Original Article

Mutational Analysis of Androgen Receptor Gene in Two Families with Androgen Insensitivity

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Abstract

Background: Androgen insensitivity syndrome (AIS) is a rare X-linked disorder due to mutations in the androgen receptor (*AR*) gene causing end-organ resistance to the androgenic hormone. **Subjects and Methods:** Genetic studies were carried out in two families by karyotype and targeted exome sequencing of the *AR* gene. **Results:** Two novel missense mutations were identified, p.L822P and p.P392S, in two families with complete androgen insensitivity (CAIS) and partial androgen insensitivity (PAIS), respectively. Both had 46, XY karyotype. The mother was a heterozygous carrier in PAIS and negative in CAIS. These two were novel mutations, reported for the first time, in the *AR* gene. *In silico* analysis predicted that both mutations were damaging. We reviewed the various reported Indian mutations in the *AR* gene. **Conclusion:** *AR* gene mutations cause a wide spectrum of disorders from CAIS to male infertility or primary amenorrhea. Early diagnosis is essential for gender assignment and further management, family counseling, and prenatal diagnosis.

Keywords: Androgen insensitivity syndrome, androgen receptor gene, mutation, X-linked recessive disorder, XY karyotype

Introduction

Androgen insensitivity syndrome (AIS; testicular feminization; OMIM #300068) is a rare X-linked disorder presenting with variable defects in virilization. The incidence is reported to be 1 in 20,000–99,000 in genetic males and 0.8%–2.4% in phenotypic females with an inguinal hernia.[1] A spectrum of phenotypic abnormalities is seen ranging from phenotypic women with complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAIS), and disorders of sex development (DSW) to normal but infertile men.[2] Lack of virilization can be CAIS or PAIS or mild AIS depending on the residual receptor function. Loss-of-function mutations in the intracellular androgen receptor (AR OMIM #313700) gene result in androgen resistance. Androgen hormone is important for the normal development of internal and external male genitalia in a fetus. XY fetus secretes active androgen hormone and directs the gonad to become a testis (sex determination), leading to a male phenotype. However, when hormone resistance in an XY man or boy occurs, virilization is affected. Complete androgen insensitivity manifests in the form of an XY female. Partial insensitivity cause varying degrees of virilization of the external genitalia from ambiguous genitalia, hypospadias, and cryptorchidism to male infertility.

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In the present study, two novel mutations in AIS in two unrelated Indian families were presented, thus expanding the spectrum of mutations in the *AR* gene with AIS.

Subjects and Methods

Case 1

A nonconsanguineous couple was referred for genetic counseling whose first child was treated for a bilateral inguinal hernia. At 6 months of age, the child was operated for bilateral inguinal masses and histopathology revealed the presence of testicular tissue. No further evaluation was performed. On evaluation, the child was phenotypically a female. Ultrasound examination of the abdomen revealed absent uterus and ovaries. Karyotype of the patient was that of a normal male, 46, XY. A diagnosis of AIS was suspected by clinical presentations, ultrasonography, and karyotype. The present age of the child is 4 years with appropriate growth for

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age. Targeted exome analysis was performed for the *AR* gene which revealed a novel pathogenic p.L822P mutation on X-chromosome. Sanger sequencing in the mother was normal indicating a sporadic occurrence of the mutation in the child. None of the family members were diagnosed to have disorders related to abnormal sexual development.

Case 2

A 1-month-old child was seen with a complaint of ambiguous genitalia. This was the first born baby to nonconsanguineous parents, the mother being hypothyroid and on treatment. There was no family history of any affected persons from the maternal and paternal side. The mother of the child had no brothers, and she is the only daughter to her parents. Clinically, the child had micro penis with a penoscrotal hypospadias and cryptorchidism. Plasma follicle-stimulating hormone was 1.9 IU/l and luteinizing hormone was 3.8 IU/l ($n < 5$). Plasma testosterone at 1 month of age was 11.2 nmol/l (normal 4–10 nmol/l). Karyotype was 46, XY. Mutation analysis for *AR* gene revealed a novel p.P392S mutation in the *AR* gene on X-chromosome. Maternal genotype revealed that she is the carrier for the same mutation.

Methods

DNA was extracted from peripheral blood leukocytes to perform targeted gene capture using a custom-made capture kit. The libraries were sequenced to mean >80–100X coverage on Illumina sequencing platform. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using BWA program and analyzed using Picard and GATK-Lite toolkit to identify variants in the exome relevant to the clinical human reference genome. Annotation of the variant was performed against the Ensembl Release 75 gene model. Clinically, the relevant mutations were annotated using published variants in literature and a set of variant database including ClinVar, OMIM, GWAS, HGMD, and SwissVar.

Results

Targeted mutation analysis of the *AR* gene revealed a novel homozygous missense mutation in exon 7 of the *AR* gene on chromosome X that results in the amino acid substitution of proline for leucine at codon 822 (c.2465T>C; p.L822P) [Figure 1] in Case 1 who was a phenotypical female with 46, XY. A novel mutation p.P392S (c.C1174T) was detected in exon 1 (N-ter Domain) in Case 2 who presented with PAIS. Case 1 was sporadic as the mother was negative for the mutation [Figure 2], while Case 2 was inherited where the mother was a carrier for the *AR* gene. Reviewing the reported mutations in Indian patients, the majority were in the ligand-binding domain (LBD) region [Table 1]. In our patients, one was in LBD and another in N-ter domain.

Discussion

AIS is an X-linked disorder, and spontaneous mutations can give rise to disease without any family history. The spontaneous mutation rate in complete AIS is about 30% – a finding consistent with other X-linked recessive disorders such

Table 1: Published data on AR gene mutations from India including the two novel mutations from the present cases

Author	cDNA	Amino acid change	LBD	DBD
Sharma&Tangaraj	c. 2058G > A	p.Ala566Thr		DBD
VasuVR *et al.*	c. 2762delc		LBD	
VasuVR *et al.*	c. 2925C > TSubsti		LBD	
VasuVR *et al.*	IVS 5+1G > A		LBD	
Abhilash VG *et al.*	c.C1713> G	p.His571Glu		DBD
Abhilash VG *et al.*	c.A1715> G	p.Tyr572Cys		DBD
Abhilash VG *et al.*	c.G2599> A	p.Val867Met	LBD	
Singh R *et al.*	c. 2329G > C	p.C601S	LBD	
Rajendersingh *et al.*	c. 2578T	p.L859F	LBD	
Rajendersingh *et al.*		p.L712V	LBD	
Rajendersingh *et al.*	c. 2650G > A	p.G708E	LBD	
Rajendersingh *et al.*	c. 2369_2370insG	p.Cys669Trp fs	LBD	
Sharma *et al.*	c. 2205C > A	p.R615S	LBD	
M.R Nagraj *et al.*		p.M742I	LBD	
M.R Nagraj *et al.*		p.V746M	LBD	
Sunilkumar *et al.*	c. 2754> T		LBD	
IramShabir *et al.*		p.T105R		NTD
Kulshreshtha B *et al.*		p.A596T		DBD
Radharamadevi (Present case)	c.C1174T	p. 392s		NTD
Radharamadevi	c. 2465T > C	p.L822P	LBD	

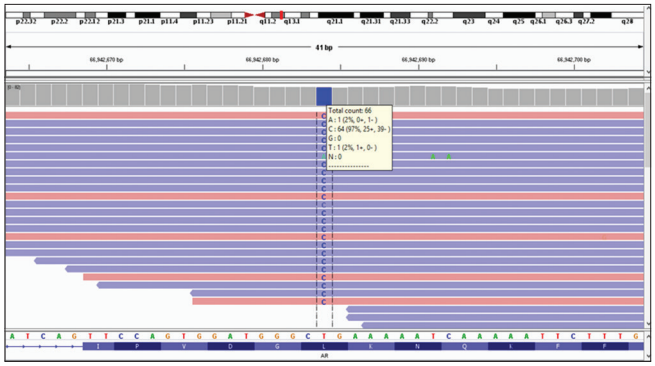

Figure 1: Integrative Genomics Viewer (IGV) of androgen receptor gene with p.L822P homozygous mutation in Case 1 (by exome)

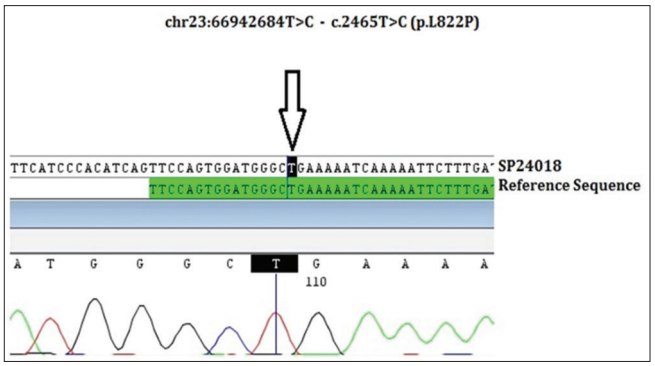

Figure 2: Mutation analysis by Sanger sequencing of the mother sample (Case 1) showing the absence of carrier status for p.L822P

as hemophilia and Duchenne muscular dystrophy.[3] Case 1 is a spontaneous mutation as the mother is tested negative for the mutation. Two-third of cases of AIS are inherited from the mother and one-third of cases come from a spontaneous mutation in the egg. *De novo* mutations occur at a high rate within the *AR* gene; a high proportion arises after the zygote stage. Thus, only direct analysis of the underlying mutation of the *AR* gene in the proband and his or her family can provide the basis for genetic counseling. This is important in counseling as the recurrence of the disease is negligible in sporadic cases. However, in the second family, the mother is a carrier and recurrence is 50% if the fetus carries a 46, XY karyotype.

CAIS presents as inguinal hernia or labial swelling containing testis in female infants. Bilateral inguinal hernias are rare in female infants and should raise the suspicion of CAIS. The incidence of CAIS in females with a bilateral inguinal hernia is 1%–2%.[4] The levels of gonadotropin and testosterone concentrations are less suggestive of hormone resistance when CAIS presents in infancy.[5] Serum testosterone concentrations surge during the first few months of life which is called "mini-puberty" and hence cannot be used to diagnose CAIS in many infants.[6] Concentrations of testosterone and anti-Mullerian hormones are inversely associated during male puberty and is not noticed during the so-called mini-puberty of infancy. Measurement of serum anti-Mullerian hormone in infants not only suggests the presence of testes in complete AIS but also suggests that concentrations are higher with the syndrome than in male infants.[6,7] This is due to nonexpression of AR in the Sertoli cells of infant testes. This gets exaggerated in CAIS and differentiates it from complete gonadal dysgenesis, a disorder of impaired Sertoli cell function.

The *AR* gene is localized to the XqI1.12 chromosome and belongs to the nuclear receptor superfamily and has four functional domains encoded by eight exons the N-terminal transactivation domain, a DNA-binding domain, a hinge region, and a C-terminal LBD. Transactivation activity of the AR is induced by the binding of androgen to its LBD, and mutations of the ligand-binding region result in the receptor unable to bind the ligand completely, resulting in CAIS.[8,9] The reported mutations in CAIS and PAIS in Indian patients are mostly missense mutations distributed throughout the eight exons of the *AR* gene localized between amino acid residues 688 and 712, 739 and 784, and 827 and 870, which include these two domains, the DNA-binding domains and LBDs, where their functional effect is great because of the ordered structure of these domains.[10] In this study, in the first patient with CAIS, the mutation p.L822P is found in the LBD and the mutation is not associated with the development of Mullerian structures as evidenced by absent uterus and fallopian tubes. In the second patient with 46, XY karyotype, with undervirilized external genitalia and reared as a male, novel mutation p.P392S (c.C1174T) was detected in exon 1 (N-ter domain), who presented with PAIS. The various mutations reported from India are quite heterogeneous with no recurrent common mutation in the *AR* gene. The majority of the mutations are detected in PAIS; a few are from CAIS and one from primary amenorrhea. However, there are no definite genotype correlations observed between the different phenotypes.[11-20]

Age of diagnosis of AIS can be anywhere between birth and even before birth to adulthood. Diagnosis is suspected in the fetus when there is a discrepancy between the findings of 46, XY karyotype on amniocentesis and the presence of female external genitalia on prenatal ultrasound examination. Most of the reported cases are adolescents and the present two cases are identified in infancy. Early diagnosis is critical in a newborn with ambiguous genitalia where gender assignment is crucial. A child with DSW phenotype should undergo adequate endocrine and genetic testing for a definitive diagnosis before gender is assigned or surgeries performed. The development of bilateral, inguinal hernia in phenotypic female infants often raises the question of CAIS. When an infant undergoes early gonadectomy, puberty should be induced with estrogen replacement similar to the treatment of Turner syndrome. The advantage of delaying gonadectomy is that puberty occurs spontaneously with growth spurt and breast development without attaining menarche. These individuals are taller compared to those who had early gonadectomy.[21,22] The risk of gonadal tumor is very low in childhood. Specific analyses in large sample groups suggest a germ-cell tumor risk as low as 0.8%–2%, especially before puberty.[23] In our Case 1, the phenotypic female underwent gonadectomy at 8 months of age and reared as a female. She should be given hormone replacement during adolescence for breast development. In Case 2, the child was assigned male gender with testosterone supplementation and underwent correction of the penoscrotal hypospadias at 3 years of age. Gynecomastia often occurs in adolescence and requires breast reduction mammoplasty.

Genetic counseling

AR mutations are inherited and transmitted in an X-linked manner. Fifty percent of XY-offspring is affected and 50% for an XX offspring is a healthy carrier. In sporadic cases, the risk of transmission is negligible. Germline mosaicism cannot be excluded in any case of *de novo* mutation of the *AR* gene, and one should be cautious in counseling these families.

Conclusion

AIS should be diagnosed at an early age and to be differentiated from other disorders of sex. Gender identification is crucial, especially in neonates with ambiguous genitalia. Mutations in the *AR* gene are highly heterogeneous among Indian patients with AIS.

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Conflicts of interest

There are no conflicts of interest.

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