

***SRY* (Sex Determining Regions in Y) Basis of Sex Reversal in XY Females**

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ABSTRACT 46,XY sex reversal condition is known to be caused by the point mutations or deletions in *SRY* gene at Yp11.3. Individuals with 46,XY status are phenotypically females with gonadal dysgenesis and poorly developed secondary sexual characters. In the present study, it is aimed to report the *SRY* basis of sex reversal in 25 XY female probands aged 4 to 39 years. The karyotypes in 21 were 46,XY and 45,X/46,XY in 4. The presence of the *SRY* gene was determined in 20 probands (80%), out of which 18 had 46,XY and 2 had 45,X/46,XY karyotypes. *SRY* gene was not detected in 5 probands (20%), among them 3 had 46,XY and 2 had 45,X/46,XY karyotypes and in these 5 case, *SRY* gene was considered to be deleted. The present study confirmed the importance of the genetic evaluation of the *SRY* gene in females with XY status for further management.

INTRODUCTION

46,XY sex reversal (OMIM 400044) condition is known to be caused by the point mutations or deletions in the *SRY* gene (OMIM 480000) positioned at Yp11.3. Individuals with 46,XY status are phenotypically females with complete gonadal dysgenesis; poorly developed secondary sexual characters; amenorrhea; bilateral 'streak gonads' and underdeveloped uterus, fallopian tubes and female external genitalia (Berkovitz et al. 1991).

SRY is the testis-determining factor (TDF) on the Y chromosome (Sinclair et al. 1990; Koopman et al. 1991). Failure of expression of the *SRY* gene during the 6th to 7th week of embryogenesis could result in the formation of the dysgenetic streak ovaries devoid of the germ cells (Lim et al. 1998). *SRY* gene expression initiates the bi-potential gonads to differentiate

into the testis and in the absence of the expression from the *SRY* gene; the bi-potential gonads differentiate into inappropriate female gonads. *SRY* encodes for a polypeptide of 204 amino acids in length. *SRY* protein contains a central high-mobility group (HMG) box domain, an amino terminal (N-terminal) and carboxy-terminal (C-terminal) regions (Clepet et al. 1993; Su and Lu 1993). Mutations are either single nucleotide substitution or deletion of *SRY* gene. These mutations affect the DNA binding and DNA bending properties of the *SRY* gene, which lead to the non-functional *SRY* gene (Sanchez-Moreno et al. 2008; Mitchell and Harley 2002). It is seen, that to date, 86 mutations have been compiled within the HMG box region of *SRY* gene (Human Gene Mutation Database- HGMD 2011). In literature, it is reported that the incidence of *SRY* mutation is 10 to 15% in 46,XY female condition (Giuffre et al. 2004). In the present study, it is aimed to report the *SRY* basis of sex reversal in cytogenetically confirmed XY females.

MATERIAL AND METHOD

Twenty five (25) female probands were referred for the genetic analysis and counseling to Division of Human Genetics, St. John's Medical College, Bangalore, India. Their age ranged

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from 4 to 39 years. The reasons for the referral were primary amenorrhea, poorly developed secondary sexual characters and genital ambiguity. Parental consent and institutional ethical clearance were obtained. For the probands, one each normal male and female were the control samples. The karyotype was confirmed with cytogenetic analysis [PHA (Phyto Haem Agglutinin) stimulated, 72 hours peripheral lymphocyte culture technique with GTG (Giemsa-Trypsin-Giemsa) banding (Arakaki and Sparkes 1963; Seabright 1971).

Molecular genetic analysis was based on the phenol-chloroform DNA extraction method (Thangaraj et al. 2002b). DNA was quantified using nano drop Thermascientific. Primer sequences were obtained (Singh et al. 2006). PCR (polymerase chain reaction) conditions and primer concentrations were standardized using Gradient Veriti PCR machine. PCR reagents included PCR Buffer (10X), MgCl₂ (Magnesium Chloride) (25mM), dNTPs (deoxy Nucleotide Tri Phosphates) (10mM), AmpliTaq Gold DNA polymerase (5units/ul) and 4 ul of genomic DNA. All the PCR reagents were procured from Applied Biosystems (USA). PCR conditions were 94°C for 12 min (94°C for 1 min, 65°C for 1 min, 72°C for 2 min) X 32 cycles, 72°C for 10 min. DNA sequencing was carried out in fully automated 3700 DNA sequencer (Bigdye™ chain terminator method) (Thangaraj et al. 2003b).

The 27 samples were screened for the *SRY* gene. Using specific primers, the entire *SRY* gene was amplified with PCR. Gel electrophoresis showed amplification of *SRY* gene of 609bp in 20 samples and in the control male sample. Further DNA sequencing using BigDye™ chain terminator and automated DNA sequencer, in the cases without amplification, a short repetitive DNA sequence of about 100 to 170bp was observed. For the cases, with positive *SRY* gene amplification, DNA sequences were compared and edited from reference sequence down loaded from <http://www.ensembl.org> (Ensembl gene ID

ENSG00000184895 and Ensembl Transcript ID ENST00000383070). Sequences of normal male control was edited and compared with the reference sequences and the XY female samples using AutoAssembler software—version 2.1 (Perkin-Elmer, Foster City, CA, USA) and CodonCode Aligner version 2.0.5 (CodonCode Corporation, Dedham, MA, USA).

RESULTS

Genetic analysis includes the cytogenetics for the karyotype and the molecular for the *SRY* gene. = The karyotypes were 46,XY in 21 probands and 45,X/46,XY in 4. = Amplification of *SRY* gene of 609bp was observed in 20 samples and in control male sample and the amplification was absent in 5 samples and in the control female sample.

= In the 5 cases without amplification, short repetitive DNA sequences of about 100 to 170bp were observed. Their presence confirmed the absence of the *SRY* gene and instead, the presence of the few non-coding sequences in that region (Fig. 1).

Hence, in these 5 probands, *SRY* gene was considered to be deleted. = In the 20 *SRY* positive cases, 18 had 46,XY karyotype and 2 had 45,X/46,XY mosaicism. Among the 5 *SRY* deletion cases, 3 had 46,XY karyotype and 2 showed 45,X/46,XY mosaicism. = In the 20 cases with amplification, sequence changes were not observed. These 20 cases with intact *SRY* gene did not manifest mutation of either single nucleotide substitution or deletion.

DISCUSSION

In 1990, Berta et al. and Jager et al. presented compelling evidence that the mutation in one type of XY female gonadal dysgenesis is not on the X; but on the Y chromosome. In the human sex-determining region in a 35-kb interval near the pseudoautosomal boundary of Y chromosome, there is the candidate gene for the

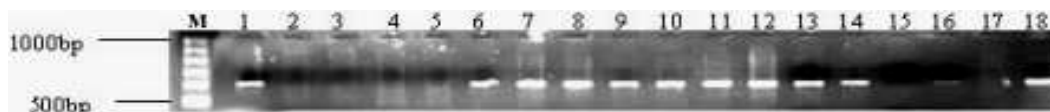


Fig. 1. Gel electrophoresis of the PCR amplicons of the *SRY* gene. Lane M: 100 bp ladder. Lanes 1, 6, 7, 8, 9, 10, 11, 13, 14 and 16 the *SRY* amplification of 609bp. In lane 2,3,4,5, 15 *SRY* amplification of the XY females was not seen. In lane 17 normal female with no amplification and in lane 18 amplification of normal male is shown

testis-determining factor, termed *SRY*, which is conserved and specific to the Y in all mammals (Sinclair et al. 1990). Chérfas (1991) stated that *SRY* stands for 'sex-determining region Y.'

It is known that *SRY* gene in Y initiates the sex determination in males. *SRY* activates a cascade of genes; so that the embryonic gonads develop into a testis. Fetal testicular Sertoli cells produce Mullerian Inhibitory substance responsible for the involution of the derivatives of Mullerian ducts, the uterus and fallopian tubes. Fetal testicular Leydig cells produce testosterone from cholesterol by the sequential action of a series of enzymes and the subsequent differentiation of the male external genitalia also requires the action of dihydrotestosterone from the testosterone. Perturbations in the enzymes in the classic pathway or in an alternative pathway of testicular androgen biosynthesis could result in genetic males with disordered sexual development and incompletely developed ('ambiguous') external genitalia (Fluck et al. 2011).

Cytogenetic Analysis: In 46,XY females, the reported cytogenetic forms could be 46,XY pure cell line as well as 45,X/46,XY mosaic cell lines. A structurally abnormal Y chromosome is not uncommon in XY gonadal dysgenesis; hence, the loss of the structurally abnormal Y results in the 45,X cell line. The predominance of the X or XY cell lines determines the gonadal differentiation into a testis or a streak gonad (Rimoin et al. 2006). In the present study, the karyotype profile was 46,XY in 21 (84%) and mosaicism (45,X/46,XY) in 4 (16%). In 4 cases with mosaicism, 2 showed the predominance of XY cell lines.

***SRY* Gene Mutation Incidence:** A mutation in *SRY* gene in 1 out of 12 (8.3%) sex-reversed XY females with gonadal dysgenesis was demonstrated and they did not have any large deletions of the short arm of the Y. (Jager et al. 1990) In a combined study, it is seen, that 3 (60%) of 5 patients with 46,XY complete gonadal dysgenesis had mutations in *SRY*; whereas only 5 (12.2%) of the 41 subjects with various forms of 46,XY gonadal dysgenesis had mutation for *SRY* (Berta et al. 1990; Hawkins et al. 1992). Nussbaum et al (2007) stated that in female patients with XY gonadal dysgenesis, approximately 10 to 15% may have the point mutations, deletions or translocations in *SRY*, which become the common cause for the sex reversal in them. In the present study, the occurrence of

the *SRY* deletion was in 5 out of 25 (20%) cases with sex-reversed XY females and the occurrence was within the reported range of 10 to 20% of *SRY* gene perturbations in XY females.

***SRY* Gene Analysis:** A 230-kb (kilobase) segment of the human Y chromosome thought to contain some or all of TDF gene was cloned. (Page et al. 1987) The cloned region spanned the deletion in a female who carried all but 160kb of the Y.

Jager et al. (1990) found a 4-nucleotide deletion in the part of the *SRY* gene that encoded a conserved DNA-binding motif. A frameshift presumably led to a non-functional protein. Mutation has occurred de novo, because the father had a normal *SRY* sequence. The de novo G-to-A mutation led to a change from methionine to isoleucine at a residue that lies within the putative DNA-binding motif of *SRY* and was identical in all *SRY* and *SRY* related genes.

Point mutations in the region of the *SRY* gene encoding the high mobility group (HMG) box in 5 XY females were detected. (The HMG box is related to that present in the T-cell-specific, DNA binding protein TCF1 [Transcription Factor 1]). (Harley et al. 1992) In 4 cases, the binding activity of mutant *SRY* protein for the AACAAAG core sequence was negligible; in the 5th case, DNA binding was reduced. In the *SRY* gene analysis in a 46,XY female, Muller et al (1992) demonstrated an A-to-T transversion of nucleotide 684 in the open reading frame, resulting in a change of lysine (AAG) to a stop codon (UAG) and the patient had gonadoblastoma. A XY sex-reversed female with pure gonadal dysgenesis who harbored a de novo nonsense mutation in the *SRY* gene, which resulted directly in the formation of a stop codon in the putative DNA-binding motif was described. (McElreavey et al. 1999) A C-to-T transition at nucleotide 687 changed a glutamine codon (CAG) to a termination codon (TAG). The patient, referred to as the 'propositus,' was a phenotypic female who presented at age 20 years for primary amenorrhea. Treatment with estrogen induced menstruation and slight enlargement of the breasts, which were underdeveloped. Laparotomy showed 2 streak gonads without germ cells.

In the present study, with direct DNA sequencing, only the *SRY* coding region was sequenced. In 5 *SRY* deletion cases without amplification, short repetitive DNA sequences of

about 100 to 170bp were observed and their presence confirmed the absence of the *SRY* gene and the presence of the few non-coding sequences in that region.

A family of 5 XY individuals in 2 generations with a single base pair substitution resulting in an amino acid change in the conserved domain of the *SRY* open reading frame was described. (Vilain et al. 1992) A G-to-C change at nucleotide 588 resulted in substitution of leucine for valine. Three of the individuals were XY sex-reversed females and 2 were XY males. One of the males had 8 children; all were phenotypic females, 2 of whom were sex-reversed XY females carrying the mutation mentioned. Several models were proposed to explain association between a sequence variant in *SRY* and 2 alternative sex phenotypes. These included the existence of alleles at an unlinked locus. In the present study, the family history was found to be normal.

Genetic Counselling

The probands and the parents are counselled about the genetic diagnosis and referred to the multiteam specialty of Endocrinology, Obstetrics and Gynecology, Psychiatry and Surgery.

On follow up, it was observed that one of the probands underwent gonadectomy; another proband had clitorodectomy and the proband with Hodgkins lymphoma on treatment has become asymptomatic.

CONCLUSION

46,XY female condition is a heterogeneous disorder, because of which establishing a definition, which will encompass all manifestations (clinical, biochemical, physical, medical, genetic), becomes a difficult task. The present study confirmed the importance of the genotypic evaluation of patients with 46,XY status in females for further management.

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