The Journal of Obstetrics and Gynecology of India (March–April 2024) 74(2):136–143
https://doi.org/10.1007/s13224-023-01876-3

ORIGINAL ARTICLE

In Silico Analysis of Functional SNPs in Genes of Complete Androgen Insensitivity Syndrome (CAIS): A Retrospective, Case–Control Study

Shalaka S. Ramgir[1] · Sivakumar Annamalai[2] · V. G. Abilash[3]

Received: 28 November 2022 / Accepted: 4 October 2023 / Published online: 6 December 2023

Abstract

Background Complete androgen insensitivity syndrome (CAIS) is one of the categories of androgen insensitivity syndrome (AIS) described as complete failure of the cell to react to androgens with external genitalia of a normal female. People with AIS condition are genetically male, with XY karyotype in each cell, but their bodies are unable to respond to male sex hormones (called androgens). It is associated with infertility as well as developing cancerous conditions. The genetic association of CAIS involves polymorphism of genes such as NR5A1, SOX9, SRD5A2, CBX2, GATA4, and SRY. Their mutation and participation in genetics of CAIS can be studied by Single Nucleotide polymorphism (SNP) analysis which is a way to detect genetic variations. SNP in coding region leads to synonymous and non-synonymous mutations. Hence, this study highlights analysis of SNPs associated with CAIS. Our aim is to study SNP analysis of NR5A1, SOX9, SRD5A2, CBX2, GATA4, SRY genes in Complete Androgen Insensitivity Syndrome.

Methods SIFT and Polyphen analysis was performed for all the genes and samples were subjected for PCR-SSCP technique.

Results SNPs were analyzed for the genes associated with CAIS. Benign and damaging SNPs were identified. DNA Samples were amplified using PCR technique and they will be analyzed using Single-strand conformation polymorphism (SSCP).

Conclusions As SNPs have decreased stability, damaging and benign character, they can be used as candidate hallmarks in study of Complete Androgen Insensitivity Syndrome.

Keywords Complete androgen insensitivity syndrome · SRD5A2 · CBX2 · SIFT · Polyphen · Single-strand conformation polymorphism

Introduction

Congenital atypical sex problems are called disorders of sex development (DSD) when anatomical, chromosomal, or gonadal aspects are affected [1]. Testicular Feminisation syndrome also known as Complete Androgen Insensitivity Syndrome (CAIS) (OMIM: 300068) is a category of DSD in which affected individuals have female external genitalia since birth with 46, XY karyotype caused by the cell's absolute resistance to androgens [2]. When an XY zygote instructs the bipotential gonad to convert into a testis (sex determination), the testis in turn secretes enough active testosterone to produce the male phenotype. This is all that is necessary for male fetal development [3]. Complete androgen insensitivity syndrome (CAIS) can be suspected at the adolescent age when primary amenorrhea is reported or earlier as testis is found in a female infant who is undergoing repair for inguinal hernia [3].

In CAIS, regression of Mullerian ducts occurs which results in a blind-ending vagina and the absence of a uterus [3, 4]. The disorder presents itself in X-linked recessive inheritance. The main gene involved is the AR gene which encodes for Androgen Receptors. In CAIS, there is the insensitivity of these receptors which leads to female phenotype

Shalaka Sudhir Ramgir is an Assistant professor; Sivakumar Annamalai is an Quality Assurance-Executive; Abilash V.G. is an associate professor.

✉ Shalaka S. Ramgir
shalaka.ramgir@gmail.com

[1] Assistant professor at Symbiosis Institute of Health Sciences (SIHS), Symbiosis International (Deemed University), Mulshi, Lavale, Maharashtra 412115, India

[2] Quality Assurance-Executive, GLR Laboratories Pvt. Ltd., Chennai, Tamilnadu 600068, India

[3] Associate Professor, School of Biosciences and Technology, Vellore Institute of Technology (VIT), Vellore, Tamilnadu 632014, India

[5]. The pathophysiology of CAIS with fetal sex development is mentioned in Fig. 1 [6]. In addition to the AR gene, NR5A1 (Nuclear Receptor Subfamily Five Group A member 1 gene) is a crucial transcriptional regulator of genes located in the hypothalamic-pituitary-steroidogenic axis and is thought to play an important role in the pathophysiology of androgen insensitivity syndrome [7, 8]. The gonadal development is controlled by Steroidogenic factor 1 (SF1), which is encoded by the NR5A1 gene [7]. On chromosome 9q33 at position 30, an autosomal gene NR5A1 gene is located [8]. Sertoli and Leydig cells of the developing testis, along with Sertoli cells of the prepubertal and adult testis, as well as various cell types in the fetal, postnatal, prepubertal, and mature ovary, expressed by the NR5A1 gene which has a flexible hinge region, a ligand-binding domain (LBD) as two zinc fingers, and AF-1 and AF-2 as two activation function domains [9].

To analyze the role of the NR5A1 gene in the pathogenesis of Testicular feminization syndrome, we carried out a mutation analysis of the NR5A1 gene with the help of bioinformatics tools and molecular biology techniques. The phenotype of the host organism is impacted by SNPs (Single Nucleotide Polymorphisms) that occur in the coding area and may result in a substitution of an amino acid which results in protein production [10]. Bioinformatics tools allow for studying the intensity of mutations and also allow for predicting the effect of substitution on phenotype. With the help of bioinformatics tools, we carried out a mutation analysis of the NR5A1 gene. We also studied other genes SOX9, SRD5A2, CBX2, GATA4, and SRY (along with NR5A1) which are also sometimes involved in CAIS.

A protein that is produced by the SOX9 gene is crucial for embryonic development. The SOX9 protein is particularly important for the skeleton and reproductive system to mature. The SOX9 protein regulates the activity of other genes by binding to particular DNA sequences. Together with the expression pattern of SOX9 in developing bone and testis, SOX9 is proven to be necessary for normal progress of the testis in males and of bone in both males and females.

The sex-determining region (SRY) gene determines the DNA-binding protein known as the testis-determining factor (TDF). The TDF protein is responsible for the onset of male sex determination in humans. This protein is involved in the synthesis of androgens, which are hormones that control sexual development in males. In particular, this protein is in charge of the process by which testosterone is transformed into the stronger androgen, i.e., dihydrotestosterone (DHT) in the tissues of the male reproductive system.

Through chromatin remodeling and histone modification, the polycomb multiprotein complex, which is encoded by the SRY gene, is essential for maintaining many genes' transcriptionally repressed status during growth. Male-to-female gonadal sex reversal is the result of this gene's disruption in mice. Gonadal dysgenesis is also linked to SRY gene mutation.

To perform mutation analysis, we retrieved data from dbSNP which is hosted by the national center for biotechnology Information. To evaluate the tolerance index, we performed SIFT (Sorting Intolerant from Tolerant) analysis. Further Polyphen 2 analysis was done to predict the possible impact of amino acid substitution for all genes. We also carried out a molecular analysis of the NR5A1 gene using the PCR-SSCP technique.

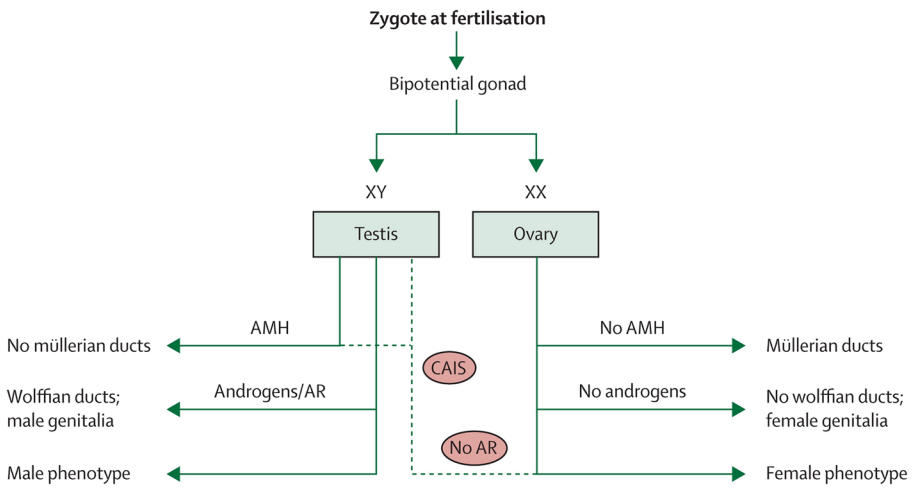

Fig. 1 Pathway indicating pathogenesis of CAIS with fetal sex growth [18]. *AR* Androgen receptor, *CAIS* Complete androgen insensitivity syndrome, *AMH*Antimüllerian hormone

Methods

This retrospective, case–control study includes samples from CAIS (46, XY) patients (n = 22) recruited from the Institute of Obstetrics and Gynaecology (IOG), Chennai, Tamilnadu, India, and approved by the Institutional ethical community, Vellore Institute of technology, Vellore, India. The controls (n = 24) (Female = 12 Male = 12) were selected as people having normal sexual development with the absence of any fertility problems. The data collection sheet included information such as age, genotypic makeup, phenotype, and menses.

Genotypic Determination

2 ml of intravenous blood was collected in an EDTA vacutainer and sampled from all the patients and controls. The DNA was extracted using standard lab protocol and quantified by UV spectrophotometer and as well as qualified by (0.8%) Agarose Gel Electrophoresis. Amplification of DNA was carried out using Eppendorf master cycler gradient with 5′GCAGAGTCACGTGGGGGCAGAG3′ and 5′GAAGGAGGCTGGCCATTAGAG3′ as forward and reverse primer, respectively. 20 µl of PCR mixture was used which contain 10 pmol concentration of primer, 5 µl of Milli-Q water, and 10 µl of master mix (ampliqon). 3µL of DNA was amplified for 35 cycles with denaturation at 95 °C for 45 s, annealing at 56 °C for 50 s, and extension at 72 °C for 1 min by PCR in thermal cycling. PCR products were visualized under a UV transilluminator and photographed with a Gel Dock system. Screening for mutation is being done by using single-strand confirmation polymorphism. Amplified PCR products will be subjected to a protocol of Single-strand conformation polymorphism (SSCP), i.e., fixation, washing (two times), fixation, sensitizing, developing, and finally the termination.

In-Silico Analysis

Data Retrieval

Single Nucleotide Polymorphism (SNP) was retrieved using a database hosted by the National Centre for Biotechnological Information (NCBI). All the pathogenic SNPs were retrieved for the NR5A1 gene. To study the impact of other genes in CAIS pathogenesis, we also retrieved pathogenic SNPs for SOX9, CBX2, GATA2, SRD5A2, and SRY genes which are also reported to have contributed in Complete Androgen Insensitivity Syndrome. NCBI server can be reached at (http://www.ncbi.nlm.nih.gov/snp/).

Estimation of Missense Mutation of SNPs Using a Sequence Homology Tool (SIFT)

SIFT (Sorting Intolerant From Tolerant) prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences, collected through PSI-BLAST. SIFT can be applied to naturally occurring nonsynonymous polymorphisms or laboratory-induced missense mutations. It is a multistep procedure in which the respective protein sequence, (1) looks for similar sequences, (2) chooses closely related sequences that may share similar functions, (3) obtains multiple alignments of these chosen sequences, and (4) calculates normalized probabilities for all possible substitutions at each position from the alignment. Substitutions of a teach position with normalized probabilities less than a tolerance index of 0.05 are predicted to be intolerant or deleterious; those greater than or equal to 0.05 are predicted to be tolerated [11–13].

Predicting Effect of a Single-Residue Substitution on Protein Function Using Polyphen-2 Web SERVER

PolyPhen 2 (Polymorphism Phenotyping version 2) is a tool that predicts the possible impact of an amino acid substitution on the structure and function of a human protein. The PolyPhen-2Web interface can be reached at http://genetics.bwh.harvard.edu/pph2/. The input form at this URL allows querying for a single individual amino acid substitution or a coding, non-synonymous SNP annotated in the dbSNP database. The prediction is based on several sequence, phylogenetic, and structural features characterizing the substitution. For a given amino acid substitution in a protein, PolyPhen-2 extracts various sequence and structure-based features of the substitution site and feeds them to a probabilistic classifier [14].

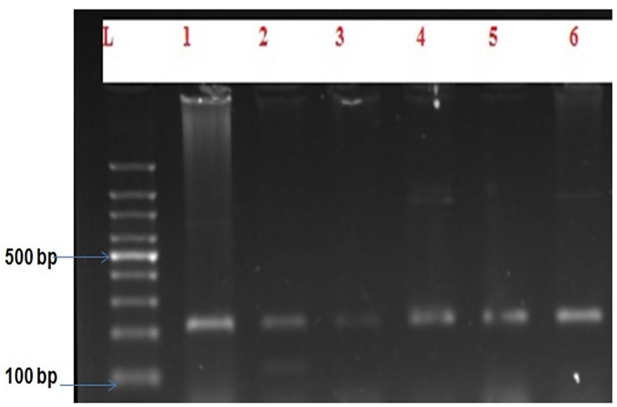

Fig. 2 Example of Exon 4 of NR5A1 gene with single primer PCR with base pair size is 286 (Lane L- 100 bp ladder, lane 1- Control DNA, lane 2- female DNA, lane 3- Water, lane 4- DNA of Patient CAIS1, lane 5- DNA of Patient CAIS 2, lane 6- DNA of Patient CAIS 3)

Table 1 SIFT analysis of SNPs associated with different genes of Complete androgen insensitivity syndrome

SNP	Nucleotide change	Amino acid change	Amino Acid	Using homologues in the protein alignment	
				Prediction	Score
a. NR5A1 gene					
rs104894119	G/T	R92Q	R	Tolerated	1
			Q	Damaging	0
rs104894120	G/A	L437Q	L	Tolerated	1
			Q	Damaging	0
rs104894125	T/A	M78I	M	Tolerated	1
			I	Damaging	0
rs104894126	C/A	G91S	G	Tolerated	1
			S	Damaging	0
rs121918655	C/T	D293N	D	Tolerated	1
			N	Damaging	0
rs863224904	T/G	Y404K	Y	Tolerated	1
			K	Damaging	0
rs104894118	G/T	R225L	R	Tolerated	1
			L	Damaging	0
rs104894123	C/A	C16T	C	Tolerated	1
			T	Damaging	0.4
rs104894124	G/A	V15M	V	Tolerated	1
			M	Damaging	0.03
rs121918654	GC/AA	G35E	G	Tolerated	0.14
			E	Damaging	0.3
rs121918656	G/A	M1I	M	Tolerated	1
			I	Damaging	0
b. SOX9 gene					
rs28940282	C/T	H165Y	H	Tolerated	1
			Y	Damaging	0
rs104894647	A/G	K173E	K	Tolerated	1
rs137853128			E	Damaging	0
rs137853129	C/A	A76E	A	Tolerated	1
			E	Damaging	0
rs137853130	C/G	F154L	F	Tolerated	1
			L	Damaging	0.3
rs193920972	G/A	A158T	A	Tolerated	1
			T	Damaging	0
rs202115157	G/A	A21T	A	Tolerated	1
			T	Damaging	0
	G/A	K173L	K	Tolerated	1
			L	Damaging	0.4
c. SRY gene					
rs104894956	T/C	F109S	F	Tolerated	1
			S	Damaging	0
rs104894957	G/C	V60L	V	Tolerated	1
			L	Damaging	0
rs104894959	C/G	I90M	I	Tolerated	1
			M	Damaging	0
rs104894961	G/A	C253S	C	Tolerated	1
			S	Damaging	0
rs104894962	G/A	K405E	K	Tolerated	1
			E	Damaging	0

Table 1 (continued)

SNP	Nucleotide change	Amino acid change	Amino Acid	Using homologues in the protein alignment	
				Prediction	Score
rs104894963	G/A	P217A	P	Tolerated	0.5
			A	Tolerated	0.14
rs104894964	T/C	K106I	K	Tolerated	1
			I	Damaging	0
rs104894960	A/T	K106I	K	Tolerated	1
			I	Damaging	0
rs104894965	G/A	M64I	M	Tolerated	1
			I	Damaging	0
d. CBX2 gene					
rs121908255	C/T	P98L	P	Tolerated	0.63
			L	Tolerated	0.26
rs121908256	G/A	R443H	R	Tolerated	0.21
			H	Tolerated	0.34
e. GATA4 gene					
rs56208331	G/A	D426N	D	Tolerated	1
			N	Damaging	0
rs56298569	C/A	Q317E	Q	Tolerated	1
			E	Damaging	0
rs104894073	G/A	G297S	G	Tolerated	1
			S	Damaging	0
rs104894074	C/T	S52F	S	Tolerated	0.38
			F	Damaging	0
rs115099192	C/A	P408Q	P	Tolerated	1
			Q	Damaging	0
rs146017816	C/T	A443V	A	Tolerated	1
			V	Damaging	0
rs368489876	G/A	E360K	E	Tolerated	1
			K	Damaging	0
rs387906770	C/T	R43W	R	Tolerated	1
			W	Damaging	0
rs387906771	C/T	T281M	T	Tolerated	1
			M	Damaging	0
f. SRD5A2 gene					
rs9332964	G/A	R227Q	R	Tolerated	1
			Q	Tolerated	0.12
rs121434244	C/T	R246W	R	Tolerated	1
			W	Damaging	0
rs121434245	T/A	L55Q	L	Tolerated	1
			Q	Damaging	0
rs121434246	G/A	G115D	G	Tolerated	0.4
			D	Damaging	0
rs121434248	C/T	R227T	R	Tolerated	1
			T	Damaging	0
rs121434249	G/A	A228T	A	Tolerated	1
			T	Damaging	0
rs121434250	G/A	G196S	G	Tolerated	1
			S	Damaging	0
rs121434251	A/G	H231R	H	Tolerated	1

Table 1 (continued)

SNP	Nucleotide change	Amino acid change	Amino Acid	Using homologues in the protein alignment	
				Prediction	Score
			R	Damaging	0
rs121434252	C/G	P212R	P	Tolerated	1
			R	Damaging	0.03
rs121434253	G/T	E197D	E	Tolerated	1
			D	Damaging	0

a Nucleotide change: g- guanine, a- adenine, t- thymine, c-cytosine

b Amino acid denoted with one letter symbol as per IUPAC-IUB commission on biochemical nomenclature

*Tolerated: The sift score > 0.05

**Damaging: The sift score ≤ 0.05 are determined by the algorithm to damaging or deleterious amino acid substitutions

Results

Molecular Analysis

Exon 4 of the NR5A1 gene was amplified using a Polymerase chain reaction with a base pair size is 286 (Fig. 2). Patient details were studied and observed.

SIFT Analysis

The SIFT server was filled with SNP IDs with amino acid changes linked to missense SNPs. It is estimated that substitutions at each location with normalized probabilities below a tolerance index of 0.05 are either intolerant or harmful, but substitutions at those positions with normalized probabilities at or above 0.05 are accepted. The results of SIFT analysis are described in Table 1. According to the tolerance index, SNPs in the genes NR5A1, SOX9, CBX2, SRY, GATA4, and SRD5A2 have tolerance indices that are less than 0 and are therefore detrimental.

Polyphen Analysis

Polyphen server works with UNIPROT gene ID. Polyphen score of 0 indicates benign and values near to 1 are predicted to be probably or possibly damaging. Table 2 shows 2 benign SNPs for the NR5A1 gene, SOX9 shows 2 benign SNPs, SRY gene shows 1 benign SNPs, CBX2 gene shows both the benign SNPs, GATA4 and SRD5A2 gene shows one benign SNPs each, respectively.

Discussion

To diagnose DSD patients with cases of regular testosterone secretion, despite AR mutations and to identify their female relatives who may be susceptible to acquiring primary ovarian insufficiency and provide them with reproductive counseling, it is critical to analyze the phenotypic manifestation of NR5A1 mutations [8]. Complete androgen insensitivity syndrome results in a complete female phenotype with primary amenorrhea. In a previous study, it is concluded that CAIS without AR gene mutation remains a tough challenge because of possible mutations in NR5A1 and SOX9 genes. Therefore, it is important to analyze other gene mutations other than the AR gene [15]. The management of CAIS focuses on appropriate hormone replacement. An understanding of the current limitations of cancer screening in women who have had their gonads removed, as well as the choices available if vaginal expansion is required, if their gonads have been separated [5]. A study performed by S. F. Ahmed et al. categorizes the genes according to their role in gonadal development and DSDs. According to a study, low-frequency gonadal development genes—CBX2, GATA-4, essential genes in CNV arrays—SOX3, SOX9, and DMRT1 were categorized [1]. The AR gene is frequently mutated in androgen insensitivity syndrome. One such study is conducted where reports were showing (p.F804S) mutation in the AR gene [16]. A variety of reproductive phenotypes, including male infertility, are frequently observed in cases of adrenal insufficiency, although disruption of SF-1 is rarely linked to this condition [17]. A study by Ralf Werner, et al. suggested that the androgen receptor has undergone a variety of mutations that have been studied, providing insight into the intricate pathways of intracellular processing and signal transduction that use the androgen receptor [6]. A study conducted by Nowacka-Woszuk et al. suggested that SSCP is the best method to check polymorphisms. They carried out a study with SOX9 and SRY genes on suspected androgen insensitivity syndrome. Negative SSCP results ruled out the possibility of AIS. It indicates the important role of SOX9 and SRY in androgen insensitivity syndrome [18]. To consider health issues and facilities to be provided, Jennifer M. Beale, et al. conducted a study in which it is suggested that health care input when required should be provided by a multidisciplinary

Table 2 Polyphen analysis for different genes involved in complete androgen insensitivity syndrome

SNP	Nucleotide change	Amino acid change	Amino acid	Prediction	Score
a. NR5A1 gene					
rs104894119	G/T	R92Q	R	Probably damaging	0.932
rs104894125	T/A	M78I	M	Probably damaging	0.998
rs104894126	C/A	G91S	G	Probably damaging	0.984
rs863224904	T/G	Y404K	Y	Probably damaging	1
rs104894118	G/T	R225L	R	Probably damaging	1
rs121918654	GC/AA	G35E	G	Probably damaging	0.981
rs387906690	C/T	P131L	P	Benign	0.001
rs775441984	G/A	E51K	E	Benign	0.337
b. SOX9 gene					
rs28940282	C/T	H165Y	H	Possibly damaging	0.666
rs104894647	A/G	K173E	K	Probably damaging	0.891
rs137853129	C/G	F154L	F	Possibly damaging	0.315
rs929651	G/A	A371A	A	Benign	0.083
rs80338688	C/A	Y440T	Y	Benign	0.276
c. SRY gene					
rs104894956	T/C	F109S	F	Probably damaging	1
rs104894957	G/C	V60L	V	Possibly damaging	0.883
rs104894959	C/G	I90M	I	Probably damaging	1
rs104894964	T/C	K106I	K	Possibly damaging	0.7
rs104894960	A/T	K106I	K	Probably damaging	1
rs104894965	G/A	M64I	M	Possibly damaging	0.875
rs104894958	C/T	Q93T	Q	Benign	0.02
d. CBX2 gene					
rs121908255	C/T	P98L	P	Benign	0.154
rs121908256	G/A	R443H	R	Benign	0.000
e. GATA4 gene					
rs115099192	C/A	P408Q	P	Probably damaging	0.991
rs368489876	G/A	E360K	E	Possibly damaging	0.549
rs387906769	C/T	P163S	P	Benign	0.256
f. SRD5A2 gene					
rs9332964	G/A	R227Q	R	Possibly damaging	0.519
rs121434245	T/A	L55Q	L	Probably damaging	0.950
rs121434248	C/T	R227T	R	Probably damaging	0.971
rs121434249	G/A	A228T	A	Probably damaging	1
rs121434251	A/G	H231R	H	Probably damaging	1
rs121434253	G/T	E197D	E	Probably damaging	0.999
rs104893667	C/G	Y26T	Y	Benign	0.057

a Nucleotide change: g- guanine, a- adenine, t- thymine, c-cytosine

b Amino acid denoted with one letter symbol as per IUPAC-IUB commission on biochemical nomenclature

team who have appropriate expertise. Holistic care should include the consideration of the risk of cancer, prevention of osteoporosis, advice on hormones, sexual health, and fertility options, and ongoing support to optimize quality of life and well-being [19].

Another example of an SRY study reports a novel SRY sporadic mutation due to a single nucleotide insertion at position 230 (c.230_23LinsA) was identified as the cause of the disease in this patient. Target region captured next-generation sequencing was found to be an effective method for the molecular genetic testing of complete gonadal dysgenesis (46, XY CGD) [20]. Genes in male sexual growth if get mutated then result in dysfunction of androgen receptors and therefore result in female phenotype.

Throughout childhood and adolescence, CAIS therapy continues to present a unique challenge, in particular regarding the gonadectomy timing, hormone therapy types, and psychological problems [21]. Mutation analysis of these

genes would help in the prediction of gene mutations and increases the chances of accurate diagnosis. Therefore, helps in providing good health care and management options for affected individuals.

Conclusion

Through integrated bioinformatics and molecular analysis, we found mutations in NR5A1 along with SRD5A2, CBX2, GATA4, SRY, and SOX9 genes which showed damaging and benign features in Complete Androgen Insensitivity Syndrome (CAIS). Hence, these mutational analysis findings provided additional diagnostic value for CAIS, including important insights into the molecular pathogenesis of CAIS. However, more studies are required to confirm these findings.

Acknowledgements The authors would like to thank the management of Vellore Institute of technology for providing excellent facilities to carry out this study.

Funding None.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethics Approval All processes executed in research regarding human individuals had been approved by the Institutional ethical community, Vellore Institute of Technology, Vellore, India.

Informed consent Informed consent was obtained from all the individual participants included in this study.

References

1. Dong Y, Yi Y, Hu H, Zhang R, et al. A novel mutation in the SRY gene of a Chinese 46, XY female patient with unilateral mixed germ cell tumor. J Reprod Contracept. 2016;27:82–8.
2. Hornig NC, Holterhus PM. Molecular basis of androgen insensitivity syndromes. Mol Cell Endocrinol. 2021;523: 111146.
3. Tadokoro R, Bunch T, Schwabe JW, Hughes IA, Murphy JC. Comparison of the molecular consequences of different mutations at residue 754 and 690 of the androgen receptor (AR) and androgen insensitivity syndrome (AIS) phenotype. Clin Endocrinol. 2009;71:253–60.
4. Mongan NP, Tadokoro-Cuccaro R, Bunch T, Hughes IA. Androgen insensitivity syndrome. Best Pract Res Clin Endocrinol Metab. 2015;29:569–80.
5. Infante JB, Alvelos MI, Bastos M, Carrilho F. Lemos MC Complete androgen insensitivity syndrome caused by a novel splice donor site mutation and activation of a cryptic splice donor site in the androgen receptor gene. J Steroid Biochem Mol Biol. 2016;155:63–6.
6. Hughes IA, Werner R, Bunch T, Hiort O. Androgen insensitivity syndrome. Semin Reprod Med. 2012;30(05):432–42.
7. Wu JY, McGown IN, Lin L, Achermann JC, et al. A novel NR5A1 variant in an infant with elevated testosterone from an Australasian cohort of 46, XY patients with disorders of sex development. Clin Endocrinol. 2013;78:545–50.
8. Fabbri HC, de Andrade JG, Soardi FC, de Calais FL, et al. Cys-65Tyr mutation in NR5A1 gene in three 46, XY siblings with normal testosterone levels and their mother with primary ovarian insufficiency. BMC Med Genet. 2014;15:1.
9. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4:1073–81.
10. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;76(1):7–20.
11. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. 2001;11:863–74.
12. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31:3812–4.
13. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet. 2006;7:61–80.
14. Rajasekaran R, Sudandiradoss C, Doss CG, Sethumadhavan R. Identification and in silico analysis of functional SNPs of the BRCA1 gene. Genomics. 2007;90:447–52.
15. Jääskeläinen J. Molecular biology of androgen insensitivity. Mol Cell Endocrinol. 2012;352:4–12.
16. Wang S, Xu H, An W, Zhu D, Li D. Mutational analysis of the androgen receptor gene in two Chinese families with complete androgen insensitivity syndrome. Exp Ther Med. 2016;11:2277–83.
17. Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. Best Pract Res Clin Endocrinol Metab. 2015;29:607–19.
18. Nowacka-Woszuk J, Nizanski W, Klimowicz M, Switonski M. Normal male chromosome complement and a lack of the SRY and SOX9 gene mutations in a male pseudohermaphrodite dog. Anim Reprod Sci. 2007;98:371–6.
19. Beale JM, Creighton SM. Long-term health issues related to disorders or differences in sex development/intersex. Maturitas. 2016;94:143–8.
20. Hines M, Ahmed SF, Hughes IA. Psychological outcomes and gender-related development in complete androgen insensitivity syndrome. Arch Sex Behav. 2003;32:93–101.
21. Lanciotti L, Cofini M, Leonardi A, Bertozzi M, et al. Different clinical presentations and management in complete androgen insensitivity syndrome (CAIS). Int J Environ Res Public Health. 2019;16(7):1268.