Genotype-phenotype correlation of NR5A1/SF1 mutations by functional in-vitro studies

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BACKGROUND:

Disorders of sex development (DSD) are congenital conditions in which the chromosomal, gonadal or anatomical sex is atypical[1]. The transcription factor **Steroidogenetic Factor 1 (SF1, Nuclear Receptor Subfamily 5 Group A member 1)** is one of the main regulators of embryonic gonadal development[2] *(fig. 1)*. Until now, more than 50 SF1 mutations have been described in patients with XY and XX DSD variable phenotypes due to different severity of gonadal dysgenesis such as complete, partial and mild gonadal dysgenesis, hypospadias with partial gonadal dysgenesis, infertility and bilateral anorchia[3]. So far, genotype-phenotype correlations could not been demonstrated.

OBJECTIVE: To investigate genotype-phenotype correlation of SF1 misssense mutations by in vitro studies

METHODS:

Heterozygous SF1 missense mutations located in different structural regions of XY patients displaying phenotypes ranging from infertility to complete gonadal dysgenesis were choosen *(fig. 2)*. Their ability to activate central factors of gonadal determination and development *(SOX9, CYP11A1 and AMH)* have been examined by dual glo luciferase assays in a homogeneous experimental set up. For the testis specific enhancer (TESCO) synergy with the known cofactor SOX9 was investigated. The human transcription factors were cloned in a pcDNA3-vector. The transfection (Fugene 6) assays were performed in human embryonal kidney (HEK293) and mouse sertoli (TM4) cells.

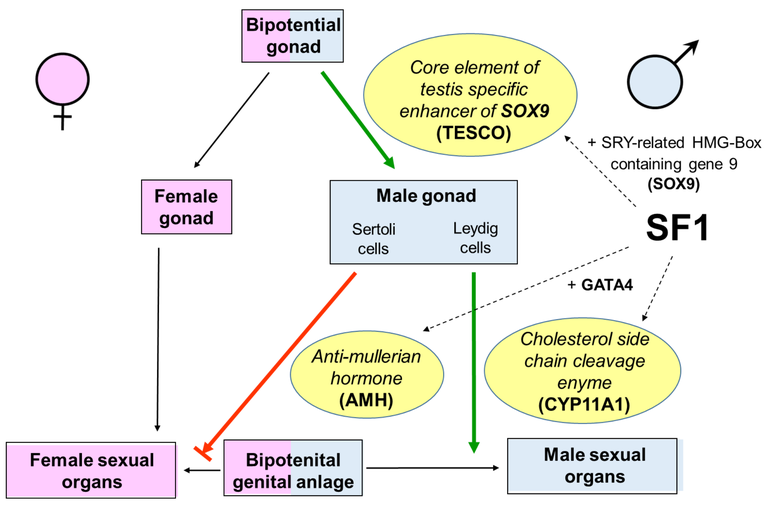


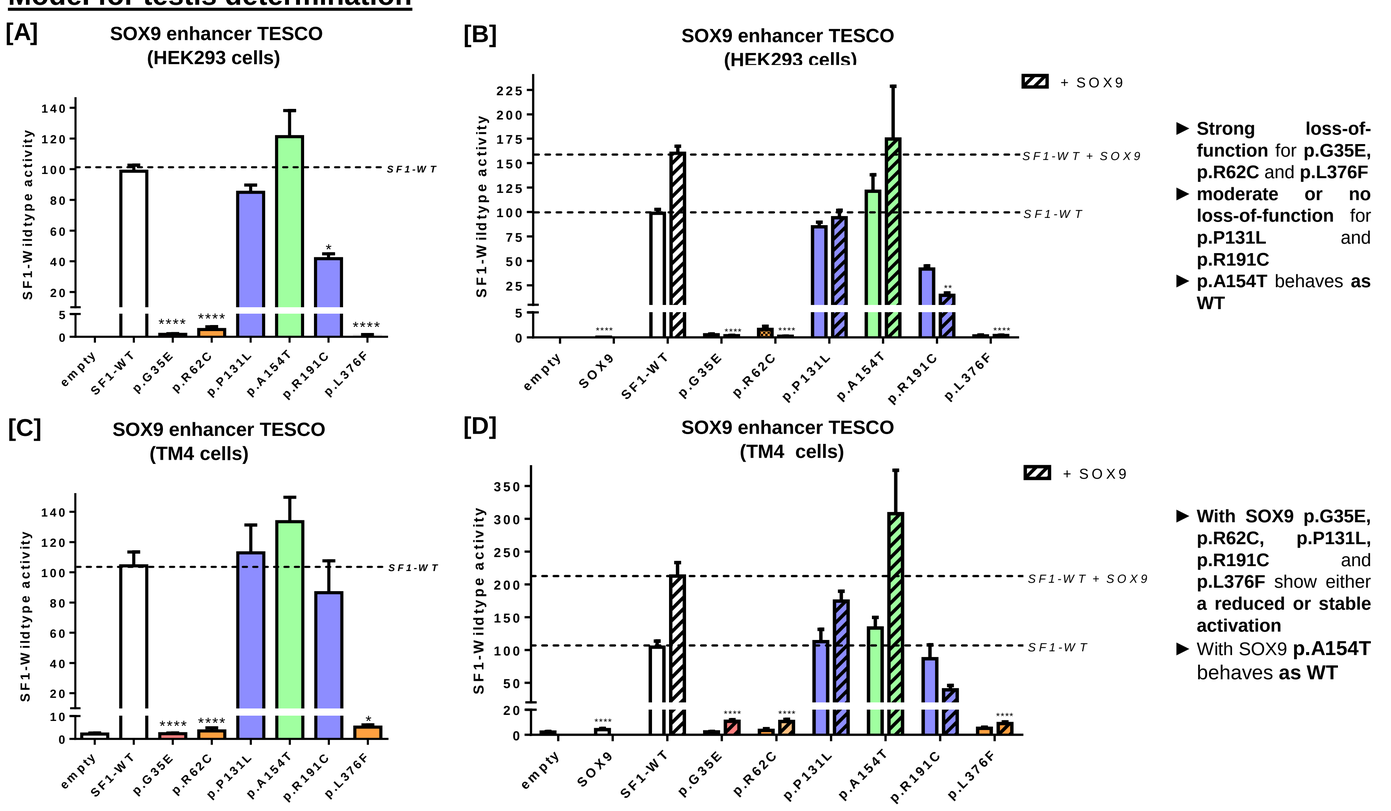
***Fig. 1:* Central factors of gonadal development.** The transcription factor SF1 acts via TESCO[4], AMH[5] and CYP11A1 accordingly[6]

Mutation	p.G35E[7]	p.R62C[8]	p.P131L[9]	p.A154T[8]	p.R191C[9]	p.L376F[10]
XY DSD diagnosis	**Complete gonadal dysgenesis**	**Partial gonadal dysgenesis**	**Infertility**	**Penile hypospadia**	**Infertility**	**Partial gonadal dysgenesis**
Age at diagnosis (in years)	1/12	6 6/12	41	4	25	14
Phenotype	Female, Streak gonads, Uterus	Male, Bilateral inguinal testis	Male, Scrotal testis	Male, Scrotal testis	Male, Scrotal testis	Female, Bilateral abdominal dysgenetic testis, no uterus
Sertoli cell function	FSH 17,8→38,0 mIU/ml (↑)	FSH 1.3→9.1 U/l (↑) Inhibin B 15,5 ng/l (↓) AMH <0,10 ng/ml (↓)	Sperm concentration 0 x 10^6/ml (↓)	FSH 1.2 →8.7 U/l (N) Inhibin B 70.4 ng/l (N) AMH <20 ng/ml (N)	FSH 18,8 IU/l (↑) Inhibin B <15 pg/ml (↓) Sperm concentration 0,3 x 10^6/ml (↓)	FSH 35,9 IU/L (↑) Inhibin B <10 pg/ml (↓)
Leydig cell function	T* not measurable LH 1,2→8,6 mIU/ml (↑)	T 0.1→1.8 ng/ml (↓) LH 0.5→2.2 U/l (N)	NA	T 0.18(N)→1.6 ng/ml (↓) LH 0.6→2.1U/l (N)	T 5,7 ng/ml (N) LH 10,7 IU/l (↑)	T 2,1 ng/ml (N) LH* 2,3 IU/L (N)

***Fig. 2:* Localisation of SF1 mutations and phenotype at age of diagnosis.** NA (not available), N (normal), ↓ decreased in comparison to reference, →/* after GnRH/hCG-stimulation. *SF1 structure:* DNA-binding domain (DBD), Hinge region,Ligand-binding domain (LBD). G35, R62, P131, R191, L376 are highly conserved, but A154T only partially

RESULTS:

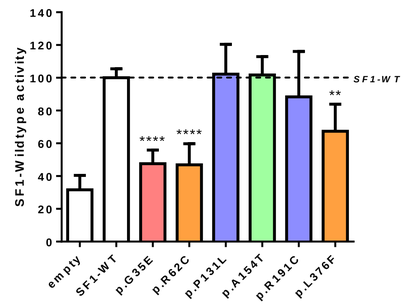
Model for testis determination



- **Strong loss-of-function** for **p.G35E, p.R62C** and **p.L376F**
- **moderate or no loss-of-function** for **p.P131L** and **p.R191C**
- **p.A154T** behaves **as WT**

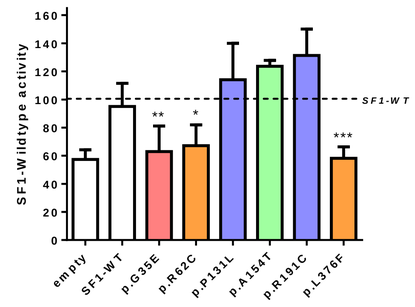
- **With SOX9 p.G35E, p.R62C, p.P131L, p.R191C** and **p.L376F** show either **a reduced or stable activation**
- With SOX9 **p.A154T** behaves **as WT**

Model for Leydig cell function



- **Moderate loss-of-function** for **p.G35E, p.R62C** and **p.L376F**
- **no loss-of-function** for **p.P131L, p.A154T** and **p.R191C**

Model for Sertoli cell function



- **Mild loss-of-function** for **p.G35E, p.R62C** and **p.L376F**
- **no loss-of-function** for **p.P131L, p.A154T** and **p.R191C**

***Figure 3:* Activity of SF1-WT and SF1-mutants on [A-D] SOX9 enhancer (TESCO), [E] CYP11A1-, and [F] AMH-promoter in human embryonal kidney (HEK293) and mouse sertoli (TM4) cells.** Relative wildtype-activity (WTA) of at least 3 independent experiments is shown as medians with interquartile range. Significance levels are calculated (Kruskal-Wallis-Test and Dunn`s Test) with SF1-WT as reference.
Significance levels:
*** = p<0,05, ** = p<0,01, *** = p<0,001, **** = p<0,0001**

CONCLUSION:

→ **In vitro Analysis of SOX9 enhancer TESCO as key regulator of gonadal determination and Sertoli cell development allows correlation of genotype with phenotype in patients with SF1-mutations**
- Mutations in DNA-binding domain (DBD)/ ligand binding domain (LBD) leading to *severe gonadal dysgenesis* show *a severe impairment of the SOX9 enhancer TESCO activation*
- In contrast, mutations in hinge region (HR) leading to male *infertility* also show only *partial impairment of TESCO activation*
- The effects of TESCO impairment cannot be rescued by self activation of TESCO by SOX9

→ **CYP11A1 reflects the phenotypes due to impairment of Leydig cell function**
- Mutatios in DBD/LBD leading to *severe gonadal dysgenesis* show also an *impairment of CYP11A1 activation*
- in contrast, mutations in the HR of patients with the milder phenotype of infertility result only in mild impairment.

→ **AMH was not found being a useful tool for later embryonal Sertoli cell function**

→ **Functional studies of SF1 mutations using SOX9 enhancer TESCO and CYP11A1 promoter in TM4 cells can be helpful as predictive models for phenotypes in vitro.**

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