A mutation in WT1 (Wilms’ Tumor Suppressor 1) Associated with 46, XX TDSD

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Scientific context

- **Human Sex Determination, WT1 and DSD**
  - In males, the SRY protein in synergy with NR5A1, upregulates SOX9 expression leading to Sertoli cell differentiation (1).
  - Ovary development is controlled by RSP01/WNT4/β-catenin and FOXL2 pathways (1).
  - Mammalian sex determination is regulated by two mutually antagonistic pathways (2).
  - DSD (Disorder/Differences of Sex Development) refers to congenital conditions with atypical development of chromosomal, gonadal, or anatomic sex (3).
  - 46,XX DSD includes an individual with ovotestis (ovotesticular DSD (OTDSD)) or testis (testicular DSD (TSDD)).
  - Most individuals with 46,XX DSD carry SRY, that results in development of testis (4).
  - Other causes include rearrangements involving SOX9 or SOX3 loci (5).
  - Syndromic forms of 46,XX DSD-OTDSD have been reported due to mutations of WNT4 and RSP01 (6).

Clinical features & sequencing

- **Patient: 46,XX TDSD Egyptian ancestry**
  - Normal plump
  - Mild microcephaly (~4.5 SD)
  - No nephroblastoma
  - Dysgenetic Testis
  - Perineum length 9 cm
  - Labiolabial fold, single opening
  - Small uterus (ablation), pubic bone size
  - Mildly prominent Suprapennals by pelvic US

What is the effect of the mutation R495G on the biological activity of WT1 protein and on sex determination pathways?

**Results**

- **OVER-ACTIVATION OF MALE PATHWAY ?**
  - WT1 (Wilms’ tumor suppressor 1) encodes a key developmental regulator with four C-terminal zinc fingers.
  - WT1 is essential for development of the kidneys, bipotential gonad and testis (7).
  - Two different isoforms of WT1 (+KTS and -KTS) have distinct functions during gonad development. The -KTS isoform binds the promoter of SRY and NR5A1 whilst +KTS binds RNA and increases the stability of SRY transcript (8).
  - WT1 gene deletions are associated with genitourinary anomalies and a predisposition to Wilms’ tumor, whereas heterozygous missense mutations give rise to Denys-Drash syndrome (7).

- **UNDER-ACTIVATION OF FEMALE PATHWAY ?**
  - R495G shows a significant quantitative reduction in the transactivation of FOXL2 promoter in transient transactivation assays.
  - Loss of protein-protein interaction between GATA4 and R495G whereas there is a strong interaction with WT1-wt.
  - R495G alters the GATA4/FOG2 mediated regulation of SOX9 via Testo enhancer element.

**Conclusions & perspectives**

The WT1p.R495G protein aberrantly regulated/interacted with genes/proteins known to be involved in both male and female gonadal development. R495G results in:

- Dysregulation of SOX9 expression via Testo enhancer
- Disruption of the protein-protein interaction between WT1 and GATA4
- Overexpression of male pathway in a granulosa cell line
- Under activation of FOXL2 promoter

First time that a mutation has been identified in WT1, associated with 46,XX TDSD. These data resemble our recent discovery of a recurrent NR5A1 mutation (R92W) associated with 46,XX OTDSD/TDSD (9).

- RNA-seq underway to fully understand the complete extent of transcriptome modulation by WT1p.R495G
- A mouse model carrying WT1p.R495G knock-in underneath to understand the mechanism of testis-formation in XX chromosomal context

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