Complete Androgen Insensitivity Syndrome Due To a Novel N-terminal Domain Mutation of Androgen Receptor Gene: Clinical Profile and Psychological Evaluation of a New Patient in India- Clinical Case report

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ABSTRACT

Introduction: Mutations in *AR* gene results in androgen insensitivity syndrome and is broadly categorised as- Partial androgen insensitivity (PAIS), mild androgen insensitivity (MAIS) and complete androgen insensitivity (CAIS). We present a 19 year old patient. She had gonadectomy done at 6 years of age. Therefore endocrine (hormonal profile) evaluation now could not be used to make a definitive diagnosis. Molecular analysis of *AR* gene revealed a novel mutation in the patient. **Case Presentation:** A child with female phenotype, presented with bilateral inguinal hernia at the age of 5 years. She was born full term at home and is the elder of the two siblings. Clinical examination revealed bilateral descended testes in the labial folds and separate urethral and vaginal opening. Hormonal profile at 5 years of age revealed LH, FSH, Testosterone and Estradiol to be within normal limits. She had gonadectomy done at 6 years of age. Psychological assessment of the patient revealed female gender identity which is concordant with her sex of rearing. WHO questionnaire indicated medium quality of life. Molecular analysis of *SRD5A2* gene showed a normal sequence. A novel mutation of p.T105R in exon 1 of *AR* gene confirmed the diagnosis of AIS. **Conclusion:** We describe for the first time a missense novel mutation of p.T105R in exon 1 of *AR* gene. The definitive diagnosis of this patient was confirmed by molecular analysis.

Key words: Androgen-Insensitivity Syndrome, Androgen Receptor gene, Gender Identity, N-terminal domain

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Conflict of Interest: None

INTRODUCTION

Androgens are essential for male sexual development as well as for differentiation. Their physiological functions are mediated through androgen receptor (AR), a member of steroid nuclear receptor family. Gene encoding for androgen receptor is located on chromosome Xq11-12 and is inherited in an X-linked recessive manner [1]. AR is a 110 KD single chain protein encoded by 8 exons and contains three main domains: N-terminal domain (NTD) is coded by exon 1, DNA binding domain consists of 2 zinc finger motifs and coded by exon 2-3 and Ligand binding domain (LBD) is coded by exon 4-8 [2]. Mutations in *AR* gene result in varying degree of androgen insensitivity (AIS). Based on the degree of masculinization, AIS is broadly categorised in two groups-Partial androgen insensitivity (PAIS) and complete androgen insensitivity (CAIS). Patients with CAIS (also known as testicular feminizing syndrome) have female like external genitalia. Testes may be present in the inguinal region or in the labial folds. If undiagnosed during infancy, they present with inguinal hernia during childhood or with primary amenorrhoea at puberty. They have normal breast development and sparse or absent pubic and axillary hair growth [3].

More than 500 different mutations in *AR* gene have been described to be associated with AIS (http://www.mcgill.ca/androgendb/). Majority of these mutations are single base substitution, while few insertions and deletions have been described. The distribution of mutations is unequal. Mutation hot-spots are mainly located on exon 4-8, while relatively less mutation are present on exon1. Here we describe a novel mutation of T105R in exon1 of *AR* gene in a patient with CAIS.

CASE PRESENTATION:

A phenotypic female child presented with bilateral inguinal hernia at the age of 5 years (III.27). She was born full term at home and is the elder of the two siblings (Figure 1.I). Clinical examination revealed bilateral descended testes in the labial folds and separate urethral and vaginal opening. No uterus and mullerian structures were observed by ultra-sonography and CT scan. Genitogram showed a well formed vaginal pouch and absent cervical indentation.

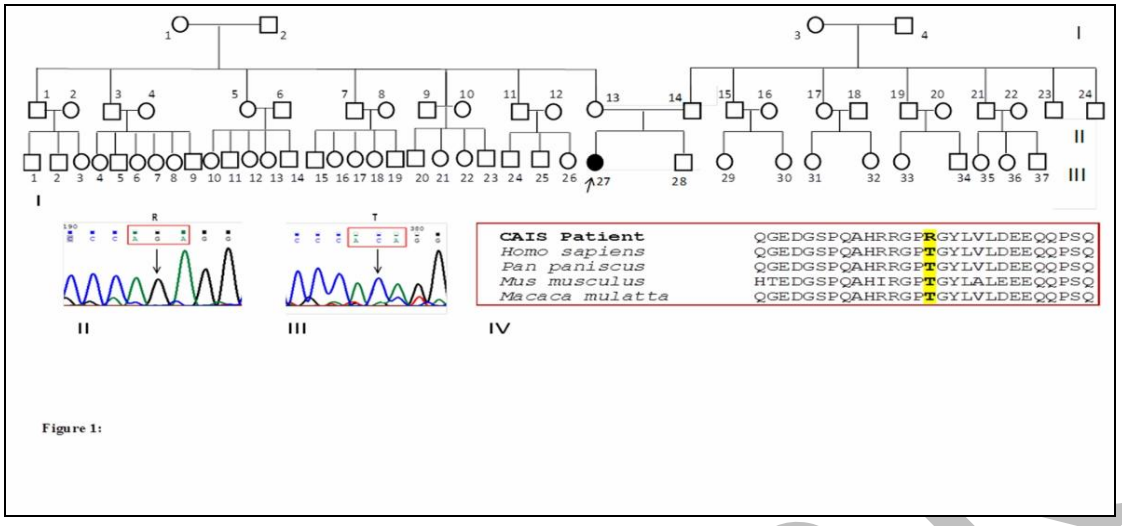

Figure 1: (I) Three generation pedigree. (II) Electropherogram showing sequence variation in patient (AGA). (III) Normal sequence in parents (ACA). (IV) Sequence alignment of various species showing the conserved Threonine residue at p.105 (exon1) of AR protein.

The cytogenetic studies carried by Giemsa staining on 72 hour peripheral blood culture revealed 46, XY karyotype of the patient. After several sessions of discussion with parents a female sex of rearing was decided and vaginoplasty was planned. Bilateral gonadectomy was done at the age of 6 years. Hormonal levels at time of surgery were LH < 0.7mIU/ ml, FSH < 0.3mIU/ml, Testosterone (T) < 0.025ng/ml and Estradiol < 20pg/ml respectively.

Histopathology revealed normal testicular tissue. At the age of 8.5 years, clitoral-vaginoplasty was done. Hormonal profile after surgery, at the age of 13 years was LH 25.15mIU/ ml, FSH 95.39mIU/ ml, T 0.027ng/ml respectively. Subsequently she was advised to take estrogen. Axillary and pubic hair was absent and she is now 19 years old. This report is a part of an on-going study on 46, XY DSD patients [4].

Detailed psychological assessment of patient was done at the age of 17 yrs. Psychological assessment was carried out using the following assessment tools: 1) Malin's Intelligence Scale for Indian Children 2) Strength and difficulties questionnaire 3) Gender Identity/ Gender Dysphoria questionnaire for adolescents and adults 4) Coopersmith Self-esteem inventory 5) WHO Quality of Life [5].The assessment was completed over multiple sessions. Eye-to-eye contact could be established and rapport could be formed.

The patient was communicative, cooperative and compliant with test instructions. Attention could be aroused and sustained for the required period of assessment.

Malin's Intelligence scale for Indian children was administered to assess intellectual functioning. IQ was found to be in the range of 90-94 indicating population average level of intellectual functioning. The Strength and Difficulties Questionnaire was applied to assess for adjustment and behavioural problems in children. The symptom scores were calculated and classified according to 3 categories, normal and abnormal for each of the symptom scales.

Table 1 gives a detailed description of patient`s psychological report.

Table 1: Detailed psychological assessment scores and interpretation of the patient with CAIS

Tests	Variable	Score	Interpretation
Malin's Intelligence Scale for Indian Children	IQ	90-94 (Range)	Population Average Intellectual Functioning
Strengths and Difficulties Questionnaire	Emotional Symptoms Conduct Problems Hyperactivity Peer Problems Prosocial Total Difficulties	2 0 2 2 10 6	Normal Normal Normal Normal Normal Normal
Gender Identity/Gender Dysphoria for adolescents and adults	Gender Identity Gender Dysphoria	4.84	Female Gender Identity No Gender Dysphoria
Coopersmith Self Esteem Inventory	General Self Social Self Peers Home Parents School Academic Total Self esteem score	15 5 6 7 68	 (50^{th} percentile) Medium self esteem
WHO QOL-BREF	Physical health Psychological Social relationships Environment	56 69 56 56	Scored in a positive direction with higher scores indicating a higher quality of life (range of scores: 0-100)

The patient's scores were within the normal ranges across each of the symptoms scales namely; emotional symptoms, conduct problems, hyperactivity and peer problems. The Coopersmith Self Esteem Inventory (School Version) was administered to assess for self-esteem. The total self-esteem score of 66 corresponded to a percentile value of 50 which implies a medium level of self-esteem. The score on the lie scale was high which indicates a disclosure bias or in other words she was not forthcoming with information or probably did not report as many problems as she experienced in actuality.

Gender Identity/Gender Dysphoria was assessed using Gender identity/Gender dysphoria questionnaire for adolescents and adults. The patient's score was 4.85 which is above the cut-off score 3.00 (scores above the cut-off indicate absence of gender dysphoria). The gender identity of the patient was female which was concordant with her sex of rearing. WHO-QOL-BREF was administered to assess quality of life across 4 domains namely: physical health, psychological health, social relationships and environment. The patient scores were in the mid interquartile range indicating medium quality of life.

Genomic DNA was extracted from 3ml peripheral blood by standard phenol-chloroform method. Polymerase chain reaction (PCR) was carried out for *AR* gene and *SRD5A2* gene. Amplification of *SRD5A2* gene was carried out as described earlier [4]. Eleven sets of primers for *AR* gene were designed using online UCSC Genome Bioinformatics Site.

The sequence primers, for amplification of exon 1 (*AR* gene) were F 5` GACTACCGCATCATCACAGC 3` and R 5` TTCGGATACTGCTTCCTGCT 3`, The products were sequenced directly by Big Dye v3.1 cycle sequencing chemistry on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Sequence Alignment and analysis was carried out on ClustalW 1.83 and European Molecular Biology Laboratory (EMBL). Variant sequence was compared with the human reference sequence of *AR* gene (NC_000023.10) and *SRD5A2* gene (NC_000002.11) provided by the National Center for Biotechnology Information (NCBI). Sequence analysis showed single base substitution of C with G at position 838 on exon 1 of *AR* gene. This hemizygous missense mutation resulted in the replacement of Threonine (T) with Arginine (R) at 105 amino acid position in exon 1 (Figure 1.II).

All other exons of *AR* and *SRD5A2* gene showed normal sequence. This mutation was confirmed by sequence analysis of two new PCR reactions carried out in forward and reverse directions from two different reaction mixtures. This mutation was not present in both parents (II.13, II.14) or in any of the control sample (Figure 1.III). The functional impact of the hemizygous missense mutation was assessed by Sorting Intolerant From Tolerant (SIFT) online analysis tool. *In Silico* online functional analysis showed this mutation as disease causing and possibly damaging with the PISC score of 0.85.

The study was approved by Institute Ethics Committee and is according to the ethical guidelines of the 1975 declaration of Helsinki written informed consent was taken from parents (II.13, II.14) and asent from patient (III.27).

DISCUSSION

Our patient was first evaluated at the age of 5 years (III.27). Hormonal profile at this time revealed LH, FSH, Testosterone and Estradiol to be within normal limits. She had gonadectomy done at 6 years of age. Therefore, endocrine (hormonal profile) evaluation now could not be used to make a definitive diagnosis. Psychological assessment of the patient revealed female gender identity which is concordant with her sex of rearing.

WHO-QOL-BREF scores were in the mid interquartile range indicating medium quality of life. Molecular analysis of *SRD5A2* gene showed a normal sequence. A novel mutation of p.T105R in exon 1 of *AR* gene confirmed the diagnosis of CAIS. AR like other members of the steroid receptor family has four main domains [6]. The NTD encoded by exon 1 is involved in transcription activation of genes, exon 2 and 3 code for DNA binding domain, 3` end of exon 4 code for hinge region and 5` end of exon 4 to exon 8 code for LBD [7]. Although exon 1 codes for more than half of AR protein, number of patients reported with mutations in this region is relatively less. Exon 1 also contain polyglutamine and polyglycine repeat which was found to be in normal range in our patient.

Several mutations have been described in *AR* genes which result in different phenotypes of AIS [8]. Our study revealed a substitution of nucleotide C with G at position c.314. This results in novel missense mutation of T>R at 105 amino acid position. The amino acid in this region of protein sequence is highly

conserved across various species (figure 1.IV) indicating the conserved nature of the residue. Both T and R are hydrophilic residues capable of hydrogen bonding. The longer side chain of R residue may hamper the normal folding of the protein thus responsible for CAIS. However further *in vitro* studies on expression and binding assays may provide an essential link between the functional and structural behaviour of this mutation in the AR protein.

Different mutations of exon 1 in *AR* gene have been reported earlier leading to CAIS [9, 10]. A novel mutation of S176A mutation in exon 1 of *AR* gene in one patient from china was shown to contribute to the mild androgen insensitivity syndrome [11]. To the best of our knowledge the mutation described in our patient has not been described earlier in patients with CAIS.

CONCLUSION:

We describe a novel mutation of p.T105R in exon 1 of AR gene in a patient with CAIS. Molecular assessment of AR gene helped in the definitive diagnosis in our patient who had undergone gonadectomy inn childhood.

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Acknowledgements:

We are thankful to Prof (Dr) Y.D.Sharma (Head, Department of Biotechnology, AIIMS, New Delhi, India) for sequencing of these samples. This work was supported for fellowship grant (SRF) by Department of Science and technology (DST, Inspire Program, N0. IF10520) for Iram Shabir.