Short Report

Novel mutation in the nuclear receptor subfamily 0, group B, member 1 (*NR0B1*) gene associated with intrafamilial heterogeneity in three boys with X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism from India

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ABSTRACT

Background. **Nuclear receptor subfamily 0, group B, member 1 (*NR0B1*) gene previously known as *DAX1* is a transcription factor that plays a key role in the development of hypothalamo–pituitary–gonadal and adrenal axis. Primary adrenal failure may result from metabolic, infection, autoimmune or developmental causes resulting in a life-threatening condition needing immediate intervention. This study aimed to analyse *NROB1* (*DAX1*) gene mutation resulting in adrenal hypoplasia congenita (AHC) in three brothers presenting with hypogonadotropic hypogonadism and primary adrenal failure either in infancy or in early childhood.**

Methods. **We studied three boys with primary adrenal failure and hypogonadotropic hypogonadism presenting at different ages at the Paediatric Endocrinology Clinic. Mutational analysis of *NROB1* gene was carried out by bidirectional sequencing.**

Results. **All the three boys had deletion of G in exon 1 at position 189 (c.189_189delG) of the gene resulting in frame shift mutation (Y64Tfs*21).**

Conclusion. **Novel mutation in *NROB1* detected by this study explained the cause of hypogonadotropic hypogonadism with primary adrenal failure in this Indian family. Intrafamilial variability was seen in this family. Early diagnosis by genetic testing, genetic counselling and family screening can help to manage this life-threatening condition.**

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INTRODUCTION

Congenital adrenal hypoplasia (AHC) is a genetic disorder of either autosomal recessive (OMIM#240 200) or an X-linked inheritance (OMIM #300 200). In both inheritance patterns, primary adrenal failure occurs due to defective development of the adrenal gland. In X-linked type, mutations result in permanent lack of adult cortical zone with compensatory changes in residual foetal cells[1] leading to cytomegalic adrenocortical hypoplasia compared to autosomal recessive or sporadic types where there is lack or paucity of both foetal and permanent cortex. Hypogonadotropic hypogonadism and primary adrenal failure are the key features of the X-linked type AHC due to defective development and functioning of the hypothalamic–pituitary–gonadal and adrenal axis. The presentation is variable; about 60% present with adrenal failure by the third week of life and 40% of them later in childhood. Very rarely those with partial hypogonadism and delayed onset adrenal failure present in adulthood.

The incidence of X-linked AHC is not clearly known. The estimated occurrence may lie between 1:140 000 and 1:1 200 000 children.[2] The *NR0B1* gene previously known as *DAX1* is mapped on Xp21.2. This gene has two exons with one intervening intron (OMIM database). *NR0B1* is an important orphan nuclear receptor involved in normal development and functioning of the hypothalamic–pituitary–gonadal and adrenal axis[3,4] The product of this gene is a transcriptional factor with the unique feature of lacking the zinc finger DNA binding domain.[5] The gene product plays an important role in development and functioning of the target tissues, namely adrenals and testes. Mutations in the *NR0B1* either results in no protein formation or may result in truncated protein when translated. Large deletions have also been reported.[6,7]

THE CASES

Patient 1

The proband came for genetic evaluation at 20 years of age. He was the eldest of three siblings and was well until 3 years of age when he presented with chronic vomiting, skin hyperpigmentation, and failure to thrive. He was hospitalized for the same and found to have hyponatraemia 120 mmol/L (135–145 mmol/L), hyperkalaemia 7.5 mmol/L (3.5–4.5 mmol/L) and hyperreninaemia 79.4 ng/ml/hour (up to 4.0 ng/ml/hour). A diagnosis of primary adrenal insufficiency was made, and he was started on hydrocortisone and fludrocortisone supplementation with marked improvement. The course was complicated by an Addisonian crisis at 10 years of age. At 15 years of age, he failed to develop secondary sexual characters. The gonadotropin levels were: follicle-stimulating hormone (FSH) 0.915 mIU/ml (0.5–3.3 mIU/ml); low luteinizing hormone (LH) 0.137 mIU/ml (0.5–3.3 mIU/ml) and testosterone (<20.0 ng/dl) but normal 17-OH progesterone <0.1 ng/ml (0.0–2.0 ng/ml) which suggest hypogonadotropic hypogonadism with a clinical diagnosis of AHC (Table I). Compliance with replacement was poor as evident by high adrenocorticotropic hormone (ACTH) 952 pg/ml (0.0–46 pg/ml) and growth velocity remained poor. At that time, his age was 17 years and 4 months, and height 147 cm with standard deviation score (SDS) 3.5. The corresponding bone age was 13 years and 6 months (Greulich–Pyle method; G&P), and SDS for mid-parental height (MPH) was 0.8. Family history was positive as two more siblings were affected (Fig. 1).

Patient 2

The younger brother was 18 years old and first visited the hospital at 5 years with poor appetite, poor weight gain and increased skin

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TABLE I. Clinical and biochemical profiles of the patients

Clinical feature	Patient 1	Patient 2	Patient 3
Onset of primary adrenal failure (years)	3	15	14
Age of detection of growth failure (years)	4	12	14
Hypoglycaemia	No	No	No
Hypotension	Yes	No	No
Adrenal crisis	Yes	No	No
Skin pigmentation	Yes	Yes	Yes
Growth retardation	Yes	Yes	Yes
Delayed puberty	Yes	Yes	Yes
Pubic hair (Tanner stage)	1	1	1
Testicular volume	2 ml	2 ml	1 ml
Triggering factor	Febrile illness	None	Febrile illness
Final height/mid-parental height*	156/157.5	152/157.5	151/157.5
Adrenocorticotropic hormone (ACTH)	>1250	1250	>1250
Testosterone	<20 (at 14 years)	<20 (at 18 years)	<20 (at 15 years)
Luteinizing hormone (LH)/follicle-stimulating hormone (FSH)	0.137/0.915	0.10/0.10	0.19/0.82
Plasma rennin activity (upright)	211	170.9	82.0

* Parents' height: father 160 cm, mother 155 cm Normal ranges: ACTH 10–60 pg/ml (a.m.), testosterone level ≥100 ng/dl (at 14 years), LH up to 3.8 mIU/ml (1.6–9 years), FSH up to 1.9 mIU/ml (4–9 years), plasma renin activity (upright) 4.4–46.1 ng/ml/hour

pigmentation. His height was 100.5 cm (SDS<1.5). His biochemical profile was normal except for poor response to ACTH stimulation as evident by basal serum cortisol 8.17 µg/dl and 1-hour level of 8.5 µg/dl at the age of 15 years confirming primary adrenal insufficiency. At 18 years, his hormonal profile showed luteinizing hormone (<0.1 mIU/ml), follicle-stimulating hormone (<0.1 mIU/ml) and testosterone (<20.0 ng/dl) suggesting hypogonadotropic hypogonadism.

Patient 3

The youngest of three siblings was asymptomatic when seen for the first time at our centre at the age of 15 years with a height of 140.5 cm ("3.0 SDS) with a history of upper respiratory tract infection and episodes of vomiting for which he was admitted to a hospital elsewhere. Physical examination showed hyperpigmented lips, perioral region and bilateral pinna and features of delayed puberty with a testicular volume of 1 ml. Laboratory evaluation showed hyponatraemia, hypocortisolaemia and low gonadotropins indicating primary adrenal failure with hypogonadotropic hypogonadism. He was managed with hydrocortisone and fludrocortisone.

METHODS

As the clinical diagnosis of AHC was evident in all three, diagnostic *NR0B1* gene analysis was initiated after approval of the institutional review board and taking informed consent from the patients. The genomic DNA was isolated from peripheral blood using Qiagen DNA blood kit. Amplification of exon-1 and exon-2 including flanking intronic region of *NR0B1* was done using a specific set of designed primers. Primers used were as follows: Exon1AF-AATAGGTCCCAGGAGGCAGC; exon1AR; AAAGCAGCAGCGGTACAGGAGTG; Exon1BF: GACGCTGGGTCCGTGCTG; exon1BR: GACTTCCACAGTCTCGAACTGC; Exon1CF-AGTACTTGCCCTGCTTCCAG; exon1CR-TTCACCTTTGCCCCGACACT; Exon2F-AAGCTAGCAAAGGACTCTGTG; exon2R-TATTTTACACTCTTTTGCCCA. Bi-directional sequencing was carried out on an ABI3500 sequencer. Sequences were compared

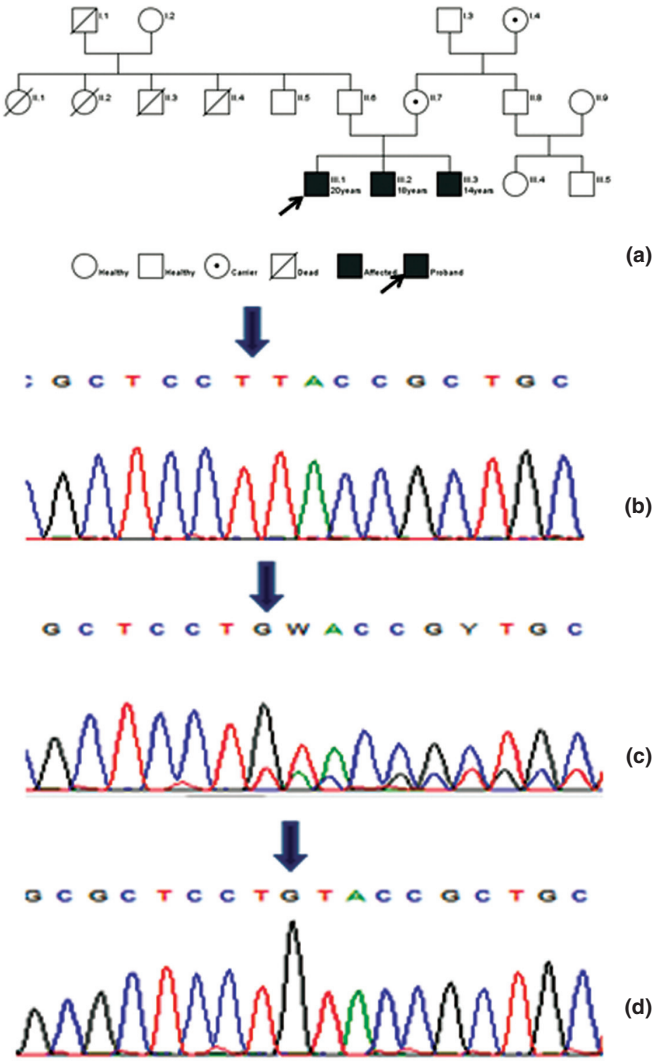

FIG 1. (a) Pedigree; (b) chromatograms showing homozygous frameshift mutation in exon-1 (189_189DelG), arrow placed at deleted G; (c) heterozygous mutation; (d) normal

with the normal reference sequence. Results of molecular testing were interpreted with the patient's clinical and biochemical profile.

RESULTS

Deletion of G at position 189 (c.189_189delG) resulting in frameshift mutation (Y64Tfs*21) in exon1 of the *DAX1* gene leading to premature termination of codon at 84 amino acid position was identified. All three brothers are hemizygous resulting in a truncated protein with 83 amino acids (Fig. 1b–d). Mother and maternal grandmother were heterozygous for the same mutation. The asymptomatic maternal uncle showed wild-type allele. Bioinformatics tools; Mutation taster and Provean were used to predict the nature of the mutation and its consequences. Both bioinformatics tools predicted the mutation as deleterious, the best possible protein structure model showed an alignment score of 63%, but there is existence of nonsense-mediated decay pathways, hence it is difficult to predict the structure and functionality of a mutated protein. Thus, no forward analysis was carried out.

DISCUSSION

AHC is a genetic disorder inherited either as X-linked recessive or as an autosomal recessive pattern. In both inheritance patterns, primary adrenal failure occurs due to defective development of the adrenal gland. The other genetic disorders causing adrenal failure are congenital adrenal hyperplasia with an elevation of 17-OH progesterone, inherited in autosomal recessive manner, X-linked adrenoleukodystrophy associated with white matter disease, behaviour problem and a decline in cognition, and finally AHC with an anatomically small adult cortex of the adrenal gland.

More than 248 (HGMD database) mutations in the *DAX1* gene have been reported in the literature, either one amino change, duplication, partial or complete gene deletion[8] rendering the protein non-functional resulting in hypogonadotropic hypogonadism.

Intrafamilial heterogeneity was observed in all the three brothers. Primary adrenal failure was observed at different time periods requiring hydrocortisone and fludrocortisone supplementation. However, impaired development of secondary sexual characters due to hypogonadotropic hypogonadism was observed at puberty in all three brothers requiring testosterone replacement therapy. In a previous study, no genotype–phenotype correlation for the hypogonadism was observed.[9]

This novel frameshift mutation does not exist in any database and is reported for the first time in the literature. This mutation analysis provides confirmation of diagnosis and this information can be used for early detection of the disorder and genetic counselling in the family. A larger study for identification of mutations across suspected cases of hypogonadotropic hypogonadism with severe or undiagnosed adrenal insufficiency will help in early detection and management of this life-threatening condition.

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Conflicts of interest. None declared

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