New disease

Novel mutation of *SRD5A2* gene in a patient with 5 α -reductase 2 deficiency from India

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Summary

Master N had genital malformation at birth and had bilateral gonads in the labial fold. He was reared as a boy and corrective surgery was done at the age of 4 years and was reassessed at the age of 14 years. His testosterone/dihydrotestosterone (DHT) was 11.8 (reference range ≤ 10). Molecular analysis of *SRD5A2* gene indicated the presence of a novel heterozygous missense mutation of p.A52T in exon 1, which was also detected in mother. The father, sister and maternal grandfather were found to have normal *SRD5A2* gene sequence. We also detected an intronic (1–2) homozygous T>C transition in patient, whereas both parents were found to have the same transition in heterozygous form. Although 5 α -steroid reductase 2 deficiency is an autosomal-recessive disorder, in this case, it appears that there may be a dominant inheritance because only one identified mutation was present which was passed from mother to son.

BACKGROUND

The 5 α -reductase 2 isoenzyme is responsible for the conversion of testosterone (T) to a more potent androgen-dihydrotestosterone (DHT). Both these androgens bind to the same androgen receptor for different physiological activities, but with different affinities. T is responsible for Wolffian duct masculinisation whereas virilisation of external genitalia, prostate and urethra is under the influence of DHT.¹ Defects in the conversion of T to DHT by enzyme 5 α -reductase 2 leads to 5 α -steroid reductase 2 deficiency (5 α SRD2) which is an autosomal recessive disorder and was first described by Imperato-McGinley *et al* in 1974.² Mutations in this gene results in decreased synthesis of DHT, which is responsible for varying degrees of genital ambiguity with clitoral-like phallus, severely bifid scrotum, perineoscrotal hypospadias and rudimentary prostate.^{3–4} Usually, these patients are reared as girls at birth and they develop male gender identity at puberty. Their clinical profile, and a strong male gender behaviour contribute to the sex reassignment as evident from previous studies.^{5–6} Clinically, it is difficult to diagnose 5 α SRD2 as the features overlap with androgen insensitivity syndrome (AIS).⁷ However, elevated T/DHT ratio to some extent and molecular analysis of *SRD5A2* gene proves to be a useful marker. Novel heterozygous missense mutation p.Q56H and p.V89L polymorphism on exon 1 and homozygous missense p.R246Q mutation on exon 5 have been reported in few case reports with 5 α SRD2 in Indian population.^{8–9} A novel heterozygous missense mutation of p.Ala52Thr and an intronic (1–2) transition of T>C are rare compound mutations found in our patient with 5 α SRD2.

CASE PRESENTATION

Master N (III.6) was the third child born of a non-consanguineous marriage. Genital malformation, penoscrotal hypospadias (PH) was noted at birth and was

referred for surgical correction. Physical examination revealed clitoris-like phallus with single opening and bilateral gonads were palpable in the labial fold. Genitogram done with contrast injected through this single opening revealed a long urethra and urinary bladder. Karyotype revealed 46, XY pattern. Application of testosterone cream locally leads to increase in the phallus length (SPL~3 cm). Subsequently, urethroplasty was done and he was reared as male. He was referred to endocrinology at the age of 9 years. Evaluation at that time revealed a prepubertal child without any gender dysphoria. Now at the age of 14 years, height (154 cm) and weight (45 kg), he has no gynaecomastia; pubic hair is Tanner stage III; few axillary hair and no facial or chest hair.

There is no history of sexual ambiguity, infertility, consanguinity or immigration in the family.

INVESTIGATIONS

This is part of an on-going study 'To assess the molecular genetics of 46, XY disorders of sexual development (DSD)'. The study protocol was approved by Institutional ethics committee.

Informed consent for genetic analysis was obtained as per ICMR guidelines: verbal consent from the children and written informed consent from parents and grandfather.

Blood sample (6 ml) was collected in an EDTA vial for estimation of plasma luteinizing hormone (LH), follicle stimulating hormone (FSH), T, DHT and genetic analysis from the patient. Blood sample for genetic analysis was collected from parents, sister and maternal grandfather. Electrochemiluminescence immunoassay using commercial kits was used for the estimation of LH, FSH and T (Roche, Germany). Intra-assay and interassay precisions were 4.1%, 7%, 7.5% and 4.7%, 6% and 8.5%, respectively. DHT was estimated by radioimmunoassay (Immunotech; Prague, Czech Republic) after extraction from other hormones with diethyl ether and Celite chromatography.

The intra-assay and interassay precisions were <10% and <6%, respectively.

DNA was isolated from peripheral leucocytes by phenol-chloroform extraction. DNA pellet was then resuspended in 200 µl of autoclaved sterile water. All 5 exons of *SRD5A2* gene and their flanking introns were amplified in the patient's sample by PCR, using set of primers reported previously¹⁰ and with some modifications. PCR amplifications for all primer sets were performed in a 50 µl volume containing 2 µl of 10 µM stock solution for each primer (Biochem, India), 100 ng of genomic DNA, 1 unit of Taq polymerase (Banglore Genei, Bengaluru, Karnataka, India), 0.1 mM of each deoxynucleotide triphosphate (dNTP) and 5 µl of 10×PCR buffer (with 15 mM MgCl₂) by means of 35 cycles of amplification, each consisting of 5 min denaturation at 94°C, 1 min annealing at 56–60°C, and 1 min extension at 72°C. Finally, an extension for 10 min at 72°C was performed. The band length of the PCR products was visualised on 2% agarose gel after electrophoresis. After purification, using RBC PCR purification kit, the products were sequenced with Big Dye terminator V.1.1 and V.3.1 Cycle Sequencing Kits (GCC Biotech (India) Pvt Ltd). To find out the mode of inheritance of this mutation, exon 1 was sequenced in whole family and from maternal grandfather.

All sequence variants from patient, control and relatives were compared with the human *SRD5A2* reference sequence NC_000002.11 provided by the National Center for Biotechnology Information using ClustalW2 (multiple sequence alignment programme for DNA; European Molecular Biology Laboratory (EMBL)-European Bioinformatics Institute.

In silico analysis using SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 (Polymorphism Phenotyping) online softwares were used to predict the functional importance of amino acid substitutions. PolyPhen structurally aligns the normal and substituted amino acids in the protein sequence to predict the deleterious effect of the change to the protein structure.¹¹ PolyPhen-2 is the advanced version of the earlier version which predicts the damaging effect by giving the PISC score (position-specific independent counts).¹² PolyPhen scores of above 0.85 indicate that the polymorphism is probably damaging protein function. Scores of above 0.15 are possibly damaging, and scores of less than 0.15 are classified as benign.

SIFT sorts intolerant amino acids from tolerant substitutions and is the homology-based tool that predicts whether an amino acid substitution in a protein will have any phenotypic effect.¹³ Positions with normalised probabilities less than 0.05 are predicted to be deleterious and those ≥0.05 are predicted to be tolerated.

DIFFERENTIAL DIAGNOSIS

Patient had normal 46, XY karyotype, which excludes any chromosomal disorder. Presence of normal testes in the labial folds excludes disorders of gonadal differentiation. Testosterone, LH and FSH were in normal range excluding disorders of testosterone biosynthesis and AIS. T/DHT ratio confirmed 5α SRD2 and molecular diagnosis confirmed a novel heterozygous missense mutation of p.A52T in exon 1 and an intronic (1–2) homozygous T>C transition.

Figure 1 Three generation family Pedigree. * Recently married and # still birth; Heterozygous for both A>T and intronic (1–2) T>C transition; heterozygous for intronic (1–2) T>C transition; heterozygous for both A>T and homozygous for intronic (1–2) T>C transition.

Figure 2 Electropherogram of exon 1 of *SRD5A2* gene in a family. Electropherogram showing heterozygous missense mutation with G>A, 31805817, c154, GCC>ACC, Ala52Thr in (i) patient (III.6) and (ii) mother (II.3). Electropherogram showing normal GCC sequence in (iii) father (II.10), (iv) sibling (III.5), (v) grandfather (I.2) and (vi) control.

Figure 3 Electropherogram showing intronic (1–2) region transition in a family; Electropherogram showing (i) homozygous T>C in the intronic (1–2) region, g.31805675, rs522638 in patient (III.6); (ii) heterozygous T>C transition in the intronic (1–2) region in mother (II.3) and (iii) heterozygous T>C transition in the intronic (1–2) region in father (II.10). Electropherogram showing normal sequence in (iv) sibling (III.5); (v) grandfather (I.2) and (vi) control.

TREATMENT

Patient and family were educated about the nature of disease and follow-up action.

OUTCOME AND FOLLOW-UP

A three-generation family tree of the affected patient is given in figure 1.

The hormonal profile of the patient revealed plasma LH 2.9U/l (1.7–8.6), FSH 2.03 U/l (1.5–12.4), T 3.79 ng/ml (2.4–8.3), DHT 0.32 ng/ml and T/DHT ratio as 11.8. The serum hormone of parents and sibling were in the normal range.

All 5 exons of *SRD5A2* gene was sequenced from patient's DNA sample (III.6). On exon 1, a novel heterozygous missense mutation was observed. This mutation lead a single-base substitution of A>T at codon 154. This mutation replaces alanine with threonine at amino acid position 52 (Ala52Thr). Further studies of this mutation in other family members revealed that the same heterozygous mutation was present in mother (II.3) and absent in father (II.10) and sibling (III.5). Exon 1 of *SRD5A2* gene was sequenced twice on new PCR reactions from two different sequencing companies (Macrogen, Korea;GCC Biotech, India) for the confirmation of the mutation. Maternal grandfather (I.2) was found to be normal (figure 2). Other members from the maternal side were either not available or not willing to participate in the study.

Another homozygous T>C substitution was found in the intronic (1–2) region (g.31805675, rs522638) in the patient (III.6). Both parents (II.3 and II.10) were heterozygous for this substitution, while sibling (III.5) and grandfather (I.2) had normal sequence (figure 3).

To assess the effect of mutation on protein function. In silico analysis using SIFT and PolyPhen 2 indicated that the mutation is possibly damaging with PSIC score of 1.791.

DISCUSSION

More than 55 different mutations have been reported worldwide (<http://www.hgmd.cf.ac.uk>), most of them being missense mutations. More than half of the affected individuals with 5 α SRD2 were homozygotes, whereas 40% were either compound heterozygotes or inferred compound heterozygotes.^{14 15}

Data from Indian subcontinent revealed that p.R246Q mutation in exon 5 of *SRD5A2* gene is a common variant indicating that it may have a founder effect.^{8 9} In addition to p.R246Q mutation, various homozygous and compound heterozygous mutations have also been described in Asian population with different ethnicity.^{9 10 15–21}

In our patient (III.6), a novel heterozygous mutation in exon 1 was identified in the *SRD5A2* gene. A single-base substitution of A>T at site 154 replaces alanine with threonine at position 52 (Ala 52 Thr). Various mutations in exon 1 have been found in individuals with divergent geographical and ethnic backgrounds.^{14 22–25} To the best of our knowledge, this mutation has not been reported earlier. In silico analysis of this mutation by using PolyPhen 2 and SIFT revealed the mutation as possibly damaging (PSIC score=1.791). The enzyme activity of 5 α -reductase 2 enzyme may alter as there is a change from non-polar amino acid (alanine) to polar amino acid (threonine). Kinetic studies on 5 α -reductase 2 that

were carried out previously have shown that mutations in the C-terminal half of the protein affect the binding to the cofactor NADPH, whereas mutations affecting N-terminal or both affects T binding.²⁶ Although we have not carried out an in vitro study to measure the mutant enzyme activity, the mutation found in our patient could affect the enzyme activity as was suggested by PolyPhen 2 and SIFT. Molecular analysis was carried out in parents (II.3 and II.10) and sibling (III.5) and the same heterozygous mutation of A52T was present in mother (II.3), while father (II.10) and sister (III.5) were found to be normal.

On the basis of molecular analysis, this mutation has been transmitted from mother to son. However, in the patient's mother, it is not clear whether this is transmitted from her mother (maternal grandmother I.1) or may be a de novo mutation.

Our patient (III.6) also had a homozygous T>C substitution in the intronic (1–2) region (g.31805675, rs522638). His parents (II.3 and II.10) had the same substitution in heterozygous condition, whereas sibling (III.5) and grandfather (I.2) had normal sequence. This substitution may or may not have an impact on gene expression or splicing.

We have identified a novel heterozygous mutation in exon 1 of *SRD5A2* gene from a 14-year-old patient from India with 46, XY DSD. The same heterozygous mutation was also detected in mother and the rest of the family members were found to have normal *SRD5A2* gene sequence. We also detected an intronic (1–2) homozygous T>C transition in patient, while both parents were found to have heterozygous transition. Whereas his sibling and maternal grandfather were found to be normal. All maternal family members need to be analysed for the *SRD5A2* gene to assess the inheritance pattern.

Learning points

- ▶ Children presenting with ambiguous genitalia should be investigated to rule out all the possible inborn errors of metabolic disorders.
- ▶ Gender assignment should be done after careful evaluation of phenotypic features, hormonal profile and molecular diagnosis.
- ▶ This child with 5 α -steroid reductase 2 deficiency has a novel mutation.

Competing interests None.

Patient consent Obtained.

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