The authors declare no conflict of interest

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

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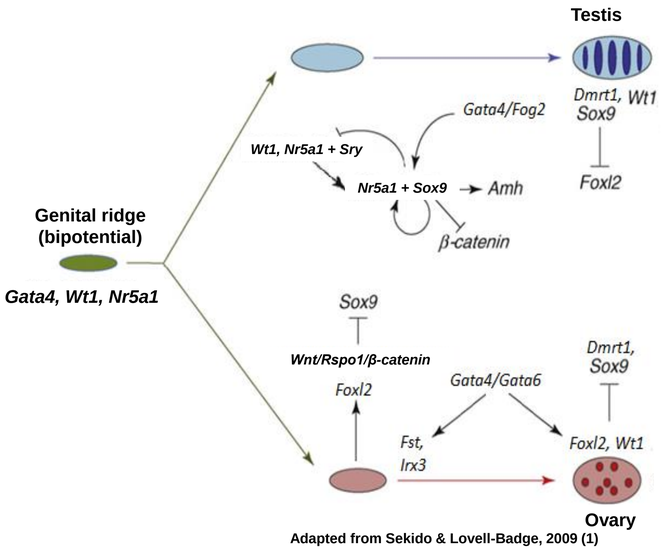
Scientific context

Human Sex Determination, *WT1* and DSD



Adapted from Sekido & Lovell-Badge, 2009 (1)

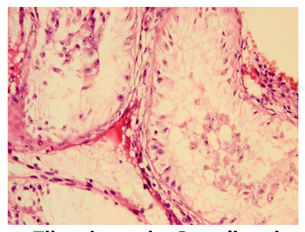
- ✓ **In males, the SRY protein in synergy with NR5A1, upregulates *SOX9* expression leading to Sertoli cell differentiation (1).**
- ✓ **Ovary development is controlled by RSPO1/WNT4/β-catenin and FOXL2 pathways (1).**
- ✓ **Mammalian sex determination is regulated by two mutually antagonistic pathways (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 (TDSD)).**
- ✓ **Most individuals with 46,XX TDSD carry *SRY*, that results in development of testis (4). Other causes include rearrangements involving *SOX9* or *SOX3* loci (5).**
- ✓ **Syndromic forms of 46,XX TDSD/OTDSD have been reported due to mutations of *WNT4* and *RSPO1* (6).**

- ✓ ***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).**

Clinical features & sequencing

Patient : 46,XX TDSD Egyptian ancestory

Normal ploïdy
Mild microcephaly (-4,5 SD)
No nephroblastoma
Dysgenetic Testis
Penile length 9 cm
Labioscrotal fold, single opening
Small Uterus (ablation), prepubertal size
Mildly prominent Suprarenals by pelvic US
SRY-Negative

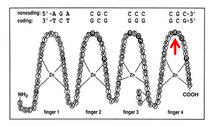


Fibrotic testis, Sertoli and Leydig-like cells, no germ cells

Whole Exome Sequencing

- ✓ Hypothesis-free exome sequencing on the proband and 50 independent cases of 46,XX TDSD and OTDSD.
- ✓ Exon enrichment using Agilent SureSelect Human All Exon V4.
- ✓ Paired-end sequencing on the Illumina HiSeq2000 platform using TruSeq v3 chemistry at an average coverage of x50.

A *de novo* missense mutation of a highly conserved arginine residue in the fourth zinc-finger of WT1 (p.Arg495Gly, R495G) identified in the proband

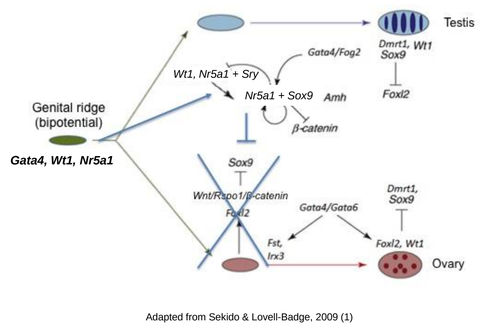


- ✓ No other potentially pathogenic mutations in known sex-determining genes.
- ✓ Normal ploidy established by high resolution aCGH and qPCR.
- ✓ The p.arg495Gly mutation absent in the dbSNP138, ExAC databases and ancestory matched controls.

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

Results

OVER- ACTIVATION OF MALE PATHWAY ?



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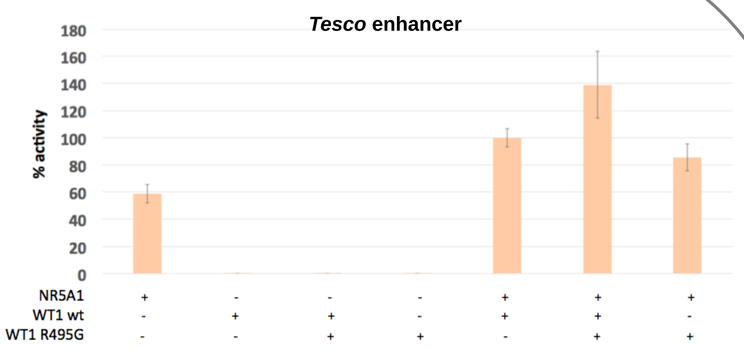
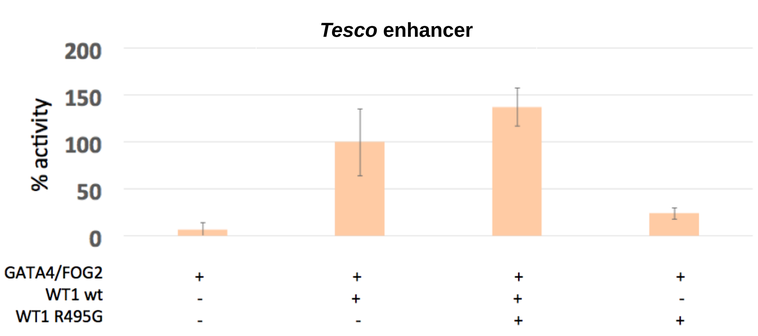
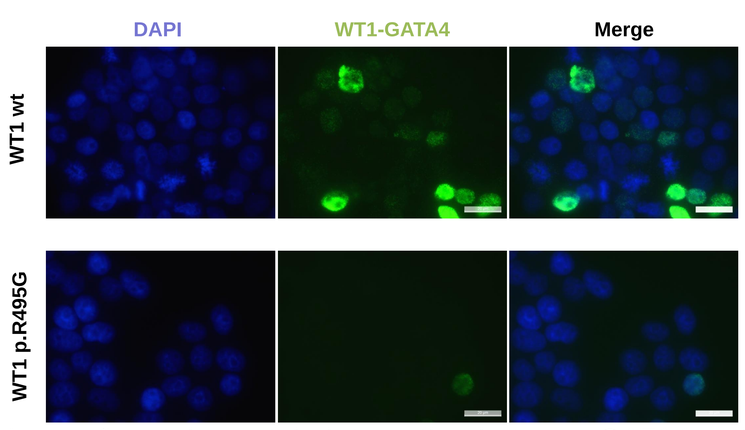


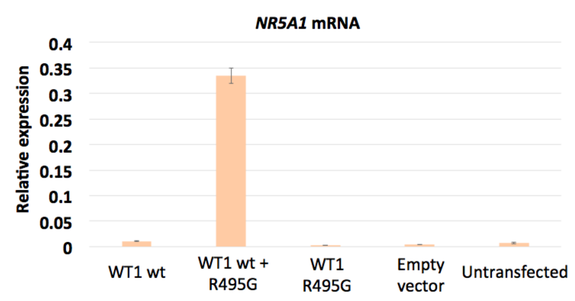
R495G does not significantly affect the NR5A1 mediated regulation of *SOX9* via *Tesco* enhancer element

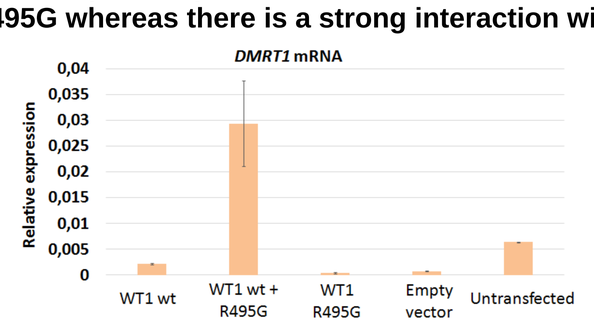


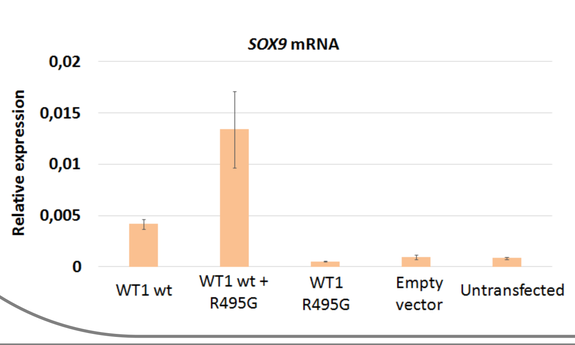
R495G negatively impacts the GATA4/FOG2 mediated regulation of *SOX9* via *Tesco* enhancer element



Loss of protein-protein interaction between GATA4 and R495G whereas there is a strong interaction with WT1-wt



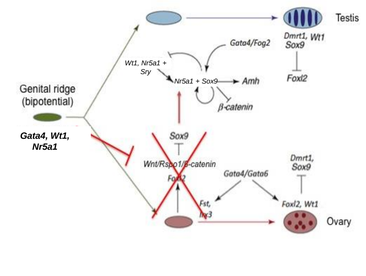


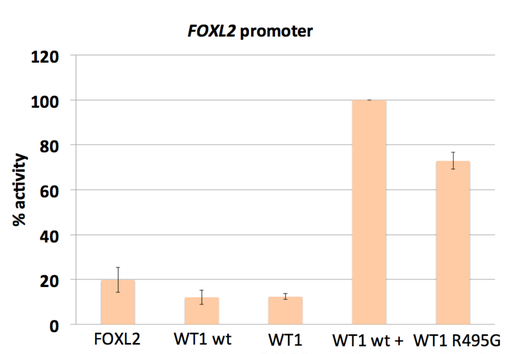


Summary

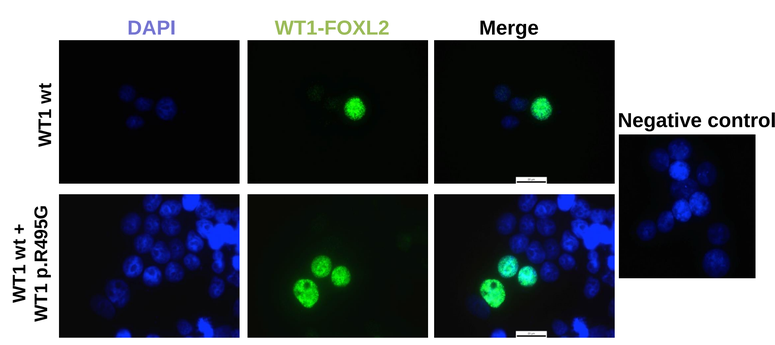
- ➢ **R495G alters the GATA4/FOG2 mediated activation of SOX9 via the *Tesco* enhancer.**
- ➢ **This is due to loss of physical binding between GATA4 and R495G**
- ➢ **Transient activation of R495G upregulates the expression of endogenous testis determining genes (*SOX9, NR5A1, DMRT1*) in a 46,XX granulosa cell line.**

UNDER-ACTIVATION OF FEMALE PATHWAY ?

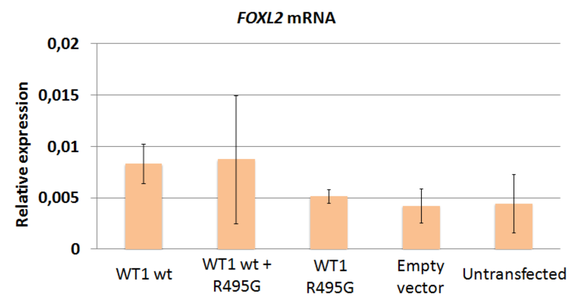




R495G shows a significant quantitative reduction in the transactivation of *FOXL2* promoter in transient transactivation assays.



Strong protein-protein interaction between wild type WT1 and FOXL2, that remains unaltered with R495G



Transient activation of R495G does not alter expression of the endogenous ovarian gene, *FOXL2*, in a 46,XX granulosa cell line.

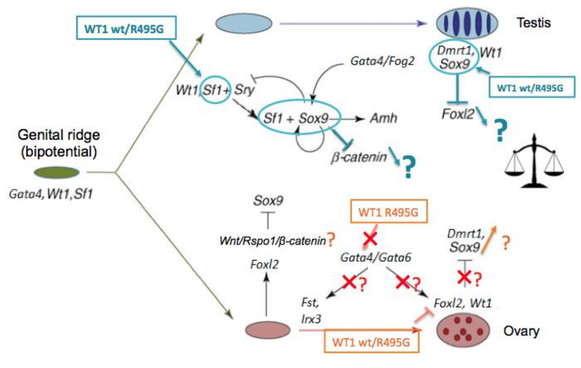
Summary

- ➢ **R495G shows a reduction in activation of the *FOXL2* promoter but the protein-protein interaction between the two remain unaltered**
- ➢ **The change in activity could be due to a hindrance in auto-activation of the *FOXL2* promoter by FOXL2/R495G complex.**
- ➢ **Transient activation of R495G does not alter the endogenous expression of ovarian genes in a 46,XX granulosa cell line.**

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 Tesco* 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 underway to understand the mechanism of testis-formation in XX chromosomal context

(1) Sekido and Lovell-Badge, 2009, *Nature*
(2) Ulhenhaut *et al.*, 2009, *Cell* and Matson *et al.*, 2011, *Nature*
(3) Bashamboo and McElreavey, 2013, *Sex Dev*
(4) Barbaro *et al.*, 2011, *Sem in Fetal&NeonatMed*
(5) Vetro *et* al., 2015, *Eur J Hum Genet*
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