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Analysis of Steroid 5-Alpha Reductase 2 (SRD5A2) Gene in Patients with 46,XY Disorder of Sex Development

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Introduction

The diagnosis of 46,XY disorder of sex development (DSD) due to 5-alpha reductase 2 (5α-RD2) deficiency has been based on testosterone:dihydrotestosterone (T/DHT) ratio, urinary steroid profiling and mutational analysis of *SRD5A2* gene. The biochemical hallmarks of 5α-RD2 deficiency include increased T/DHT ratio. However, several difficulties are observed in the DHT measurement leading to misdiagnosis. The mutational analysis of the *SRD5A2* has been proposed as the first line test in the investigation of 5α-RD2 deficiency.

Objective

The aim of this study was to screen the presence of *SRD5A2* mutations in twenty-one patients with 46,XY DSD.

Subjects and Methods

Twenty-one 46,XY DSD patients were studied. All of them were followed in the outpatient Developmental Endocrinology Unit of the Hospital das Clinicas, School of Medicine, University of Sao Paulo, Brazil.

All the patients presented ambiguous genitalia at birth. The age at hormonal evaluation range from 11 months to 35 years of age. The 46,XY DSD patients was distributed in two groups:

Group 1: six patients with known T/DHT ratio (range from 11 to 129)

Group 2: fifteen patients with unknown T/DHT ratio; 13 of them were previously orchiectomized and the hormonal profile was not available in the two other patients.

The entire coding region of *SRD5A2* gene was PCR amplified and directly sequenced using a BigDye Terminator in ABI PRISM 3130 DNA Sequencer.

In *silico* secondary structure analysis of SRD5A2 variants were done in the prediction websites PolyPhen and SIFT.

SRD5A2 CNVs was evaluated by MLPA technique.

Results

Group 1: four allelic variants of *SRD5A2* were identified in homozygous state and in one patient a compound heterozygous variant was found. *In silico* analysis of the two novel variants identified in this group, p.Trp140Glnfs*19 and p.Gly123Val, were predicted to be potentially damage. The variant p.Trp140Glnfs*19 was found in two unrelated patients with T/DHT ratio of 11 and 43. The patients in whom the mutations previously described (p.Gly123Val, p.Arg227*, and p.Gln126Arg) were identified presented the T/DHT ratio of 24, 46 and 129 respectively. The patient with a compound heterozygous allelic variants, p.Gly183Ser (damage) and p.Asp164Val (probably damage) presented T/DHT ratio of 39 (Table 1; Fig.1).

Table 1- Main clinical, hormonal and molecular data of patients with *SRD5A2* allelic variants

N	Social Sex	Age at hormonal evaluation (Ys)	T ng/dL	T/DHT ratio	*SRD5A2* allelic variants identified	Exon	*In silico* analysis	Described
1	F	14	222	24	p.Gly123Val/ p.Gly123Val	2	Probably damage	Present study
2	F	22	657	43	p.Trp140Glnfs*19/ p.Trp140Glnfs*19	2	Probably damage	Present study
3	F	18	1101	11	p.Trp140Glnfs*19/ p.Trp140Glnfs*19	2	Probably damage	Present study
4	M	19	461	46	p.Arg227*/ p.Arg227*	4	-	Previously [1]
5	F to M	23	980	129	p.Gln126Arg/ p.Gln126Arg	2	-	Previously [2]
6	F	18	1590	39	p.Gly183Ser/ p.Asp164Val	3	Damage/ Probably damage	Previously [3] + Present study
7**	F	-	-	-	p.Gly183Ser/ p.Gly183Ser	3	-	Previously [3]

F: female, M: male, Ys: years, Homo: Homozygous, Hetero: Heterozygous, Comp Hetero: Compound heterozygous

**Patient previously gonadectomized

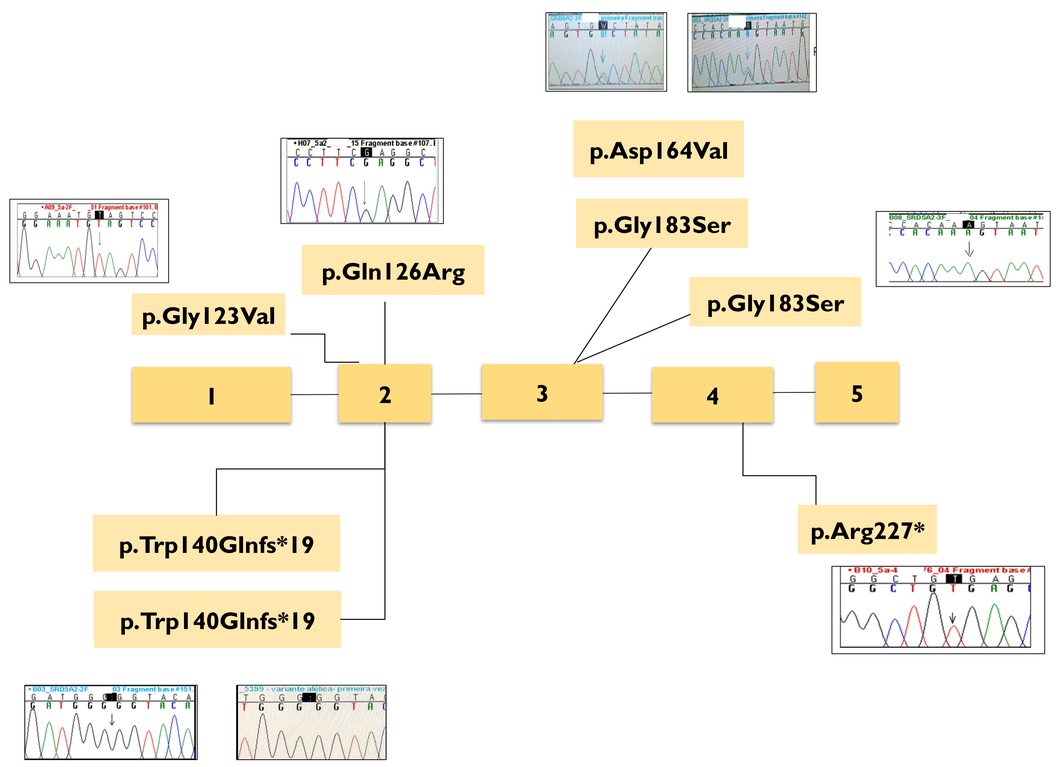

Fig. 1- Schematic representation of allelic variants in *SRD5A2* gene identified in seven 46,XY DSD patients .

SRD5A2 sequencing identified mutations or potential deleterious allelic variants in 100% (5 of 5) of patients with hormonal profile of 5α-RD2 deficiency and in one patient without hormonal profile of 5α-RD2 deficiency (T/DHT=11). In the group 2, the p.Gly183Ser mutation, was identified in homozygous state in a 46,XY DSD patient without etiologic diagnosis that was submitted in the childhood to a genital feminization surgery with bilateral gonadectomy (Table 1; Fig 1). *SRD5A2* CNV was not identified in these patients.

Conclusion

Sequencing of *SRD5A2* is a fast and effortless technique and should be used as the preferable approach for the diagnosis of 46,XY DSD due to 5α-RD2 deficiency

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