**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 atypical¹. The transcription factor Steroidogenic Factor 1 (SF1, Nuclear Receptor Subfamily 5 Group A member 1) is one of the main regulators of embryonic gonadal development² (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 anorchia³. So far, genotype-phenotype correlations could not been demonstrated.

**OBJECTIVE:** To investigate genotype-phenotype correlation of SF1 missense 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 chosen (fig. 2). Their ability to activate central factors of gonadal determination and development (SOX9, CYP11A1 and AMH) have been examined by luciferase assays in a homogeneous experimental set up. For the tests 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 embryonic kidney (HEK293) and mouse sertoli (TM4) cells.

**RESULTS:**

**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 SOX9 impairment cannot be rescued by self activation of TESCO by SOX9

→ CYP11A1 reflects the phenotypes due to impairment of Leydig cell function

- Mutations 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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