A novel androgen receptor gene mutation in a patient with congenital adrenal hyperplasia associated with penoscrotal hypospadias

VIKAS SHARMA, KUMARASAMY THANGARAJ, and AKKA JYOTHY

HYDERABAD, INDIA

Congenital adrenal hyperplasia (CAH) associated with penoscrotal hypospadias is a rare case of disorders of sex development. Here, we report clinical, genetic, biochemical, and molecular findings in a 2-year-old infant with CAH and penoscrotal hypospadias. Chromosomal analysis revealed 46,XX karyotype. Hormonal investigations indicated low levels of cortisol and elevated levels of testosterone, 17-hydroxyprogesterone, and androstenedione hormone. Molecular genetic testing of androgen receptor (*AR*) gene identified a novel homozygous missense mutation of single nucleotide transition G to A at position 2058 (GenBank accession number GU784855), resulting in amino acid interchange alanine to threonine at codon 566 in exon 2 (Ala566Thr) (GenBank Protein_id ADD26777.1). The nature of the mutation presented is in the highly conserved DNA-binding domain of the *AR* gene. The novel mutation identified in the rare genetic disorder provides additional support to the previously reported genotype-phenotype correlations, and our finding has expanded the spectrum of known mutations of the *AR* gene. (Translational Research 2014;164:149–152)

Abbreviations: *AR* = androgen receptor; CAH = congenital adrenal hyperplasia; DBD = DNA-binding domain; 17-OHP = 17-hydroxyprogesterone; PCR = polymerase chain reaction; T = testosterone

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a most prevalent form of disorders of sex development with an incidence of 1:15,000 live births worldwide.[1] The severe form of CAH, called classical CAH, is usually detected in the newborn period or in early childhood. The classical CAH is further divided into salt wasting CAH and simple virilizing CAH. Virilization occurs in females only in both forms. The milder form, called nonclassical CAH, may cause symptoms at anytime from infancy through adulthood. Salt wasting CAH can lead to significant salt loss, which, if unrecognized and not appropriately treated, may lead to severe complications. In virilizing CAH, the androgens produced in an excessive amount results in ambiguous external genitalia or virilization in the newborns. Management of these patients is a challenging aspect for health care professionals and requires holistic multidisciplinary and interdisciplinary approach.[2] In the present study, we report a novel mutation in the DNA-binding

From the Department of Biotechnology, University College of Science, Osmania University, Hyderabad, India; Centre for Cellular and Molecular Biology, Hyderabad, India; Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, India.

Submitted for publication July 17, 2013; revision submitted March 13, 2014; accepted for publication March 16, 2014.

Reprint requests: Vikas Sharma, Department of Biotechnology, University College of Science, Osmania University, Saifabad, Hyderabad 500004, India; e-mail: drvikasonline@gmail.com.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2014.03.013

domain (DBD) of the androgen receptor (*AR*) gene detected in a patient with CAH associated with penoscrotal hypospadias.

PATIENT

Subject. A 2-year-old boy was referred to the institute because of the ambiguous external genitalia. He was the only child born to a nonconsanguineous healthy parent after full-term normal vaginal delivery, and there was no history of drug affects or infections during pregnancy. The family pedigree showed no history of sex reversal or ambiguous external genitalia. He had not developed conditions such as vomiting, impaired consciousness, and severe dehydration at newborn. On examination, the height and weight were 76.2 cm and 10 kg, respectively (<third percentile for height and weight). His blood pressure was 95/50 mm Hg. He had no hyperpigmentation or dysmorphic features. The bone age was appropriate with a girl aged 4 years 6 months. No further diagnostic procedures were performed, and at this stage subject was screened for the clinical, genetic, biochemical, and molecular investigations.

METHODS AND RESULTS

Clinical profile. The study was approved by the institutional ethics committee, and informed written consent for the patient, but not for the parents, was obtained from the parents. A thorough clinical examination of the subject revealed penoscrotal hypospadias with chordee and micropenis (Prader stage 4), external genitalia was pigmented, and gonads were nonpalpable. The abdominal and retroperitoneal ultrasound examination showed the presence of ovaries and uterus, whereas the testis was absent. No other abnormalities were detected.

Biochemical analysis. The laboratory investigations showed a serum sodium level of 141 mmol/L (normal range: 135–145 mmol/L), potassium 4.8 mmol/L (normal range: 3.5–5.0 mmol/L), and chloride 98 mmol/L (normal range: 9–108 mmol/L). The hormonal profile showed elevated testosterone levels of 1500 ng/dL (normal range: 10 ng/dL) and elevated 17-hydroxyprogesterone (17-OHP) levels of l400 ng/dL (normal range: <200 ng/dL). Morning cortisol serum was 298 μg/dL (normal range: 83–441 μg/dL) and evening cortisol serum was 626 μg/dL (normal range: 138–635 μg/dL). 11-Deoxycortisol (compound S) level was 280 ng/dL (normal range: <344 ng/dL) and deoxycorticosterone level was 22 ng/dL (normal range: 30 ng/dL). Androstenedione levels were found to be more than 10 ng/dL (normal range: 3–10 ng/dL).

Cytogenetic investigation. The chromosome analysis was done by lymphocyte culture obtained from the peripheral blood of the subject. Conventional staining and the standard G-banding with Trypsin-Giemsa technique were applied.[3] The karyotype analysis was performed with Leica CW4000 Karyo imaging software (Leica Imaging Systems, Cambridge, UK). In the subject, 200 metaphases were screened and the chromosomal analysis revealed a 46,XX karyotype. On the basis of the clinical findings, with a 46,XX karyotype and elevated 17-OHP levels the likely diagnosis of CAH associated with penoscrotal hypospadias was made.

DNA extraction and mutation analysis. For mutational studies, genomic DNA was extracted from the peripheral blood sample. The sex-determining region Y (*SRY*) gene amplification was performed using specific primers and the patient was found to be *SRY* negative. The *AR* gene coding regions and flanking intronic sequences of all exons 2–8 and nonpolymorphic regions of exon 1 were also amplified by polymerase chain reaction (PCR) by use of primers.[4] Standard PCR mixture was prepared and standard PCR conditions were maintained.[5] The DNA sequencing was performed with the PCR primers using the BigDye terminator v3.1 Cycle Sequencing Kit and DNA sequences run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The cytosine-adenine-guanine (CAG) repeat in exon 1 was 24, within the normal range. The direct sequencing analysis of *AR* gene PCR products revealed the presence of G to A transition at nucleotide 2058 in exon 2 (GenBank accession number GU784855) resulting in the previously unreported alanine 566 threonine substitution (Ala566Thr) (The GenBank Protein_id ADD26777.1). The mutation was confirmed by bidirectional sequencing (Fig 1).

DISCUSSION

Disorders of sex development are among the most common birth defects in newborns. A number of genes contribute to both early and late processes of sex development. The *AR* gene is among the most mutated of the steroid receptor gene. The human *AR* gene is located on chromosome Xq11–12. Spanning 90 kb, it comprises 8 exons with 2757 bp of open reading frame within a 10.5-kb messenger RNA encoding for a modular protein of 919 amino acid residues,[6] with 3 functional domains. The N-terminal domain is encoded by exon 1, the DBD is encoded by exons 2 and 3, and the C-terminal ligand-binding domain is encoded by 5 exons 4–8.[7] In addition, exon 1 contains a highly polymorphic CAG and polyglycine repeats encoding for polyglutamine and polyglycine tract, respectively. CAG repeat variability has been associated with various androgen influenced diseases and clinical symptoms. Rocha et al[8] reported an association of CAG repeat length with genital virilization in CAH females. However, Welzel et al[9] recently reported that neither CAG nor polyglycine repeat