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Follow-up of two similar patients with Steroidogenic Factor-1 (SF-1/ NR5A1) variants in two different eras

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Introduction

Steroidogenic factor 1 (SF1)/"nuclear receptor subfamily 5 group A member 1" (NR5A1) is involved in adrenal and gonadal development, steroidogenesis and reproduction. The heterozygous changes in SF-1/NR5A1 causing 46,XY DSD were found to be very frequent, while adrenal insufficiency is rare. Furthermore, SF1 variants are responsible for premature ovarian failure and ovotesticular DSD, suggesting a pivotal role in the development of both sexes (1,2).

Methods

Genetic variants in SF1/NR5A1 were identified using whole exome sequencing (WES). For the in silico prediction of the variants, Meta-SNP was used (3). The molecular models are based on the previously elucidated crystal structure of the SF1 DNA binding domain bound to a target sequence (4). PyMol was used to introduce the variants Arg84Cys and Gly22Ser (Pymol.org). This was followed by a short (500ps) molecular dynamics simulation for the wildtype (WT) and both variant models, in order to refine the local structure around the mutagenesis. Finally, PyMol was used to visualize the models and to take pictures.

Patients

Patient 1 (*1993) presented with ambiguous genitalia (phallus 1 cm), descended gonads and 46, XY karyotype. No salt wasting crisis was present. HCG test showed no increase of testosterone and ultrasound revealed no uterus or vagina. At 3 years of age, gonadectomy and clitoroplasty were performed. Histology showed immature seminiferous tubules with normal Sertoli cells. The patient was raised as girl. Vaginoplasty was performed at the age of 16. Psychosocial development was normal with achievement of high degree of education.
Whole Exome Sequencing showed a heterozygote novel variant in NR5A1/SF1 (c.64G>A p.(Gly22Ser); Figure 1), and is compatible with a *de novo* event (Minor Allele Frequency, MAF=0).

Patient 2 (*2016) presented with penoscrotal hypospadias (phallus 2.1 cm), at ultrasound the right testis was found in the scrotum and the left retained between scrotal and inguinal position. No adrenal insufficiency was present and karyotype was 46,XY. Urine analysis suggested a testosterone synthesis defect. Testosterone injections at the age of 3, 4 and 5 months resulted in penis growth up to 3 cm. In the first year of life surgical correction of the hypospadias was performed. The patient's sex was assigned male.
Genetic studies showed a *de novo* heterozygote variant in NR5A1/SF1 (c.250C>T p.(Arg84Cys); Figure 2), which has previously been reported to cause 46,XY DSD (1).

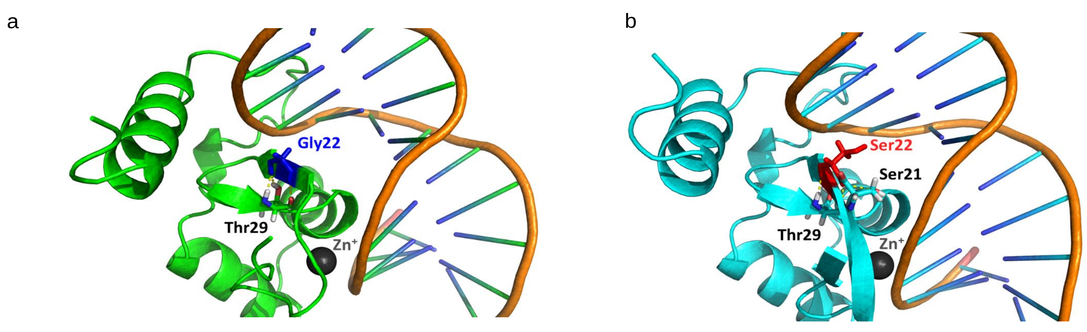

Figure 1 Close-up of the Zinc-finger domain I of SF1.
(a) The WT amino acid Gly22 is depicted in blue, variant Ser22 in red and the Zinc ions in grey. Gly22 binds Thr29 tightly and helps establish the local beta-sheet structure. **(b)** Ser22 also binds Thr29, and also its neighbour amino acid Ser21. The introduction of an amino acid with a polar side chain into the important Zinc-finger domain might influence the interaction between SF1 and genomic DNA.

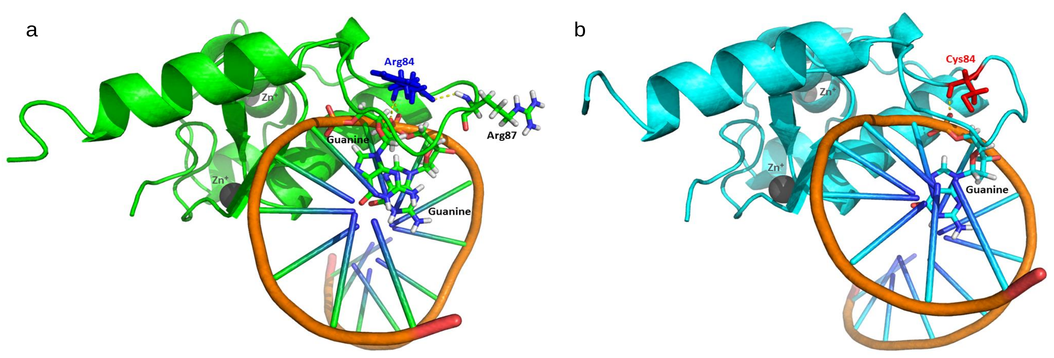

Figure 2 Polar interactions of WT Arg84 and variant Cys84.
(a) The WT amino acid Arg84 is depicted in blue, variant Cys84 in red and the Zinc ions in grey. Arg84 participates in binding the DNA by making polar contacts to two Guanines. Additionally, it stabilizes the local loop by interacting with the close amino acid Arg87 (potential with others as well). **(b)** The variant Cys84 does not participate in the stabilization of the local loop and partially looses the ability to bind the DNA, since it only makes polar contact to one Guanine.

Conclusion

The novel p.Gly22Ser/WT in patient 1 and the c.250C>T p.Arg84Cys/WT in patient 2 are the most likely genetic cause of 46,XY DSD in our patients, given the dominant negative effect of SF1 variants.
These two cases emphasize the different management of two only slightly different phenotypes in patients with NR5A1/SF1 variants. Time of diagnosis (one patient born in 1993 and one in 2016) before and after the change of policy in management of DSD cases with the advent of multidisciplinary teams had probably a stronger influence on the decision making for medical and surgical treatment and gender assignment than the phenotype.

References
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