Whole-Exome Sequencing Analysis and Oligogenic Inheritance Investigation in a Brazilian Family with a Rare *NR5A1* Variant

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Introduction

Differences of sex development (DSD) comprise heterogeneous congenital conditions with atypical chromosomal, gonadal, or anatomical features [1]. Given that a same genetic variant may sometimes lead to distinct DSD phenotypes, the involvement of multiple variants in more than one gene has been proposed. Next-generation sequencing (NGS) has highlighted the potential of oligogenic inheritance in cases lacking a monogenic explanation [2, 3].

Objectives

This study aimed to investigate the possibility of an oligogenic inheritance pattern in a Brazilian family harboring a previously known *NR5A1* pathogenic variant (c.938G>A,p.Arg313His) (Figure 1) through whole-exome sequencing (WES) analysis (CAAE: 69225123.3.0000.5404).

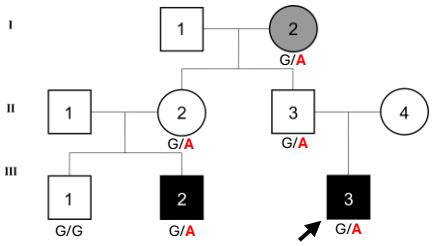


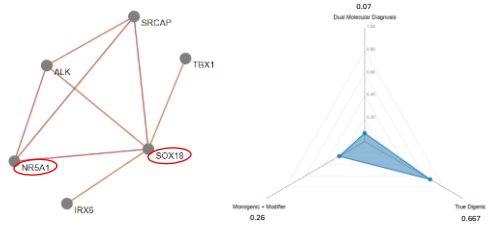
Figure 1. Pedigree of the family. Squares represent males and circles represent females. Black squares represent affected 46,XY DSD individuals, and gray circle represent a 46,XX individual with primary ovarian insufficiency. Subject I.2 is the proband's grandmother, and subject III.3 is the proband.

Methods

WES was performed on the proband and his father using the XGEN IDT Exome v2 library and NovaSeq 6000 (Illumina). Reads were aligned to GRCh38 via BWA, and variants were called with DeepVariant and filtered for MAF <0.001 (gnomAD v4.1.0) and CADD >20. Analysis was performed via Franklin (QIAGEN), using an in-house DSD gene panel and genes described in the Human Phenotype Ontology database for "ambiguous genitalia" and "abnormal external genitalia morphology". Oligogenic interaction was explored using the Oligogenic Resource for Variant Analysis (ORVAL) software. STRING and JASPER/FIMO/DBpred software's were used to determine protein interactions and motif identification, respectively.

Results

ORVAL analysis indicated that the strongest variant interaction was between *NR5A1* c.938G>A,p.(Arg313His) and *SOX18* c.664G>C,p.(Gly222Arg), with a median pathogenicity score of 0,975 (Figure 2).



Gene A	Gene B	Variant combinations			Median pathogenicity
		% Pathogenic	# Pathogenic	Total count	
NR5A1	SOX18	100.00	1	1	0.97

Figure 2. Results of the in-silico analysis in ORVAL and the digenic effect of the identified variants in *NR5A1* and *SOX18*.

While STRING indicated no protein-protein interaction, further exploration through DBpred highlighted residues that present a high affinity for DNA interaction, while JASPAR and FIMO identified four high-confidence binding sites (p <0.001) in *NR5A1* promoter region, suggesting that SOX18 may bind and act as a transcription factor (Figure 3).

>SOX18-201peptide:ENSP000
MQRSPPGYGAQDDPPARRDCAWAPGHGAAADTRGLA
AGPAALAAPAAPASPPSPQRSPPRSPEPGRYGLSPAGRGERQAADESRIRRPMNAFM
VWAKDERKRLAQQNPDLHNAVLSKMLGKAWKELNAAEKRPFVEEAERLRVQH
LRDHPNYKYRPRRKKQARKARRLEPGLLLPGLAPPQPPPEPFPAASGSARAFREL
PPLGAEFDGLGLPTPERSPLDGLEPGEAAFFPPPAAPEDCALRPFRAPYAPTELSRDPG
GCYGAPLAEALRTAPPAAPLAGLYYGTLGTPGPYPGPLSPPPEAPPLESAEPLGPAADL
WADVDLTEFDQYLNCSRTRPDAPGLPYHVALAKLGPRAMSCPEESSLISALSDASSAV
YYSACISG
##
Note: The residues
shown in**RED** color with bigger font size are **DNA-interacting** and residues shown in **BLACK** color with smaller font-size are **non-interacting**.

Figure 3. Analysis performed through DBpred, indicating the residues of the SOX18 protein with more affinity for DNA interaction [4].

Conclusion

Establishing a definitive molecular diagnosis in conditions driven by oligogenic inheritance is challenging. Functional studies could clarify if the *SOX18* variant interferes with *NR5A1* transcriptional activity. WES analysis provides valuable insights into the genetic background of these individuals and unravel the possibility of a synergistic effect between variants in genes involved in sex and gonadal development

References

[1] doi: 10.1136/adc.2006.098319. Epub 2006 Apr 19. PMID: 16624884; PMCID: PMC2082839; [2] doi: 10.1159/000445983. Epub 2016 May 12. PMID: 27169744; [3] doi: 10.1038/s41431-018-0202-7. Epub 2018 Jun 11. PMID: 29891883; PMCID: PMC6117353; [4] doi: 10.1093/bib/bbac322. PMID: 35943134.

ACKNOWLEDGMENTS

This work was supported by Coordenação de de Aperfeiçoamento de Pessoa de Nível Superior (CAPES – 3300301754D0) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – 2024/05003-2)