Complete androgen insensitivity syndrome caused by a deep intronic pseudoexon-activating mutation in the androgen receptor gene

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Background:

Complete androgen insensitivity syndrome (CAIS) is characterized by a female phenotype in a genetically male (46,XY) individual[1]. Although most CAIS patients are identified to have a mutation in *AR*, the X-linked gene encoding the androgen receptor, there are still some cases that remain without a confirming molecular genetic diagnosis[2]. Genetic screening of patients is often focused on the coding region and conserved splice sites, although mutations outside these regions are increasingly being recognized as a cause of human disorders. Eukaryotic genes contain several sequences that resemble exons but that are normally not spliced into the mature mRNA ("pseudoexons")[3]. These sequences can be activated by mutations, so that they are recognized as exons by the splicing machinery.

Aim:

- To find out the cause of CAIS in two siblings (46,XY girls) without identified mutations in the *AR* coding region or in the conserved splice sites.

Methods:

- Whole-genome sequencing (Illumina HiSeq 2000 technology; BGI, Shenzhen, China) of the patients and their healthy father (serving as a healthy control), and Sanger-sequencing to confirm the identified mutation
- Prediction of the effects of the identified mutation with Human Splicing Finder[4]
- PCR analysis and Sanger sequencing of cDNA obtained from patient fibroblasts (from the labia majora)
- RT-qPCR analysis of the total amount of *AR* expression and of the normally spliced *AR* expression in patient and control fibroblasts
- Analysis of AR protein amount by immunoprecipitation and western blot

Results:

AR cDNA analysis and whole-genome sequencing revealed aberrant splicing of the mRNA (Fig. 1A) caused by a deep intronic mutation (c.2450-118A>G) in intron 6 of *AR*. The mutation is present as hemizygous in both CAIS patients, heterozygous in the mother, and absent in the father. The mutation creates a *de novo* 5' splice site and a putative exonic splicing enhancer (ESE) motif (Fig. 1B and C), which both promote the use of an upstream cryptic 3' splice site, leading to the preferential formation of two aberrantly spliced mRNAs (Fig. 1A and 1B).

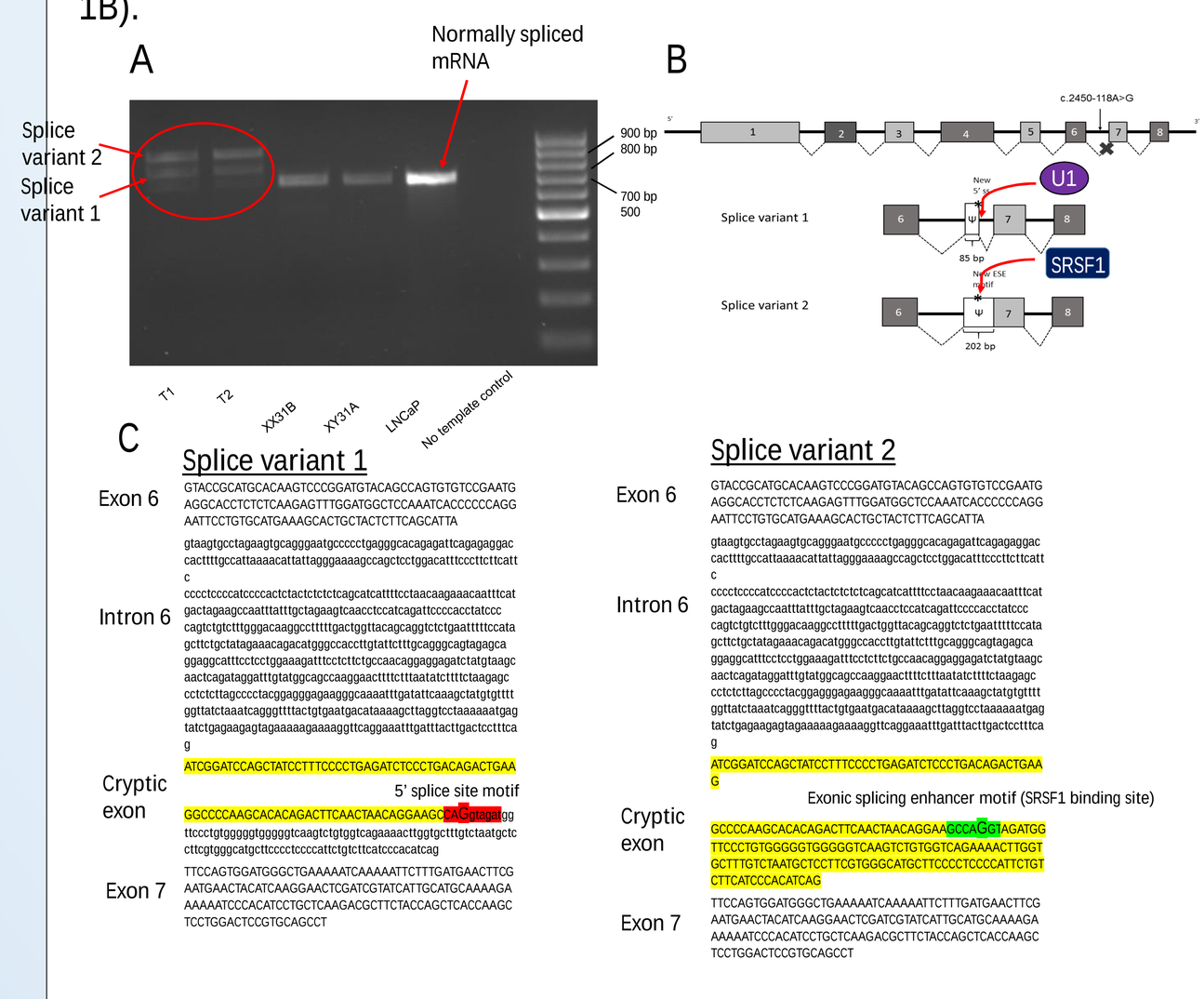

Fig. 1. A) The PCR amplification of *AR* cDNA, visualized on an agarose gel, showing the two aberrantly spliced variants (splice variant 1; 768 bp, and 2; 885 bp) amplified from the patient fibroblasts (T1 and T2) and the much fainter normally-spliced product of 683 bp which was the only product amplified from the control fibroblasts XX31B and XY31A as well as LNCaP cells. B) The schematic representation of the two aberrant mRNAs caused by the deep intronic mutation; the cryptic exonic sequences are marked with **Ψ**. C) The nucleotide sequences of exon 6, intron 6, and exon 7 including the identified mutation (in bold and bigger font size) and the cryptic exonic sequences, corresponding to the boxes marked with **Ψ** in panel B, highlighted in yellow (and red/green). The new splicing motifs created by the mutation are highlighted either in red (5' splice (donor) site) or in green (ESE motif).

Both abnormal mRNAs are predicted to lead to the inclusion of a premature stop codon after addition of 12 novel amino acids, potentially leading to nonsense-mediated decay. However, RT-qPCR analysis showed similar levels of total *AR* mRNA in patient fibroblasts when they were compared to controls (Fig. 2A), but the expression of the normally-spliced mRNA was only at approximately 10% level of control fibroblasts (Fig. 2B), and accordingly, there was no detectable AR protein in the patient fibroblasts (Fig.2C), indicating that the low amount of normal *AR* mRNA does not yield significant amounts of AR protein.

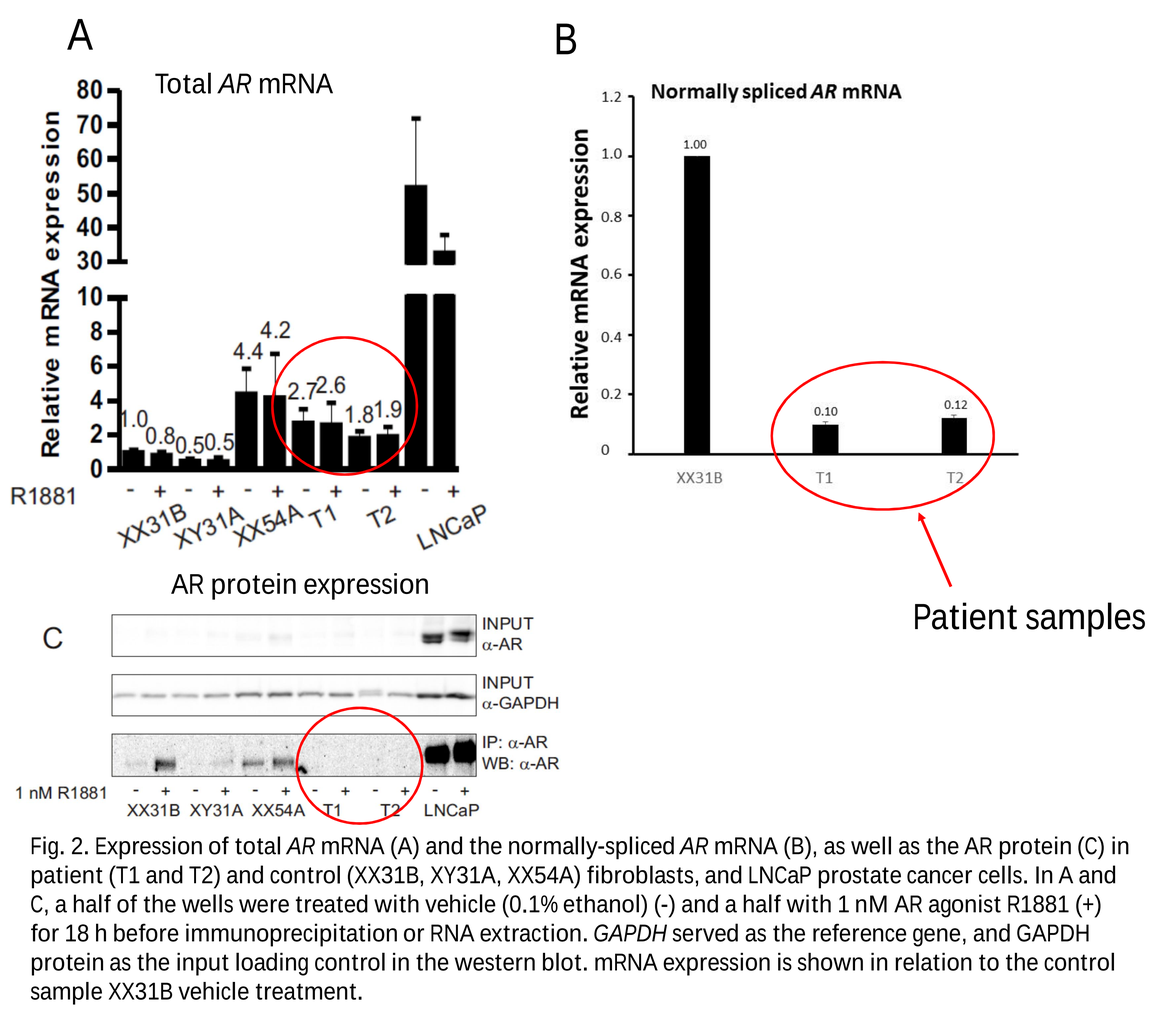

Fig. 2. Expression of total *AR* mRNA (A) and the normally-spliced *AR* mRNA (B), as well as the AR protein (C) in patient (T1 and T2) and control (XX31B, XY31A, XX54A) fibroblasts, and LNCaP prostate cancer cells. In A and C, a half of the wells were treated with vehicle (0.1% ethanol) (-) and a half with 1 nM AR agonist R1881 (+) for 18 h before immunoprecipitation or RNA extraction. *GAPDH* served as the reference gene, and GAPDH protein as the input loading control in the western blot. mRNA expression is shown in relation to the control sample XX31B vehicle treatment.

Conclusions:

- This is the first reported *AR* pseudoexon-activating mutation that leads to AIS.
- The *AR* cDNA and intronic sequences should be analyzed in CAIS patients with normal *AR* coding region and conserved splice sites.
- The precise molecular genetic diagnosis is of crucial importance for genetic counseling of the patients and their family members.
- In addition, the molecular genetic diagnosis in DSD patients is valuable in predicting the clinical course of the disease, and in decision-making related to the possible need of gonadectomy.

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Gonads & DSD
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