Exonic Splicing Silent Mutations in Patients with Androgen Insensitivity Syndrome

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Introduction

Androgen insensitivity syndrome (AIS) is a common cause of undervirilization in 46,XY patients. There are three forms of clinical presentation: Mild (MAIS) – infertility with normal male genitalia , Partial (PAIS) – variable degrees of undervirilized male genitalia and Complete (CAIS) – typical female external genitalia. This disease is caused by mutations in Androgen Receptor gene (AR). In the AR mutations database there are 1029 AR mutations described).
Silent mutations can occur in non-coding regions (outside of genes or within introns), or they may occur within exons.
Synonymous mutations can affect transcription, splicing, mRNA transport, and translation, any of which could alter phenotype, rendering the synonymous mutation non-silent. To date, some 50 genetic disorders have been linked to silent mutations, many of which also appear to interfere with intron removal.
In AIS, there is only one silent mutation (p.S889S) identified in a patient with PAIS.

Objective

To describe two silent mutations in the exonic region of AR in three families with AIS phenotype, one of them causing the partial form (PAIS) and the other the complete form (CAIS).

Material and Methods

The whole coding region of the AR gene, including exon/intron boundaries, were PCR-amplified and submitted to direct automated sequencing in ABI PRISM 3130XL. AR cDNA was obtained from reverse transcription of mRNA extracted from testicular tissue of one affected patient (Family 1) and amplified by PCR. The size of the fragment amplified was compared with a commercial pool of testes mRNA. The mutations identified were analyzed by prediction tools: Netgene2 and Human Splicing Finder.

Results

We identified two silent mutations in three families with AIS. The first mutation **(p.S889S)** in exon 8 of AR was found in a patient with atypical genitalia raised in male social sex.
The second mutation **(p.S510S)** was found in two families with CAIS. In the first family, there are 3 affected 46,XY female with normal female external genitalia, primary amenorrhea and inguinal hernia. In the other family with the same mutation the patient was a 46,XY female with normal female external genitalia, primary amenorrhea and abdominal testes. There is no familiarity between these families.
In predictive tools analysis, both mutations are classified as deleterious suggesting an alteration of Exonic splicing enhancers (ESEs).
The first silent mutation (p.S889S) was described in the literature with a functional study showing a short transcript. The patient was a male patient, with PAIS. In order to analyse the possibility of splicing alteration, we performed a AR cDNA from reverse transcription of mRNA extracted from testicular tissue of one affected patient with p.S510S AR mutation.
The cDNA sequencing showed that the AR mRNA lost 90 bp at the end of exon 1 leading to a premature stop codon.
the complete form of AIS (Figures 1 and 2).

Fig.1- Schematic representation of wild type AR (A) and a resulting protein of an AR with p.S510S mutation

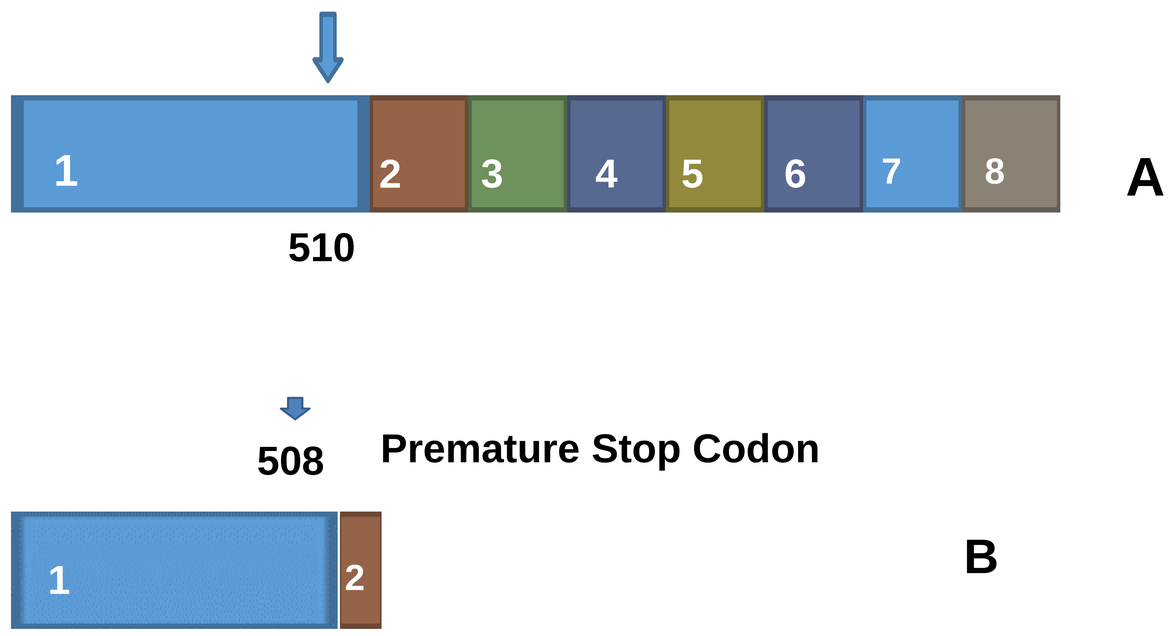
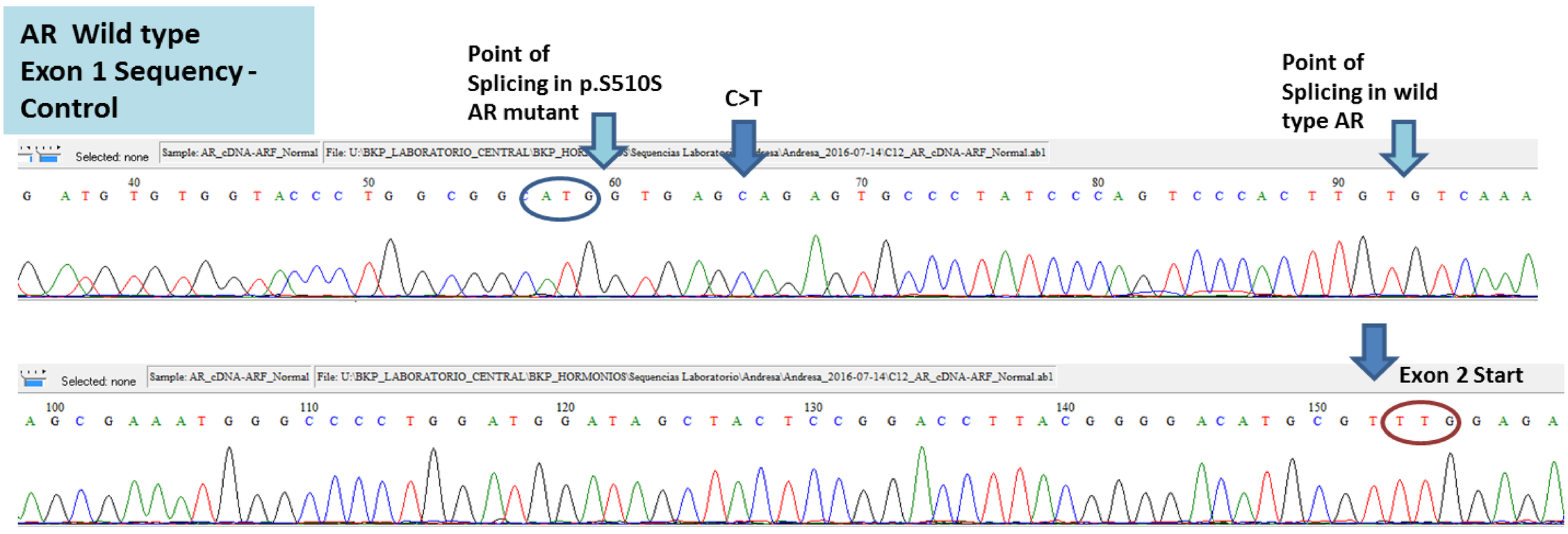


Fig.2- Eletropherogram of a wild type AR exon 1 sequencing and a AR p.S510S mutation



Conclusion

A single nucleotide change without aminoacid change can modify exonic splicing enhancers (ESEs) leading to an abnormal splicing. We identified a novel mutation in the AR gene associated an abnormal splicing resulting in a truncate protein. This is the first description of a silent mutation associated to CAIS. Our results further expand the spectrum of mutations associated with the AIS and may contribute to the understanding of the molecular mechanisms involved in the splicing defects.

References: *Melo K, JCEM 2003; Hellwinkel OJ, JCEM 2001, Gottlieb B*, Hum Mut 1999; *Sanfilipo JS*, J Pediat Adolesc Gynecol 2016

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