RESEARCH ARTICLE

Three novel and two known androgen receptor gene mutations associated with androgen insensitivity syndrome in sex-reversed XY female patients

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Abstract

Molecular characterization of 23 cytogenetically confirmed XY females was attempted by screening coding regions of *SRY* and androgen receptor (*AR*) genes. Five of the index cases showed sequence variations in various exons of the *AR* gene: a deletion (n.1911delG) and substitutions n.1761G>A and n.1317C>T in exon 1; n.3510C>T transition in exon 6 and deletion mutation (n.3672delT) in exon 7. Four mutations identified here lead to the formation of truncated receptor protein, involving a substantial loss of AR functional domains which explains the phenotype in the subjects. The n.1761G>A substitution has been previously reported in cases with mild androgen insensitivity. Although the ligand-binding domain was considered as the mutational hot spot in *AR* gene, we report here 3/5 variations in the N-terminal domain emphasizing the significance of considering the N-terminal domain of AR as well for mutation screening. Our present observation also strengthens the role of *AR* gene and its direct association with AIS.

[Balachandran S., Gunasekaran B., Arumugam B., Jayashankar M. and Santhiya S. T. 2016 Three novel and two known androgen receptor gene mutations associated with androgen insensitivity syndrome in sex-reversed XY female patients. *J. Genet.* **95**, 911–921]

Introduction

Defects in the androgen receptor gene affects the androgen-dependent male sexual development in 46,XY individuals, leading to androgen insensitivity syndrome (AIS); the incidence being 1 in 20,000–64,000 male births (Ahmed *et al.* 2000). Clinically, based on the severity of the symptoms, AIS is categorized into complete, partial and mild. Complete androgen insensitivity syndrome (CAIS), the most severe manifestation of androgen insensitivity is characterized by female phenotypic appearance with 46,XY karyotype, typical female genitalia, normal breast development, absent/sparse pubic hair, absent uterus and bilateral intra-abdominal or inguinal testis, hypoplastic or absent wolffian structures associated with normal or elevated plasma testosterone (pl-T) and high luteinizing hormone (LH) levels (Sultan *et al.* 2001; Melo *et al.* 2011). Partial androgen insensitivity (PAIS) presents with either ambiguous genitalia or male phenotype with hypospadias and micropenis. Impaired spermatogenesis and/or pubertal undervirilization in otherwise normal men is considered as the mildest form of androgen insensitivity (MAIS) (Evans *et al.* 1997; Brinkmann 2001; Galani *et al.* 2008; Raicu *et al.* 2008). Other pathological conditions associated with defective androgen receptors are spinal bulbar muscular atrophy (La Spada *et al.* 1991), male breast cancer (Wooster *et al.* 1992) and prostate cancer (Tilley *et al.* 1996).

The two most important androgens are testosterone and 5α-dihydrotestosterone, whose actions are mediated by functional androgen receptor, which upon receipt of signal activate transcription of specific genes in target tissues (Melo *et al.* 2011). The androgen receptor (*AR*) gene located at Xq11-12, has eight exons and encodes 920 amino acid residues. Akin to other members of the nuclear receptor superfamily, the *AR* gene contains four functional domains: an N-terminal domain (NTD) which serves transcriptional activation function encoded by exon 1; a central deoxyribonucleic acid (DNA) binding domain (DBD) rich in cysteine residues encoded by exons 2 and 3, a hinge region containing the nuclear targeting signal and a C-terminal

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Keywords. androgen insensitivity syndrome; androgen receptor; truncation mutation; N-terminal domain; XY sex reversal.

Table 1. Details of clinical and hormonal profiles of patients.

Case no.	Age (yrs)	Height (cm)	C/NC	SSC			Ultrasonogram impression		Provisional diagnosis	Physical features	Hormone profile[a]
				Breast	AH	PH	Uterus	Gonads			
SA10	22	170	C(III)	II	II	II	Not visualized	Atropic testis with germ cell aplasia	CGD	PA, tall stature, hoarse voice, cliteromegaly, broad feet, high arched palate	T-0.238 ng/mL **FSH-93.09 mIU/mL** TSH-1.540 mIU/mL
SA11	20	165	NC	–			Not visualized	Bilateral testis in inguinal region	PAIS	PA, tall stature, hair male pattern, hoarse voice	NA
SA16	2	70	C(III)	–	–	–	Not visualized	Testicular tissue in hernia contents	AIS	Female genitalia, swelling suggestive of testis	**Serum T-12.65 ng/mL** Free T-0.25 pg/mL
SA35	15	152	NC	III	I	I	No uterus	Ovaries not imaged, scar in inguinal region	AIS	PA, tall stature	**T-1072 ng/dL** FSH-3.09 mIU/mL
SA44	17	157	NC	I	II	II	No uterus	Testis in inguinal folds	PAIS	PA, tall stature, hoarse voice, cliteromegaly	T-2.27 pg/mL **FSH-15.13 mIU/mL**
SA68	12	150	C(II)	–	II	II	No uterus	Both sides palpable testis	PAIS	Male pattern hair distribution	**T-125.23 ng/dL**
SA77	16	137	NC	I	–	I	Poorly developed uterus	Ovaries not imaged	PA	Amenorrhoea	FSH-6.59 mIU/mL TSH-1.44 mIU/mL
SA111	24	147	NC	II	I	I	Rudimentary uterus	Ovaries not imaged	GD	PA	**T-38.45 ng/mL** **FSH-69.76 mIU/mL** **LH-33.10 mIU/mL** Estradiol-26.72 pg/mL
SA125	7	105	NC	–	–	–	Not imaged	Testis in inguinal region	PAIS	Low set ears, enlarged clitoris	T-0.025 ng/mL
SA160	15	163	C(II)	II	I		Rudimentary uterus	Ovaries not imaged	PA	PA, tall female, long hands and feet, hyperpigmentation of orbit, mouth and neck	T-1.3 pg/mL **FSH-84.16 mIU/mL** **LH-25.69 mIU/mL** PRL-12.17 ng/mL
SA179	22	167	NC	–	II	II	Not visualized	Right, left testis palpable	PAIS	PA, mustache present, hoarse voice	**T-4.19 ng/mL** FSH-5.11 mIU/mL LH-3.18 mIU/mL
SA180	17	178	NC	II	II	II	Not imaged	No ovaries	GD	PA, tall stature, hirsuitism	**T-7.25 ng/mL** **FSH-15.65 mIU/mL**
SA184	17	160	NC	I	I	I	Not imaged	No ovaries	GD	PA	T-10.6 ng/dL **FSH-72.63 mIU/mL** **LH-26.61 mIU/mL** PRL-8.01 ng/mL
SA213	21	158	C(III)	IV	I	I	Not visualized	Ovaries not imaged	AIS	PA, low set ears, stubby fingers	**T-391.70 ng/dL** FSH-10.30 mIU/mL PRL-6.13 ng/mL
SA214	19	149.5	C(III)	IV	I	I	Not visualized	Ovaries not imaged	GD	PA	**T-7.260 ng/mL** **FSH-19.32 mIU/mL** LH-4.25 mIU/mL

Table 1. (*contd*)

Case no.	Age (yrs)	Height (cm)	C/NC	SSC			Ultrasonogram impression		Provisional diagnosis	Physical features	Hormone profile[a]
				Breast	AH	PH	Uterus	Gonads			
SA219	14	150	C(II)	I	I	I	Not imaged	No ovaries	AIS	PA, mild facial dysmorphism, large mouth, slight mental retardation	T-8.87 ng/dL **FSH-143.09 mIU/mL**
SA234	1/6	–	NC	–	–	–	Not imaged	Right, left testis palpable	AG	–	T-0.025 ng/mL
SA235[b]	18	150	NC	IV	I	I	Not visualized	Left inguinal swelling, no ovaries	PA	PA, slight cliteromegaly	**Free T-5.94 pg/mL** FSH-3.8 mIU/mL **LH-34.4 mIU/mL**
SA236	15	167	C(II)	–	–	–	Not imaged	No ovaries, inguinal swelling in right side	PAIS	PA, hoarse voice, mustache development, ambiguous genitalia, cliteromegaly	Free T-5.23 ng/dL **FSH-35.27 mIU/mL** LH-15.3 mIU/mL
SA246	5	–	C(V)	–	–	–	Not imaged	Testis in right inguinal region	AIS	–	T-0.025 ng/mL
SA263	18	154	C(III)	IV	I	I	Rudimentary uterus	No ovaries	PA	PA	FSH-4.87 mIU/mL **LH-48.82 mIU/mL**
SA284	34	187	C(III)	IV	–	–	Not imaged	Testis in both inguinal folds	AIS	PA	T-0.026 ng/mL FSH-2.37 mIU/mL
SA295	17	152	C(III)	–	–	–	Rudimentary uterus	No ovaries	PA	PA	**FSH-13.80 mIU/mL** LH-4.2 mIU/mL

C, consanguineous (degree of consanguinity shown in parenthesis); NC, nonconsanguineous; SSC, secondary sexual characteristics; AH, auxillary hair; PH, pubic hair; CGD, complete gonadal dysgenesis; PAIS, partial androgen insensitivity syndrome; AIS, androgen insensitivity syndrome; PA, primary amenorrhoea; GD, gonadal dysgenesis; AG, ambiguous genitalia; T, testosterone; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin releasing hormone; TSH, thyroid stimulating hormone; NA, not available.

[a]Elevated hormone levels are indicated in bold.

[b]This case presented with 47,XY+mar karyotype.

ligand-binding domain (LBD) encoded by exons 4–8. LBD plays a critical role in nuclear localization, receptor dimerization and interaction with other proteins in addition to ligand binding (Brinkmann 2001; Raicu *et al.* 2008; Petroli *et al.* 2011).

SRY is another crucial gene that plays an important role in initiating male sexual development, mutations of which are known to cause XY sex reversal with gonadal dysgenesis and an increased risk of gonadal tumour. Mutations in *SRY* gene results in partial sex reversal implying its importance in male sex determination (McElreavy *et al.* 1992; Shahid *et al.* 2010). At birth, these patients are phenotypically normal female, however, during the pubertal age, they present with amenorrhoea and delayed secondary sexual characteristics. To date there are 60 different mutations identified within the open reading frame of *SRY* (Isidor *et al.* 2009).

In this study, we screened the *SRY* and *AR* genes in patients with gonadal dysgenesis and AIS and identified five sequence variations in *AR* gene.

Subjects and methods

Subjects and clinical history

The study adhered to the institutional ethical guidelines and was cleared by the same. Informed consent was obtained from the subjects, their family members and controls who participated in the study. Twenty-three cytogenetically

Table 2. Details of primers used for amplification of *SRY* and *AR* genes (exons 1–8).

Primer	Sequence (5′ → 3′)		Annealing temp. (°C)	Product size (bp)
SRY (I)	F-AGGGCGGAGAAATGCAAGTTTCA;	R-TGTGCCTCCTGGAAGAATGGCCA	58	474
SRY (II)	F-GCTGGGATACCAGTGGAAAATGCT;	R-TGACGGCCACAAACATAGGCAGG	58	584
AR exon 1a	F-TTCTCTGGAGCTTCCCGCA;	R-CGGCTGTGAAGGTTGCTGTT	54.5	491
AR exon 1b	F-CAGCAAGAGACTAGCCCCAG;	R-GGACACCGACACTGCCTTAC	59	504
AR exon 1c	F-ACTCCTTCAGCAACAGCAGCAC;	R-AGGGTAGACGGCAGTTCA	54.5	461
AR exon 1d	F-CCATTGGCCGAATGCAAAGG;	R-GGCTGTGAAGAGAGTGTGCC	54.5	468
AR exon 1e	F-CTGGAGAACCCGCTGGACTA;	R-GGAAAAACTTACCGCATGTCCC	57	464
AR exon 2	F-AGGCTCAGTCACACCCTACA;	R-AGGGCCTTGCCAATGACTCT	54	461
AR exon 3	F-CATCAGGATCTGAATTGTTCATCCC;	R-CCCTTGGAAGCATCAAAGAAGAAA	53	463
AR exon 4	F-ACCAGTGTTGAATGAGCACTTG;	R-TGGTCCATAGGAGCGTTCAC	53	512
AR exon 5	F-AGCAGCTCAGGGAAGTAGGG;	R-CACCATCACCACCAACCAGG	57	428
AR exon 6	F-TGCTAACCCATATCCTGGCCA;	R-GTCCAGGAGCTGGCTTTTCC	54	487
AR exon 7	F-TGACAGACTGAAGGCCCCAA;	R-GGTGGTGCCAGACTCTAGAGA	54	411
AR exon 8	F-ACCTCATGGGGGAGGACCA;	R-GAGGAAATTCCCCAAGGCACT	54.5	407

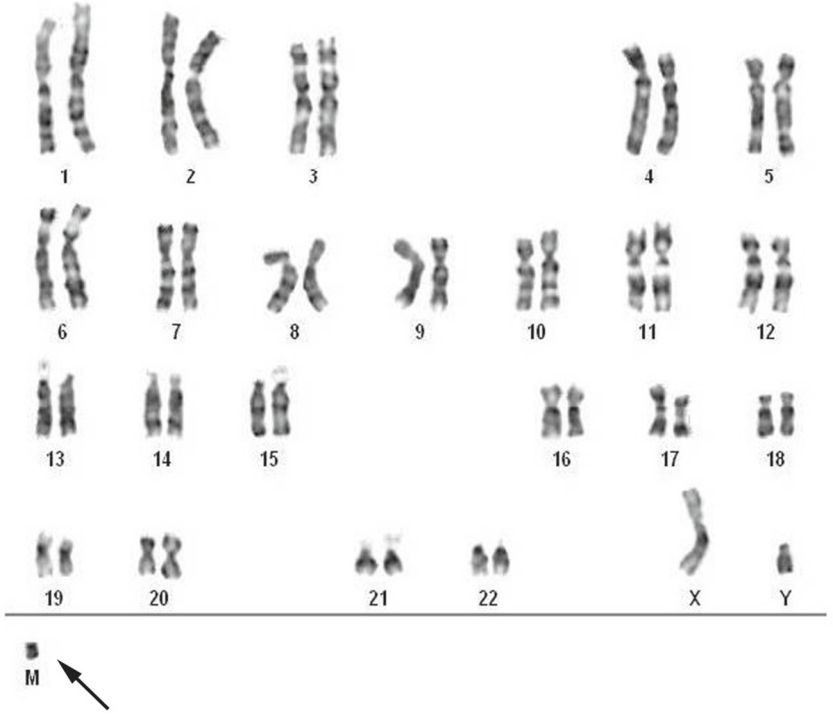


Figure 1. GTG-banded karyogram of proband (SA235P) showing 47,XY+mar karyotype.

confirmed XY females with mean age of 15.96 years were investigated. The clinical findings of the subjects are briefed in table 1. A total of 100 random healthy males were recruited in the study as normal controls.

Cytogenetic and molecular analyses

Chromosome analysis was performed on metaphases prepared from peripheral blood lymphocyte culture (Hungerford 1965), applying standard GTG banding technique (Seabright 1971). Karyotype analysis was done using Olympus microscope BX51 (Olympus Singapore Pte Ltd.) with applied spectral imaging systems karyotyping software (BandView ver. 6.0). Genomic DNA (100 ng) obtained from peripheral blood lymphocytes, using phenol–chloroform extraction was PCR amplified for the complete *SRY* (NM_003140.2) and *AR* (NM_000044.3) coding regions (exons 1>8) in PTC 200 (DNA Engine, BioRad, USA) thermal cycler. Details of primers are listed in table 2. Two sets of overlapping primers were used to amplify the single exon of *SRY* with the PCR conditions of initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 15 s, annealing at 58°C for 30 s, extension at 68°C for 1 min and final extension at 68°C for 7 min. Five sets of overlapping primers were used to amplify exon 1 and a single set of primer was used to amplify each exons 2–8 of *AR* gene as initial denaturation at 94°C for 3 min for 1 cycle, followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s at variable temperatures (table 2) and extension at 72°C for 45 s and a final extension at 72°C for 5 min. PCR reactions for *SRY* and *AR* genes were performed using Accuprime Pfx mastermix (Invitrogen, Carlsbad, USA) and Jump start red taq (Sigma, St Louis, USA), respectively, as per the manufacturer's instructions. The amplified products were visualized through 1.5% agarose gel. Purified amplicons (Qiagen PCR purification kit, Hilden, Germany) were commercially sequenced (Madras Diabetic Research Foundation, Chennai, India) and checked for variations through basic local alignment search test (BLAST) analysis.

Results

Chromosome analysis revealed the karyotype to be 46,XY in 22 of the patients studied and one subject (SA235) showed presence of an additional marker; 47,XY+mar (figure 1). All cases analysed ($n = 23$) were positive for *SRY* with no sequence variation in the coding region. Screening the complete coding region of *AR* gene (exons 1>8) revealed the following variations: patient 1 (SA235) presented with C>T transition in exon 1 (n.1317C>T) substituting codon (CAG) for glutamine at 68 to stop codon (TAG) (p.Q68X) (figure 2). Patient 2 (SA125) carried a G–A substitution in exon 1 (n.1761G>A) resulting in a change from glycine (GGG) to arginine (AGG) at codon 216 (p.G216R) (figure 3). Patient 3 (SA35) harboured deletion of a single-nucleotide G at codon 266 (n.1911delG), which resulted in a frameshift and subsequently introduced a premature stop at codon 295 (D266fs295ter) (figure 4). Patient 4 (SA213) revealed a C > T substitution in exon 6 (n.3510C>T) (figure 5) that changes the codon for glutamine at 799 (CAA) to a stop codon (TAA) (p.Q799X). Patient 5 (SA263) showed deletion of nucleotide T in exon 7 at codon 853 (n.3672delT) resulting in a frameshift and introduction of premature stop at codon 882 (p.C853fs882ter) (figure 6). All the above variations in the respective AR domains are schematically shown in figure 7. The possibility of presence of the following mutations in normal population was ruled out as follows, mutation n.1911delG was analysed in 100 random male controls through sequencing analysis and mutations n.1761G>A, n.3510C>T, n.3672delT were studied in 100 male control samples through RFLP using the restriction enzyme *Xho*I, *Nla*IV and *Bsr*BI, respectively (figure 8).

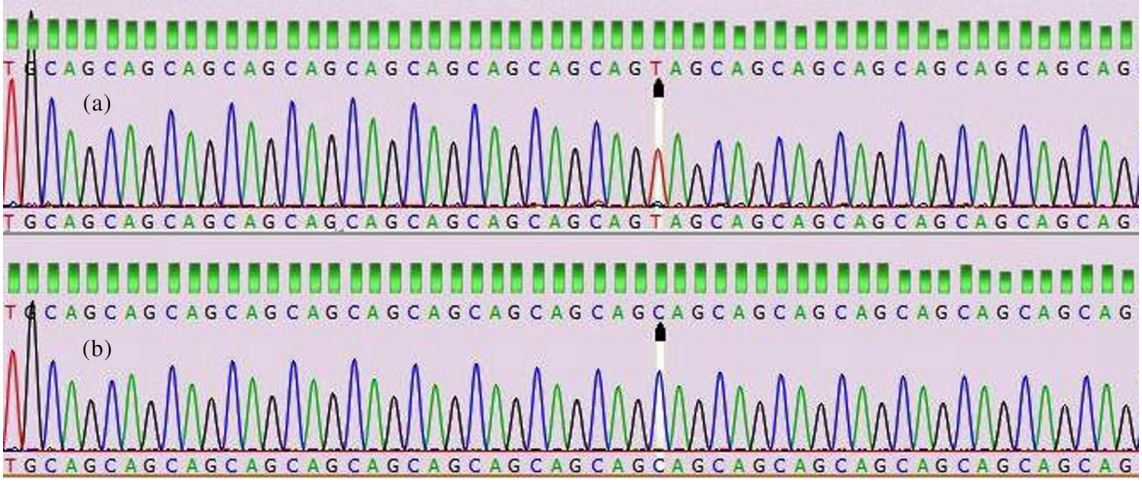


Figure 2. (a) Partial electrophoregram of *AR* gene (exon 1) of the proband (SA235) showing n.1317C>T leading to Q68X. (b) Normal control.

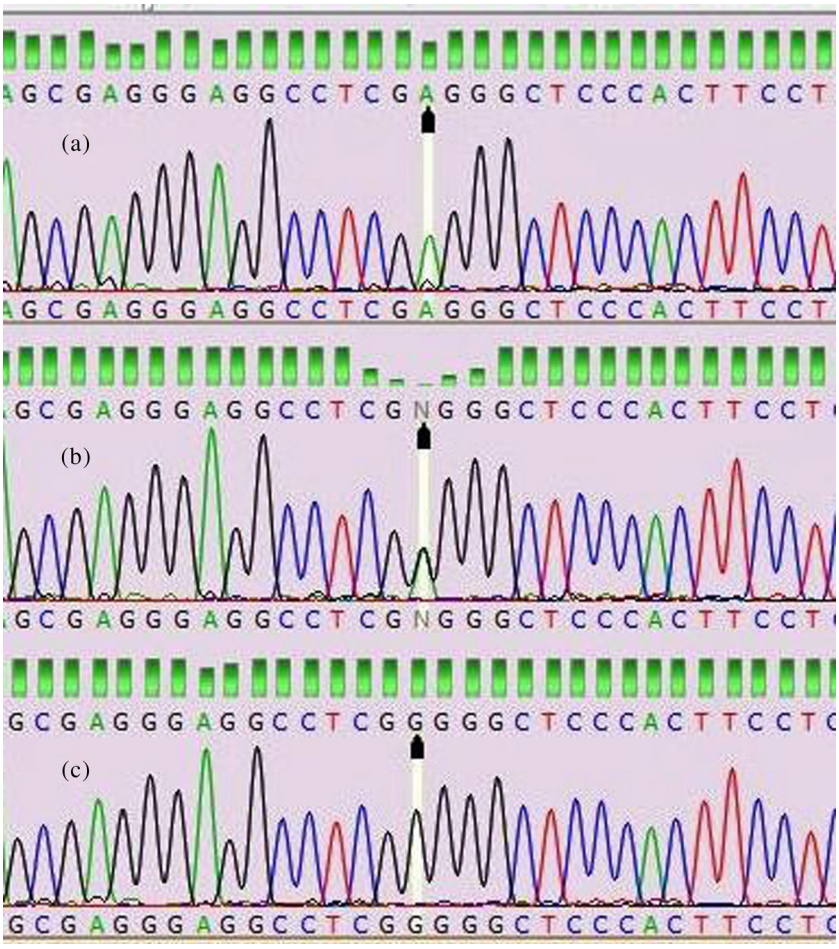


Figure 3. (a) Partial electrophoregram of *AR* gene (exon 1) of the proband (SA125) showing n.1761G>A leading to G216R. (b) Heterozygous mother. (c) Normal father.

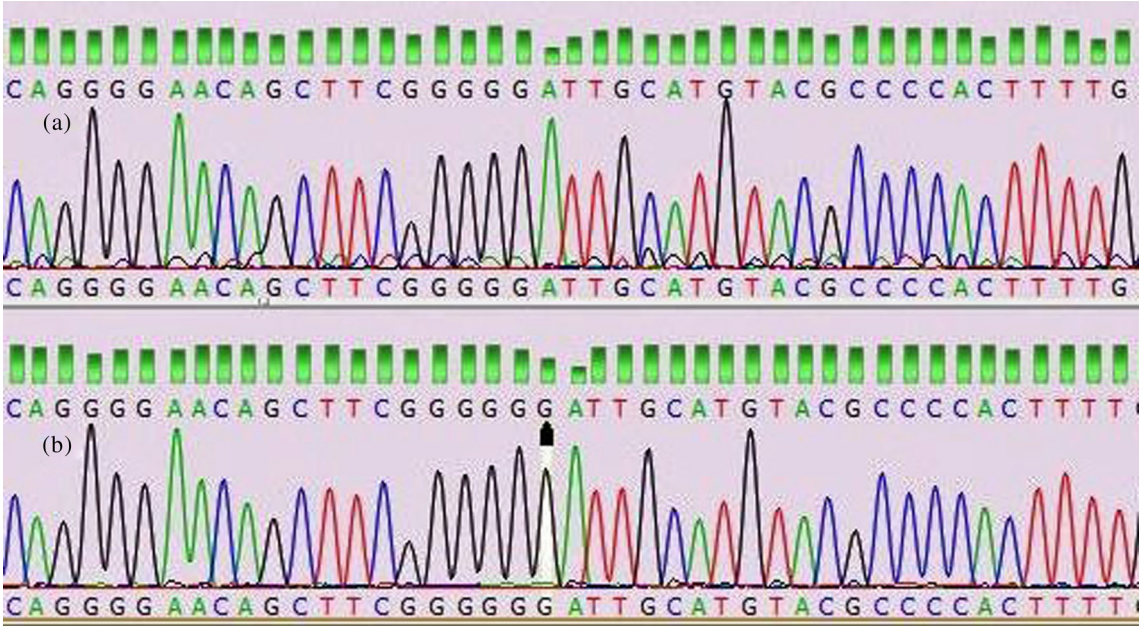


Figure 4. (a) Partial electrophoregram of *AR* gene (exon 1) of the proband (SA35) with n.1911delG leading to frameshift mutation (D266fs295ter). (b) Normal control.

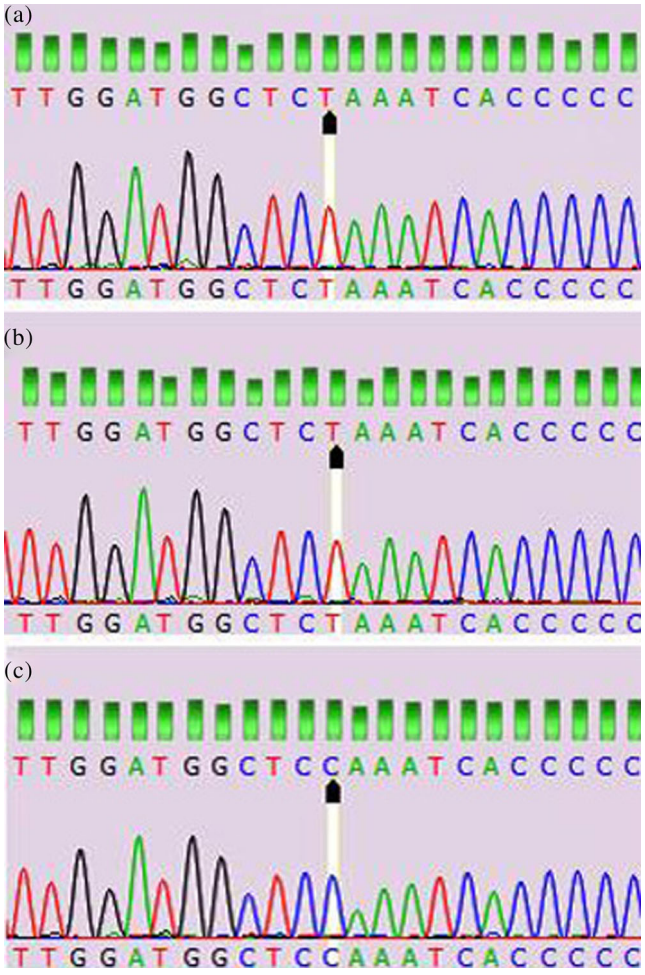


Figure 5. (a) Partial electrophoregram of *AR* gene (exon 6) of the proband (SA213) showing n.3510C>T leading to Q799X. (b) Same mutation in the sibling (SA213S). (c) Normal father.

Discussion

AIS and complete gonadal dysgenesis (CGD) associated with 46,XY karyotype in females are often diagnosed at puberty due to the absence of menarche. Unlike CGD, mixed and partial gonadal dysgenesis often present with karyotypes including mosaicism-like 45,X/46,XY (Layman *et al.* 2009; Jorgensen *et al.* 2010; Ocal *et al.* 2012). Literature suggests the incidence of *SRY* mutation to be about 10–15% in cases of XY CGD and sex reversal including point mutations, frameshifts and deletions (Shahid *et al.* 2004; Rocha *et al.* 2011). Majority of the other cases are known to carry mutations in either *SRY* regulatory elements or other genes like *AR*, *NR5A1*, *DHH*, *FGF9*, *M33*, *DMRT1*, *AMH* (antimullerian hormone) that are involved in sex differentiation pathway (Katoh-Fukui *et al.* 1998; Boyer *et al.* 2002; Canto *et al.* 2005; DiNapoli *et al.* 2006; Jorgensen *et al.* 2010) or other unknown genes (Rocha *et al.* 2011).

Molecular analysis of the subjects that are clinically diagnosed and cytogenetically validated for AIS, revealed no alterations in *SRY*, but showed five variations in the *AR* gene. The human AR is a member of the nuclear receptor superfamily, which facilitates the development of male secondary sexual characteristics as well as the growth of prostate gland (Brinkmann 2001; McEwan 2009). Among the steroid receptors, the AR records the highest density, witnessing more than 600 different mutations leading to AIS (Gottlieb *et al.* 2012). Two thirds of mutation reports are in the LBD, 20% of the mutations occur in the DBD and the NTD is said to cover less than 15% of the mutations (Gottlieb *et al.* 2004). Mutations detected in individuals with AIS range from single-base variations, nucleotide insertions or deletions, complete or partial gene deletion to intronic mutations (Brinkmann 2001). Among genetic lesions, gene deletion (either partial or complete) and intronic mutations are at low frequency, whereas missense mutations leading to variable phenotypes account for the most common molecular lesion.

The present study reports three variations in exon 1 of *AR* gene. This exon constitutes a major portion of the *AR* gene, encoding about 50% of the AR protein with two repeat regions: a CAG repeat normally varying from 11 to 31 and a CGN repeat ranging from 10 to 25. The variable length of these polymorphic regions are likely to be involved in regulation of AR activity and is implicated with AR functional defects (La Spada *et al.* 1991; Dowsing *et al.* 1999; Liu *et al.* 2008). Patient 1 harboured a C>T transition within the CAG repeat region at codon 68 converting glutamine (CAG) to stop codon (TAG) (n.1317C>T; p.Q68X) (figure 2). Mutation at this site has not been reported previously but similar premature terminating mutations within this polyglutamine stretch has been shown elsewhere (Zoppi *et al.* 1993; Philibert *et al.* 2010). Family members of patient 1 could not be studied due to unavailability of sample.

Patient 3 was identified with a novel frameshift mutation D266fs295ter that resulted from a single-nucleotide deletion (n.1911delG) at codon 266 (figure 4). This deletion leads to frameshift and introduces premature stop codon at position 295. The patient's father, mother and brother carried the normal allele indicating the mutation to be of *de novo* origin. Exon 1 encodes the NTD and it mediates the transactivation function, hence, the disruption of this region might often compromise the biological function of AR. Supporting this, Zoppi *et al.* (1993) demonstrated the possibility of translation initiation downstream of the premature termination at NTD. However, such a translation reinitiated protein containing the portions downstream of NTD still remained to cause CAIS, which might be due to the loss of regions necessary for transactivation or the loss of specific ^{23}FXXLF^{27} region that is involved in the C-terminal interaction (Thompson *et al.* 2001; He and Wilson 2003). In both the cases of premature termination within exon 1 showing typical features of CAIS, the protein would remain nonfunctional even if translation reinitiation had occurred.

G216R is a single-base alteration (n.1761G>A) that was identified in patient 2 (figure 3), which results in substitution of nonpolar (G) by a strong basic (R) residue at position 216. Molecular analysis of the patient's mother revealed

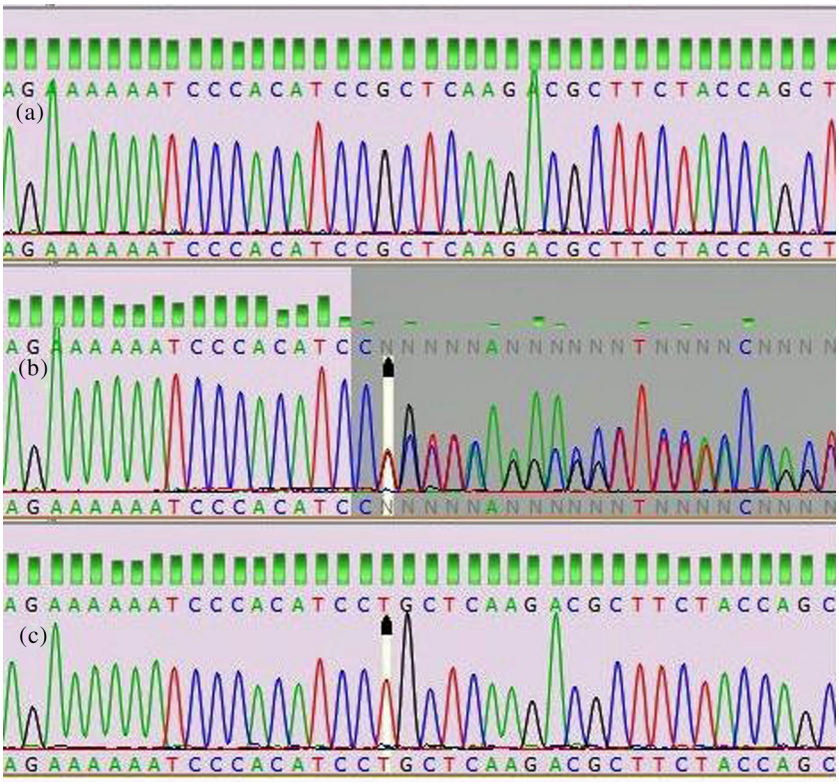


Figure 6. (a) Partial electrophoregram of *AR* gene (exon 7) of the proband (SA263) showing deletion T (n.3672delT) leading to frame shift mutation. (b) Heterozygous mother showing normal and the mutated allele as overlapping sequences from the site of mutation indicated. (c) Normal control.

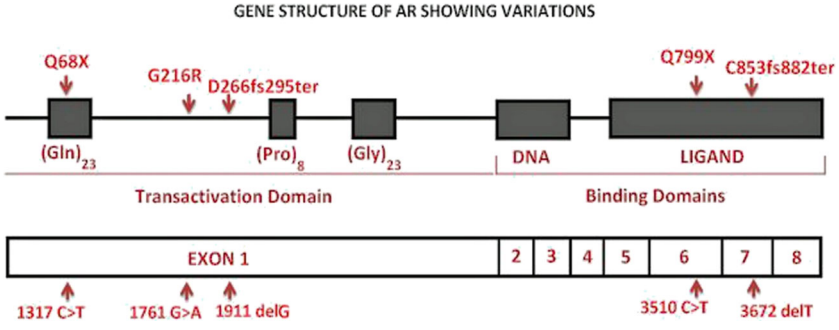
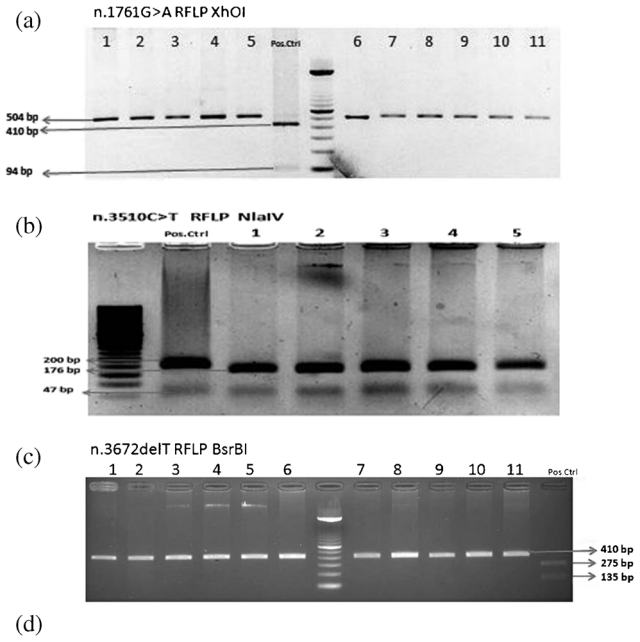


Figure 7. Gene structure of androgen receptor (*AR*) showing five mutations detected in the respective domains. Mutations p.Q68X, p.G216R and p.D266fs295ter are located in the transactivation domain (exon 1). Mutations p.Q799X and p.C853fs882ter are located in the ligand binding domain (exons 6 and 7, respectively).

her to be heterozygous for the variation, while her father carried the normal allele, thus explaining maternal inheritance. The same variation has been previously reported by Deeb *et al.* (2005) in three unrelated patients; two females with ambiguous genitalia and a male with oligozoospermia. Another study by Wang *et al.* (1998) in a male with severe oligospermia revealed this mutation to be the cause for reduced AR activity, however the same mutation was also reported in a normal male suggesting that the presence of this variation alone cannot exclude fertility. Actually, this mutation has been reported to be present with variable phenotypes (PAIS, MAIS and normal) according to the AR mutation database (Gottlieb *et al.* 2012) and also associated with differences in sex of rearing (Deeb *et al.* 2005). The proband in our study diagnosed for PAIS, presented ambiguous genitalia with cliteromegaly, her gonads were of testicular origin and raised as a female.

The above discussed three mutations are clustered in the NTD of *AR* gene. The NTD encoded by exon 1 so far was considered to carry least number of mutations, probably



(d)

AR exon	Mutation	Primer F	Primer R	PCR Size	RFLP	WT	Mutation	Pos. Control
Exon 1b	n.1761G>A, p.G216R	CAGCAAGAGACTAGCCCCAG	GGACACCGACACTGCCTTAC	504 bp	*Xho*I	uncut	410+94	SA 125
Exon 6	n.3510C>T , p.Q799X	TACCGCATGCACAAGTCCC	GTCCAGGAGCTGGCTTTTCC	247 bp	*Nla*IV	176+47+24	200+47	SA 213
Exon 7	n.3672delT, S852fs	TGACAGACTGAAGGCCCCAA	GGTGGTGCCAGACTCTAGAGA	410 bp	*Bsr*BI	uncut	275+135	SA 263

Figure 8. Representative RFLP gel pictures of mutation screening in normal males (a) showing n.1761G mutation screening by *Xho*I RFLP. (b) showing n.3510C>T mutation screening by *Nla*IV RFLP. (c) showing n.3672delT mutation screening by *Bsr*BI RFLP. (d) Table describing the primers, PCR product size, product size after RFLP in the context of WT and mutation with appropriate mutated sample as positive control.

because this exon may not have been analysed in most series. Missense mutations occurring in exon 1 are known to have a mild effect on AR function causing MAIS (Gottlieb *et al.* 2012). Single amino acid changes are considered to be rather infrequent in exon 1 of *AR* gene than stop codons, insertions or deletions as the unstructured NTD might tolerate single amino acid changes without affecting AR function (Audi *et al.* 2010). Truncation mutations in exon 1 is said to have increased by 25% (Audi *et al.* 2010; Philibert *et al.* 2010), which is reflected in this study.

Present study revealed two truncation mutations in the LBD, which is the hot spot for loss or defective functional mutations in AR. Patient 4 (SA213) harboured a truncation mutation (n.3510C>T) in exon 6 changing the codon 799 for glutamine to a stop codon (CAA>TAA) (p.Q799X) (figure 5). Even though the mother was deceased and father was found to carry normal allele, the elder sibling of the proband also harboured the same mutation suggestive of maternal inheritance. Notably, the elder sibling presented with amenorrhoea and poor secondary sexual characters like the proband, suggesting a strong correlation between the phenotype and this mutation. Moreover, Ignacak *et al.* (2004) reported the same mutation in a female with typical features of CAIS and abdominal tumour. Patient 5 (SA263) affected by CAIS, had a p.C853fs882ter mutation, where there is deletion of nucleotide T at codon 853 in exon 7 leading to frameshift and stop codon at exon 882 (figure 6). This n.3672delT novel mutation located in exon 7 was also found in her heterozygous mother demonstrating the maternal inheritance. There are similar reports of truncation mutations in exon 7 at different sites (Ahmed *et al.* 2000; Goulis *et al.* 2006; Cheikhelard *et al.* 2008) and all of these are reported to be present with CAIS. Truncation mutations in the highly conserved LBD of C-terminal results in the formation of receptor proteins, which are either incapable of binding to androgen or binding with high affinity (Imasaki *et al.* 1995; Ignacak *et al.* 2002; Goulis *et al.* 2006; Sun *et al.* 2010).

Truncation mutations are known to occur in all the exons of the *AR* gene, while missense mutations are said to be more common in the LBD. Here, we report four truncation mutations leading to truncated AR protein, two mutations in the NTD; and the other two in the LBD. Irrespective of the domain involved in mutation, it was noted that all four patients carrying truncation mutation presented features of complete androgen insensitivity, suggesting the truncated AR protein to be functionally compromised. This could be due to the loss of essential functional domain (LBD or DBD) in the truncated receptor protein (Bagatell and Bremner 2003). The fifth variation reported here is a missense mutation occurring in the NTD. A ligand-mediated N and C terminal interaction plays a critical role in stabilization and transcriptional activation of AR (Li *et al.* 2006; Centenera *et al.* 2008). Presence of a second nuclear localization

signal in the LBD has also been reported (Poukka *et al.* 2000), suggesting that the substantial loss of AR protein in our subjects might lead to a defective protein stabilization, transcriptional activation or localization of AR. Truncated receptors could easily be predicted for their fully compromised biological function or incapable of binding to androgen with high affinity leading to insufficient activity. These alterations are uniformly said to be associated with phenotype of CAIS as seen in all four patients.

In conclusion, our study has identified three novel mutations and two known variations in the *AR* gene that might add more insights into the molecular mechanisms of AIS. Moreover, this study emphasizes the importance of mutation screening in the NTD, in addition to the LBD of AR. Further, our current result strengthens the direct role of *AR* gene as the most susceptible target in association with AIS.

Acknowledgements

We thank the patients and their family members for their cooperation and acknowledge the grant support of UGC Major Research project (F. no. 39-113/2010 SR), UGC-SAP DRS1 (F.3-3/2007), UGC-infrastructure, UPE II, DST-FIST to Department of Genetics. BS is grateful to Lady Tata Memorial Trust, Mumbai and UGC-BSR for fellowship assistance.

References

Ahmed S. F., Cheng A., Dovey L., Hawkins J. R., Martin H., Rowland J. *et al.* 2000 Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. *J. Clin. Endocrinol. Metab.* **85**, 658–665.

Audi L., Fernández-Cancio M., Carrascosa A., Andaluz P., Torán N., Piró C. *et al.* 2010 Novel (60%) and recurrent (40%) androgen receptor gene mutations in series of 59 patients with a 46,XY disorder of sex development. *J. Clin. Endocrinol. Metab.* **95**, 1876–1888.

Bagatell C. J. and Bremner W. J. 2003 *Androgens in health and disease*, 1st edition. Springer Science + Business Media, New York, USA.

Boyer A., Dornan S., Daneau I., Lussier J. and Silversides D. W. 2002 Conservation of the function of DMRT1 regulatory sequences in mammalian sex differentiation. *Genesis* **34**, 236–243.

Brinkmann A. O. 2001 Molecular basis of androgen insensitivity. *Mol. Cell. Endocrinol.* **179**, 105–109.

Canto P., Vilchis F., Soderlund D., Reyes E. and Mendez J. P. 2005 A heterozygous mutation in the desert hedgehog gene in patients with mixed gonadal dysgenesis. *Mol. Hum. Reprod.* **11**, 833–836.

Centenera M. M., Harris J. M., Tilley W. D. and Butler L. M. 2008 The contribution of different androgen receptor domains to receptor dimerization and signaling. *Mol. Endocrinol* **22**, 2373–2382.

Cheikhelard A., Morel Y., Thibaud E., Lortat-Jacob S., Jaubert F., Polak M. *et al.* 2008 Long-term followup and comparison between genotype and phenotype in 29 cases of complete androgen insensitivity syndrome. *J. Urol.* **180**, 1496–1501.

Deeb A., Mason C., Lee Y. S. and Hughes I. A. 2005 Correlation between genotype, phenotype and sex of rearing in 111 patients with partial androgen insensitivity syndrome. *Clin. Endocrinol. (Oxf)* **63**, 56–62.

DiNapoli L., Batchvarov J. and Capel B. 2006 FGF9 promotes survival of germ cells in the fetal testis. *Development* **133**, 1519–1527.

Dowsing A. T., Yong E. L., Clark M., McLachlan R. I, de Kretser D. M. and Trounson A. O 1999 Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet* **354**, 640–643.

Evans B. A., Hughes I. A., Bevan C. L., Patterson M. N. and Gregory J. W. 1997 Phenotypic diversity in siblings with partial androgen insensitivity syndrome. *Arch. Dis. Child.* **76**, 529–531.

Galani A., Sofocleous C., Karahaliou F., Papathanasiou A., Kitsiou-Tzeli S. and Kalpini-Mavrou A. 2008 Sex-reversed phenotype in association with two novel mutations c.2494delA and c.T3004C in the ligand-binding domain of the androgen receptor gene. *Fertil. Steril.* **90**, e1–e4.

Gottlieb B., Beitel L. K., Wu J. H. and Trifiro M. 2004 The androgen receptor gene mutations database (ARDB): 2004 update. *Hum. Mutat.* **23**, 527–533.

Gottlieb B., Beitel L. K., Nadarajah A., Paliouras M. and Trifiro M. 2012 The androgen receptor gene mutations database: 2012 update. *Hum. Mutat.* **33**, 887–894.

Goulis D. G., Iliadou P. K., Papanicolaou A., Georgiou I., Chatzikyriakidou A., Gerou S. *et al.* 2006 R831X mutation of the androgen receptor gene in an adolescent with complete androgen insensitivity syndrome and bilateral testicular hamartomata. *Hormones (Athens)* **5**, 200–204.

He B. and Wilson E. M. 2003 Electrostatic modulation in steroid receptor recruitment of LXXLL and FXXLF motifs. *Mol. Cell Biol.* **23**, 2135–2150.

Hungerford D. A. 1965 Leucocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. *Stain Technol.* **40**, 333–338.

Ignacak M., Niedziela M. and Trzeciak W. H. 2002 Transition C2718T in the AR gene, resulting in generation of a termination codon and truncated form of the androgen receptor, causes complete androgen insensitivity syndrome. *J. Appl. Genet.* **43**, 109–114.

Ignacak M., Turek-Plewa J., Limon J. and Trzeciak W. H. 2004 A novel c.C2754>T transition in the androgen receptor gene introduces the premature termination codon Q798X and results in a truncated form of the receptor. *Gynecol. Endocrinol.* **19**, 178–181.

Imasaki K., Okabe T., Murakami H., Fujita K., Takayanagi R. and Nawata H. 1995 Premature termination mutation (772Glu–> stop) in the hormone-binding domain of the androgen receptor in a patient with the receptor-negative form of complete androgen insensitivity syndrome. *Endocr. J.* **42**, 643–648.

Isidor B., Capito C., Paris F., Baron S., Corradini N. and Cabaret B. 2009 Familial frameshift SRY mutation inherited from a mosaic father with testicular dysgenesis syndrome. *J. Clin. Endocrinol. Metab.* **94**, 3467–3471.

Jorgensen P. B., Kjartansdóttir K. R. and Fedder J. 2010 Care of women with XY karyotype: a clinical practice guideline. *Fertil. Steril.* **94**, 105–113.

Katoh-Fukui Y., Tsuchiya R., Shiroishi T., Nakahara Y., Hashimoto N., Noguchi K. *et al.* 1998 Male-to-female sex reversal in M33 mutant mice. *Nature* **393**, 688–692.

La Spada A. R., Wilson E. M., Lubahn D. B., Harding A. E. and Fischbeck K. H. 1991 Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79.

Layman L. C., Tho S. P., Clark A. D., Kulharya A. and McDonough P. G. 2009 Phenotypic spectrum of 45,X/46,XY males with a ring Y chromosome and bilaterally descended testes. *Fertil. Steril.* **91**, 791–797.

Li J., Fu J., Toumazou C., Yoon H. G. and Wong J. 2006 A role of the amino-terminal (N) and carboxyl-terminal (C) interaction in binding of androgen receptor to chromatin. *Mol. Endocrinol.* **20**, 776–785.

Liu Q., Hong J., Cui B., Zhang Y., Gu W., Chi Z. *et al.* 2008 Androgen receptor gene CAG(n) trinucleotide repeats polymorphism in Chinese women with polycystic ovary syndrome. *Endocrine* **33**, 165–170.

McEwan I. J. 2009 Nuclear receptors: one big family. *Methods Mol. Biol.* **505**, 3–18.

McElreavy K., Vilain E., Abbas N., Costa J. M., Pouleyreau N., Kucheria K. *et al.* 1992 XY sex reversal associated with a deletion 50 to the SRY "HMG box" in the testis-determining region. *Proc. Natl. Acad. Sci. USA* **89**, 11016–11020.

Melo C. O., Silva D. M. and da Cruz A. D. 2011 Challenges in clinical and laboratory diagnosis of androgen insensitivity syndrome: a case report. *J. Med. Case Rep.* **5**, 446.

Ocal G., Berberoğlu M., Sıklar Z., Ruhi H. I., Tükün A., Camtosun E. *et al.* 2012 The clinical and genetic heterogeneity of mixed gonadal dysgenesis: does "disorders of sexual development (DSD)" classification based on new Chicago consensus cover all sex chromosome DSD? *Eur. J. Pediatr.* **171**, 1497–1502.

Petroli R. J., Maciel-Guerra A. T., Soardi F. C., de Calais F. L., Guerra-Junior G. and de Mello M. P. 2011 Severe forms of partial androgen insensitivity syndrome due to p.L830F novel mutation in androgen receptor gene in a Brazilian family. *BMC Res. Notes* **4**, 173.

Philibert P., Audran F., Pienkowski C., Morange I., Kohler B., Flori E. *et al.* 2010 Complete androgen insensitivity syndrome is frequently due to premature stop codons in exon 1 of the androgen receptor gene: an international collaborative report of 13 new mutations. *Fertil. Steril.* **94**, 472–476.

Poukka H., Karvonen U., Yoshikawa N., Tanaka H., Palvimo J. J. and Janne O. A. 2000 The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. *J. Cell Sci.* **113**, 2991–3001.

Raicu F., Giuliani R., Gatta V., Palka C., Franchi P. G., Lelli-Chiesa P. *et al.* 2008 Novel mutation in the ligand-binding domain of the androgen receptor gene (l790p) associated with complete androgen insensitivity syndrome. *Asian J. Androl.* **10**, 687–691.

Rocha V. B., Guerra-Júnior G., Marques-de-Faria A. P., de Mello M. P. and Maciel-Guerra A. T. 2011 Complete gonadal dysgenesis in clinical practice: the 46,XY karyotype accounts for more than one third of cases. *Fertil. Steril.* **96**, 1431–1434.

Seabright M. 1971 A rapid banding technique for human chromosomes. *Lancet* **2**, 971–972.

Shahid M., Dhillion V. S., Jain N., Hedau S., Diwakar S., Sachdeva P. *et al.* 2004 Two new novel point mutations localized upstream and downstream of the HMG box region of the SRY gene in three Indian 46,XY females with sex reversal and gonadal tumour formation. *Mol. Hum. Reprod.* **10**, 521–526.

Shahid M., Dhillon V. S., Khalil H. S., Haque S., Batra S., Husain S. A. *et al.* 2010 A SRY-HMG box frame shift mutation inherited from a mosaic father with a mild form of testicular dysgenesis syndrome in Turner syndrome patient. *BMC Med. Genet.* **11**, 131.

Sultan C., Paris F., Terouanne B., Balaguer P., Georget V., Poujol N. *et al.* 2001 Disorders linked to insufficient androgen action in male children. *Hum. Reprod. Update* **7**, 314–322.

Sun S., Luo F., Zhou Z. and Wu W. 2010 A novel androgen receptor gene mutation in a Chinese patient with complete androgen insensitivity syndrome. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **153**, 173–175.

Thompson J., Saatcioglu F., Jänne O. A. and Palvimo J. J. 2001 Disrupted amino- and carboxyl-terminal interactions of the androgen receptor are linked to androgen insensitivity. *Mol. Endocrinol.* **15**, 923–935.

Tilley W. D., Buchanan G., Hickey T. E. and Bentel J. M. 1996 Mutations in the androgen receptor gene are associated with progression of human prostate cancer to androgen independence. *Clin. Cancer Res.* **2**, 277–285.

Wang Q., Ghadessy F. J. and Yong E. L. 1998 Analysis of the transactivation domain of the androgen receptor in patients with male infertility. *Clin. Genet.* **54**, 185–192.

Wooster R., Mangion J., Eeles R., Smith S., Dowsett M., Averill D. *et al.* 1992 A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. *Nat. Genet.* **2**, 132–134.

Zoppi S., Wilson C. M., Harbison M. D., Griffin J. E., Wilson J. D., McPhaul M. J. *et al.* 1993 Complete testicular feminization caused by an amino-terminal truncation of the androgen receptor with downstream initiation. *J. Clin. Invest.* **91**, 1105–1112.

Received 3 November 2015, in final revised form 5 April 2016; accepted 7 April 2016
Unedited version published online: 11 April 2016
Final version published online: 21 November 2016

Corresponding editor: RAJIVA RAMAN