Mutations involving nuclear receptors and their cofactors as a major cause of 46,XX DSD

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The genomic analysis of 46,XX individuals with testes (known as testicular Disorders/Differences of Sex Development (TDSD) or ovotestes (ovotesticular DSD (OTDSD)) supports the hypothesis that "pro-testis/anti-ovary" or "pro-ovary/anti-testis" genetic pathways exist. Many individuals with TDSD and a minority with OTDSD have a translocation of the testis-determining *SRY* gene usually onto one of the X-chromosomes, whereas a small proportion have chromosomal rearrangements associated with upregulation (gain-of-function) of *SOX* gene expression. Other rare forms of 46,XX DSD can occur due to mutations (loss-of-function) involving genes in the WNT4/RSPO1 signaling pathway. However, the etiology of majority of 46,XX DSD cases remains unknown. Using unbiased high throughput sequencing approaches, we are generating evidence to support a key role for nuclear receptors as pro-ovary/anti-testis factors.

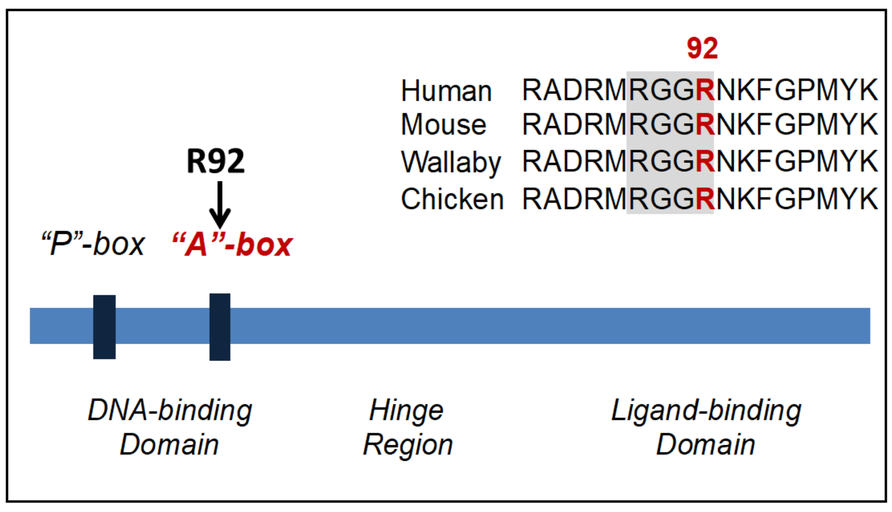

Fig 1. Schematic representation of the NR5A1 protein showing the position of the R92 residue

In an analysis of 82 cases of *SRY*-negative 46,XX TDSD and OVOTDSD cases, we identified recurrent mutations involving in the R92 residue of the nuclear receptor NR5A1 in six families with 46,XX DSD (genetic association $p=10^{-7}$, reference 1 and unpublished data).

We also identified 3 recurrent mutations in the nuclear receptor *NR2F2*, which encodes COUP-TF2 (Figure 1., genetic association $p=10^{-8}$, reference 2). In these 3 cases the affected children also had congenital heart defects (Table 1.)

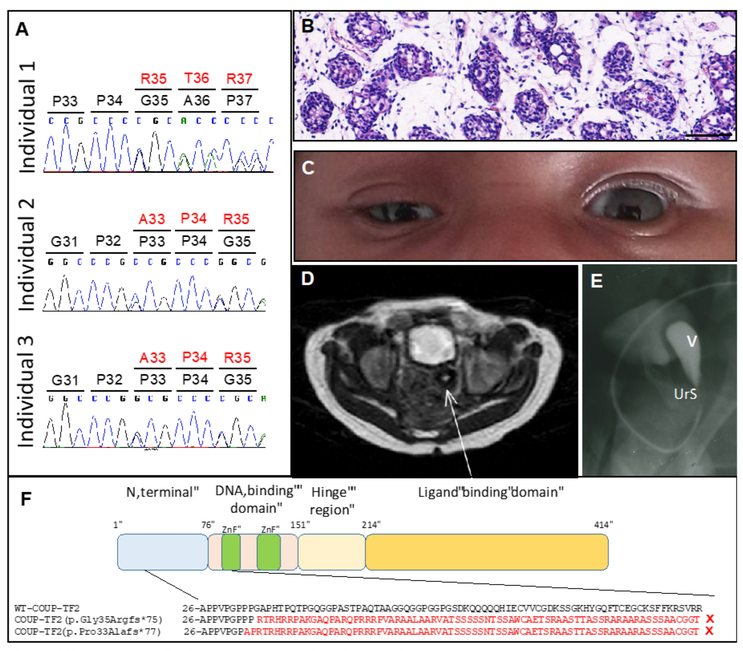

Fig 2. Identification of Three Individuals with 46,XX DSD and Mutations in *NR2F2*
(A) Representative Sanger sequence chromatograms of showing the positions of the frameshift mutations. (B) Histology of the right gonad of individual 3 showing testicular tubule-like structures surrounded by stromal-like tissue (scale bar corresponds to 100 µm) (C) Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) of individual 3. (D) Uterus (arrow) of individual 2 observed by MRI. (E) Pelvic radiography of individual 3 showing the vagina (V) and a short urogenital sinus (UrS). (F) Schematic representation of COUP-TF2 showing the main functional domains and the position and downstream consequences of the three frameshift mutations. The first zinc finger motif is highlighted in green in the sequence alignment.

Variable	Individual 1	Individual 2	Individual 3
Ancestry	Latino	Senegalese	Hungarian
Karyotype	46,XX *SRY*-negative	46,XX *SRY*-negative	46,XX *SRY*-negative
Birth weight (kg)	2.6	2.05	NA
Cardiac	Hypoplastic L heart at birth. Severely dilated R ventricle with moderate RVH and mild to moderately depressed RV systolic function	Persistent ostium secundum and ASD	VSD at birth. Spontaneous closure of VSD at 9 years of age
External genitalia	Male, hyperpigmented. Phallus 3 cm with no hypospadias. Gonads not palpable.	"Ambiguous" with phallus-like clitoris, pigmented scrotum. Gonads not palpable.	"Ambiguous" Prader IV, pigmented scrotum, phallus-like clitoris. R- palpable gonad in the inguinal canal.
Internal genitalia	Uterus not identified by US	Uterus present	R- ductus-like Wolffian structures; L - uterus, ovary, and Fallopian tube. Vagina and short urogenital sinus
Gonads	Not observed by US	Not observed by US or MRI	Pelvic ultrasound, R testis, L ovary. Histology - R gonad, testis tubules and ovarian tissue with oocytes
Other somatic anomalies	Left congenital diaphragmatic hernia	BPES	Mild learning disabilities, minor limb anomalies, hypertelorism, BPES
Endocrine data (reference values)	**Day 1**: T, 135 ng/dL (20-64; 17-OHP, 120 ng/dL (11-170); LH, 5.1 IU/L (0.02-7.0); FSH, 1.1 IU/L (0.16-4.1)	**Day 11**: T, 579 ng/dL (<130); AMH, 44 ng/mL (<4.2); Inhibin B, 263 pg/mL (<110) ; FSH, 13.2 IU/L (<10) ; LH, 9.3 IU/L (0.9-3); 17-OHP, 127 ng/dL (<270) ; **Day 15** - T, 327 ng/dL (<40); AMH, 43.1 ng/mL (<4.2); Inhibin B, 217 pg/mL (<110); FSH, 6.9 IU/L (<10); LH, 5.1 IU/L (0.9-3); estradiol <5 pg/mL **1 month** - T, 304 ng/dL (<40); AMH, 43.1 ng/mL (<4.2); Inhibin B, 230 pg/mL (<110); FSH, 9 IU/L (<10); LH, 15.5 IU/L (0.9-3); estradiol, <5 pg/mL	**Day 17**: T, 250 ng/dL (<40); 17-OHP, 185 ng/dL (40-490) **Day 30**: T, 460 ng/dL (<40) **Day 45**: T, 50 ng/dL (<40) **Day 73**: T, 110 ng/dL (<40)
Mutation	*de novo*, c.103_109delGGCGCCC p.Gly35Argfs*75	*de novo*, c.97_103delCCGCCCG p.Pro33Alafs*77	c.97_103delCCGCCCG p.Pro33Alafs*77

Table 1. Phenotypes, Genotypes, and Investigation of 3 Children with Frameshift Mutations in NR2F2

We identified both *de novo* mutations and rare variants in nuclear receptor corepressor 2 (*NCOR2)* gene, which we consider to be either pathogenic or may contribute to the development of the phenotype (Table 2). NCOR2 mediates transcriptional silencing of target genes and acts as part of a multi-subunit complex which includes histone deacetylases to modify chromatin structure. NCOR2 is also known to physically interacts with the NR2F2 protein (3).

Ancestry	Phenotype	Mutation	Zygosity	Inheritance/Minor allelic frequency
Indian	46,XX TDSD	p.I1109F	Het.	De novo/novel
Tunisian	46,XX TDSD	p.R1665H	Het.	Unknown/novel
Egyptian	46,XX TDSD	p.R1905C	Het.	Paternal/novel
North African	46,XX TDSD	p.R936W	Het.	Unknown/1:2000 north africans

Table 2. NCOR2 mutations observed in children with 46,XX TDSD. All mutations are predicted to be deleterious by SIFT, PolyPhen2 and MutationTaster.

In this study of 82 cases of *SRY*-negative 46,XX TDSD and OVOTDSD, we observed potentially pathogenic mutations in nuclear receptors or their cofactors in 13 cases (16%). At the moment it is unclear if these factors form part of a multi-protein complex or if they are acting in independent pathways. This data provides further evidence of the emerging importance of nuclear receptors in specifically establishing human ovarian identity.

References. 1. Bashamboo et al., A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. Hum Mol Genet. 2016;25:3446-3453.
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3. Johnson et al., Regulation and binding of pregnane X receptor by nuclear receptor corepressor silencing mediator of retinoid and thyroid hormone receptors (SMRT). Mol Pharmacol. 2006;69:99-108.

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