qiu Tan[1,2]**

*Asian Journal of Andrology* (2018) **20**, 308–310; doi: 10.4103/aja.aja_32_17; published online: 29 August 2017

Dear Editor,

Androgen insensitivity syndrome (AIS) is a common 46, XY disorder of sex development resulting from androgen resistance. AIS can be subdivided into three phenotypes according to the degree of external genital defects: complete AIS (CAIS), with typical female external genitalia; partial AIS, with predominantly male or ambiguous external genitalia; and mild AIS, with typical male external genitalia. CAIS is the classical manifestation of AIS. Individuals affected by CAIS typically exhibit inguinal swellings during infancy or primary amenorrhea during puberty.[1] AIS is usually caused by mutations in the androgen receptor (*AR*) gene.

The *AR* gene is located on chromosome Xq11-12 and encodes a 920-amino acid residue protein. This protein functions as a steroid-hormone-activated transcription factor and contains three major functional domains: the N-terminal domain, the DNA-binding domain, and the ligand-binding domain (LBD), which is involved in binding to androgens and relevant coactivator proteins.[2] Upon binding the hormone ligand, the androgen receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and subsequently stimulates transcription of androgen-responsive genes. To date, over 1000 AIS-causing variants in the *AR* gene have been identified.[3] Among these variants, missense mutations are the most common anomalies, while splicing mutations are rare. Here, we identified a novel mutation (c.2450-6C>G; RefSeqNM_000044.4) in the polypyrimidine tract of the *AR* gene in a CAIS patient, which led to an aberrant splicing product. This study was approved by the Institutional Ethics Committee of the Reproductive and Genetic Hospital of Citic-Xiangya, and written informed consent was obtained from the patient's parents.

The affected individual is a 17-year-old girl with primary amenorrhea and a 46, XY karyotype. There were no individuals with similar abnormalities in the patient's family (**Figure 1a**). Physical examination revealed normal female external genitalia, well-developed breasts without papilla, a short and blind-ending 7-cm vagina, and no pubic hair. Additionally, gynecological ultrasonography revealed the absence of ovaries and uterus and the presence of a dense elongated structure measuring 23 mm × 12 mm in the right groin, regarded as the ovariotestis. Moreover, an elongated cystic structure measuring 22 mm × 16 mm was observed in the left pelvic cavity. Serum hormone measurements revealed testosterone 3.86 ng $ml^{-1}$ (normal range [NR]: 1.42–9.23), follicle-stimulating hormone 2.08 mIU $ml^{-1}$ (NR: 0.95–11.95), estradiol 23.17 pmol $L^{-1}$ (NR: 11–44), and a high luteinizing hormone level 17.89 mIU $ml^{-1}$ (NR: 0.57–12.07). Histological analysis of the patient's gonadal tissues, obtained by gonadectomy, revealed prepubertal tubules and Sertoli cells within the left gonad and large areas of fibrosis occupying the right gonad. No germ cells were observed in either gonad (**Figure 1b**).

Sequence analysis of the *AR* gene in the patient's genomic DNA revealed a novel polypyrimidine tract mutation: cytosine to guanine, at position c.2450-6 (c.2450-6 C>G) in intron 6 (**Figure 1c**). The patient's mother and sister were heterozygous for this mutation, but the mutation was not present in her father or brother, or 100 unrelated normal controls. To determine the functional consequences of the mutation, the resulting messenger RNA (mRNA) transcript was analyzed. Subsequent sequence analysis of the RT-PCR (reverse transcription polymerase chain reaction) products demonstrated an insertion of 5 nucleotides in the junction between exons 6 and 7 (c.2449-c.2450 insATCAG) (**Figure 1d**). The mutation generated a new splice acceptor site upstream of the original site, resulting in incorrect pre-mRNA splicing (**Figure 1e**). The aberrant splicing transcript resulted in the introduction of a premature stop codon, thus producing a truncated protein (823 amino acids, p.Ile817Asnfs*8) (**Figure 1f**).

In the gene splicing process, the "cis" (conserved sequence) elements, which include donor sites, acceptor sites, the branch point, the polypyrimidine tract, and auxiliary elements, are the key nucleotide sequences for the correct recognition of exons.[4] In mammals, the polypyrimidine tract, which is generally rich in pyrimidines, is located in the region between the branch point and a YAG/sequence (where Y denotes pyrimidine and "/" denotes the splice site), and is essential for lariat formation and the precise joining of exons after intron excision.[5] Mutations at splice donor (5′) and acceptor (3′) sites are the most commonly occurring anomalies, whereas mutations in the polypyrimidine tract are rare.[6]

[1]Institute of Reproduction and Stem Cell Engineering, Central South University, Changsha 410078, China; [2]Reproductive and Genetic Hospital of Citic-Xiangya, Changsha 410078, China; [3]Department of Gynecology, The Third Xiangya Hospital, Central South University, Changsha 410013, China.
*These authors contributed equally to this work.
Correspondence: Dr. YQ Tan (tanyueqiu@csu.edu.cn)
Received: 30 March 2017; Accepted: 26 June 2017

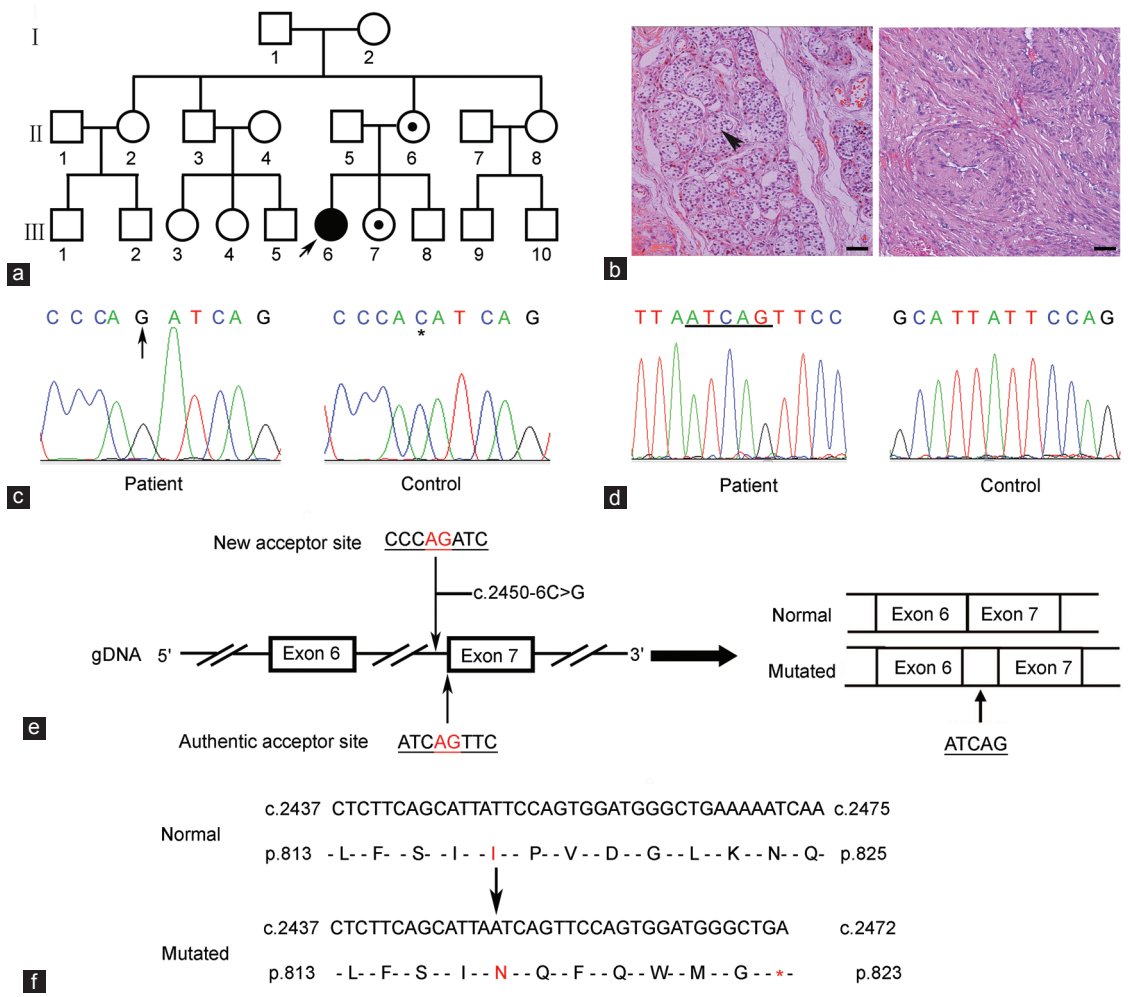


**Figure 1:** Clinical features of the patient with CAIS and identification and functional characterization of the polypyrimidine tract mutation (c.2450-6C>G). (**a**) Three-generation pedigree of the family with the proband (III-6) indicated by an arrow. Black symbols, affected individuals; open symbols, unaffected individuals; black spots, carriers; squares, males; circles, females. (**b**) Histological analysis of the gonadal tissues of the proband, obtained by gonadectomy. Left gonad (left) exhibited prepubertal tubules (arrow), massive Sertoli cells, and no germ cells. Right gonad (right) exhibited large areas of fibrosis and no germ cells. Scale bars: 25 μm both in left and right. (**c**) Genomic DNA sequence analysis of the patient (left) and a normal individual (right). The position of the mutation (c.2450-6C>G) in intron 6 of the *AR* gene is indicated by a arrow, and the normal nucleotide sequence is indicated by an asterisk. (**d**) Sequence of the RT-PCR product, demonstrating a 5-base insertion (ATCAG) at the junction between exons 6 and 7 in the patient (left) compared with control (right). (**e**) Schematic representation of the incorrect splicing and (**f**)the translation of the *AR* exons (numbered boxes). A new splice insertion site (AG) generated by the novel mutation was located upstream of the authentic splice sites, which resulted in a frameshift (I817Nfs*8). Arrow, amino acid substitution; asterisk, the premature termination codon. CAIS: complete androgen insensitivity syndrome; AR: androgen receptor; RT-PCR: reverse transcription polymerase chain reaction.

The polypyrimidine tract mutation (c.2450-6 C>G) in intron 6 of the *AR* gene in the patient formed a new conserved dinucleotide (AG) upstream of the original splice site at the 3′ terminus. Coincidentally, the dinucleotide AG is the precise site of exon ligation, which is recognized by the spliceosome. Previous studies demonstrated that whatever the mechanism of 3′ splice site selection in the bimolecular system, there was a strong preference for use of the 5′-most AG.[7,8] In this study, we verified that the polypyrimidine tract mutation indeed generated a new splice acceptor site upstream of the original site, and this site was preferentially used. This alternative splicing pathway generated an aberrant splicing product. Our report provides the first evidence of this type of mutation in the *AR* gene.

Use of the newly created alternative splice acceptor site results in an aberrant transcript that includes five nucleotides (ATCAG) inserted between exons 6 and 7. This insertion leads to a frameshift with a premature stop codon in exon 7 (p.Ile817AsnfsX8; RefProtNP_000035.2), which could induce a truncated AR protein lacking the last 107 amino acids of the LBD. We did not investigate the function of the mutant protein (p.Ile817AsnfsX8). However, De Bellis *et al.*[9] demonstrated that a mutant AR protein (p.Arg832*) resulting from a nonsense mutation (c.2494C>T) was not capable of binding androgen or activating transcripts. The mutant protein in our case was shorter than that described by De Bellis *et al.*,[9] so might be expected to exhibit the same functional defects. The LBD is essential for AR functions such as AR-LBD homodimerization. Mutations in the LBD could impair the function of the protein by disrupting dimer formation and impairing AR-induced transactivation.[10] Thus, the mutation in the present case may interfere with receptor homodimerization, resulting in CAIS.

In conclusion, a rare and novel polypyrimidine tract mutation in the *AR* gene resulting in a rare abnormal splicing mechanism was

identified in a patient with CAIS. Our study expands the spectrum of *AR* gene mutations and may increase the current understanding of the molecular mechanisms involved in splicing defects and may provide relevant information for genetic and reproductive counseling for families with androgen insensitivity syndrome.

## AUTHOR CONTRIBUTIONS

SMY, HH identified the case, conducted the genetic studies, and drafted the manuscript; CFT, JD, and DBX performed the statistical analysis and drafted the manuscript; GL and GXL participated in the experimental instruction; YQT conceived the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing interests.

## ACKNOWLEDGMENTS

This study was supported by a grant from the National Natural Science Foundation of China (No. 81471432).

## REFERENCES

1 Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, *et al*. Androgen insensitivity syndrome. *Lancet* 2012; 380: 1419–28.
2 Li L, Liu WM, Liu MX, Zheng SQ, Zhang JX, *et al*. A missense mutation in the androgen receptor gene causing androgen insensitivity syndrome in a Chinese family. *Asian J Androl* 2017; 19: 260–1.
3 Infante JB, Alvelos MI, Bastos M, Carrilho F, Lemos MC. Complete androgen insensitivity syndrome caused by a novel splice donor site mutation and activation of a cryptic splice donor site in the androgen receptor gene. *J Steroid Biochem Mol Biol* 2016; 155: 63–6.
4 Anderson K, Moore MJ. Bimolecular exon ligation by the human spliceosome. *Science* 1997; 276: 1712–6.
5 Reed R. The organization of 3' splice-site sequences in mammalian introns. *Genes Dev* 1989; 3: 2113–23.
6 Scotti MM, Swanson MS. RNA mis-splicing in disease. *Nat Rev Genet* 2016; 17: 19–32.
7 Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. *Nature* 2010; 463: 457–63.
8 Chen S, Anderson K, Moore MJ. Evidence for a linear search in bimolecular 3' splice site AG selection. *Proc Natl Acad Sci U S A* 2000; 97: 593–8.
9 De Bellis A, Quigley CA, Cariello NF, el-Awady MK, Sar M, *et al*. Single base mutations in the human androgen receptor gene causing complete androgen insensitivity: rapid detection by a modified denaturing gradient gel electrophoresis technique. *Mol Endocrinol* 1992; 6: 1909–20.
10 Nadal M, Prekovic S, Gallastegui N, Helsen C, Abella M, *et al*. Structure of the homodimeric androgen receptor ligand-binding domain. *Nat Commun* 2017; 8: 14388.