Mutations at the SF-1 ligand-binding domain can lead to different effects on DNA binding: report of two novel mutations

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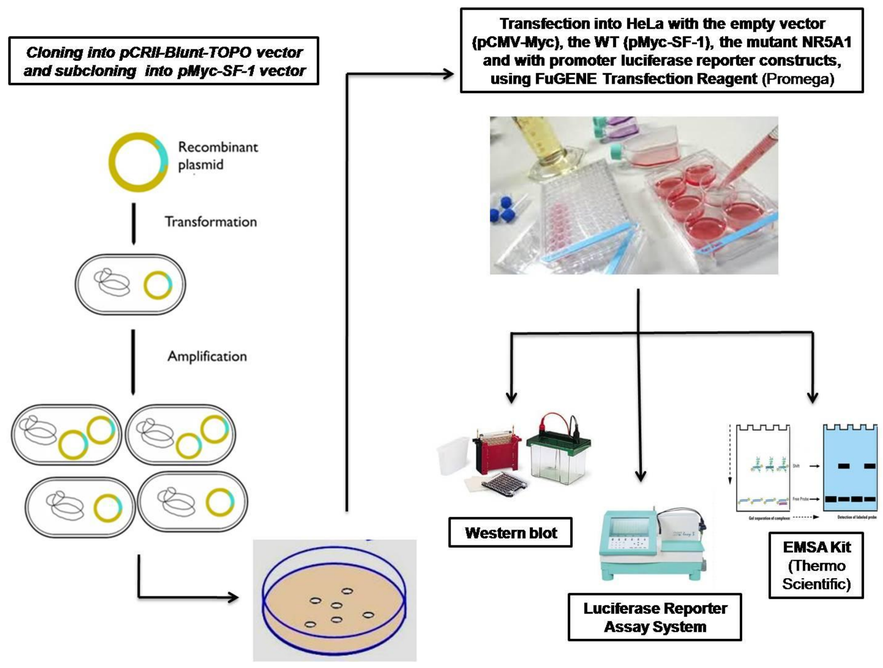
Introduction:

Steroidogenic factor-1 (SF-1), encoded by *NR5A1* gene, is a key regulator of steroidogenesis and reproductive development[1]. *NR5A1* mutations found in 46,XY patients with disorder of sex development (DSD), are associated with a range of conditions within the spectrum of phenotypes, including testicular dysgenesis, hypospadias and male infertility[2].

Fig. 1: Structure of human SF-1 is characterized by a DNA-binding domain (DBD) containing two zinc fingers, an accessory hinge region, a ligand-binding domain (LBD), and two functional activation domains, AF-1 and AF-2, located at the hinge region and at the C-terminal in LDB, respectively[3].

Methods:



Results:

Two mutations (p.C247* and p.K396Rfs*34), identified in the SF-1 ligand domain (LBD) have been analyzed to estimate their functional influence on SF-1 transcriptional activity. Luciferase reporter gene expression was reduced for both p.C247* and p.K396Rfs*34 when tested on *AHM* and *STAR* promoters. Whereas the transactivation activity for p.K396Rfs*34 was completely null, p.C247* retained a very low activity. Western blot showed that normal and mutant proteins were expressed in similar amounts. EMSA was also performed to analyze if those mutations would disturb SF-1 DNA binding ability. Results showed that the mutation p.K396Rfs*34 abolished the ability to bind DNA, whereas the formation of a protein-DNA complex was still observed for p.C247*. Clinical data of the patients are detailed in Table 1.

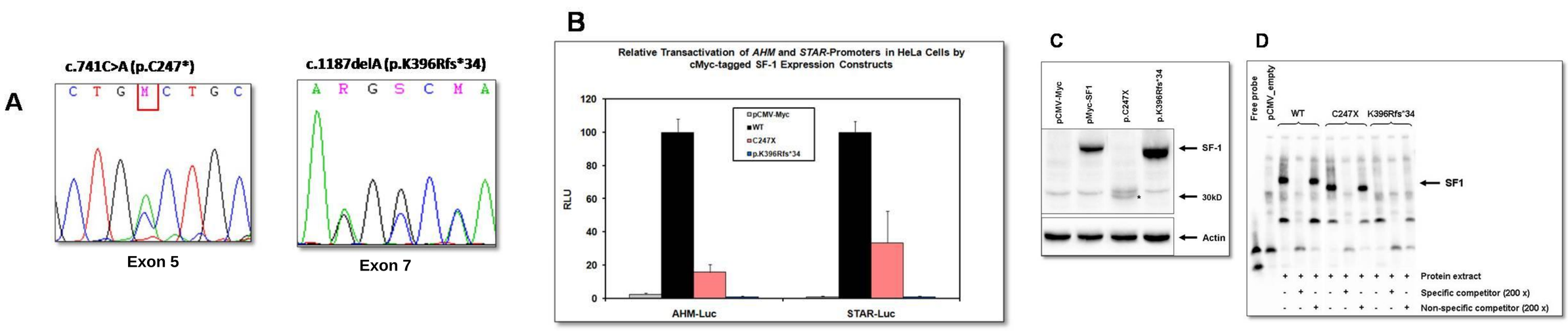


Fig. 2: A) Part of electropherogram sequences showing the mutations c.741C>A in exon 5 and c.1187delA within exon 7. B) Transactivation of *AHM* and *STAR* Promoters in HeLa cells, showing no activity of the mutant p.K396Rfs*34 and low activity of p.C247*. C) Expression levels of SF-1 protein in the Western Blot. D) EMSA assays revealed that only the SF-1 mutant p.C247* was able to bind to an SF-1 specific DNA sequence, whereas the p.K396Rfs*34, lost this ability.

Table 1: Clinical data of the patients with *NR5A1* mutations.

NR5A1 gene mutation	*Karyotype/ Assigned sex*	*PHENOTYPE*							*Family*
		Age at first visit	*Urethral opening*	*Gonadal location (R/L)*	*Basal gonadotrophins*	*Basal testosteone*	*Testosterone response to hCG testing*	*Gonadal histology (R/L)*	
p.C247*	46,XY/Female to male	20 years	Penoscrotal	IC / LSF	Elevated FSH, normal-high LH	Normal	NP	N/A	NP
p.K396Rfs*34	46,XY/ Male	7.8 years	Perineal	LSF / LSF	Prepubertal	Prepubertal	No response	Dysgenetic testes bilaterally	M=N

R, Right; L, Left; IC, Inguinal Channel; LSF, Labioscrotal folds; NP, Not performed; M, Mother; N, Normal.

Conclusion:

It is already known that, mutations at SF-1 LBD, may result in variable effects depending on their location and alterations in the ligand specificity/recognition[4]. This was also observed here, once both mutations localized in the LBD had completely different effects on DNA binding. However, both patients present partial gonadal dysgenesis, suggesting that the genotype-phenotype correlation, especially for mutations within the LBD, remains elusive. SF-1 function/regulation is very complex and must be increasingly studied, mainly because the number of different phenotypes correlated with mutations on this gene has been constantly increased.

DISCLOSURE STATEMENT: The authors have nothing to disclose.

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Supported by: FAPESP