Asian Biomedicine Vol. 5 No. 6 December 2011; 889-892 **DOI: 10.5372/1905-7415.0505.042**

Clinical report

Homozygous complete deletion of *CYP21A2* causes a simple virilizing phenotype in an Azeri child

Bahareh Rabbani[a], Mohammad Taghi Akbari[a], Nejat Mahdieh[a,b], Ehya Zaridust[c,d], Mohammad Taghi Haghi Ashtiani[c,d], Hsien-Hsiung Lee[e], Richard Auchus[f], Ali Rabbani[b,c,d]

[a]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-331, [b]Growth and Development Research Center, Tehran University of Medical Sciences, [c]Pediatrics Center of Excellence, Tehran University of Medical Sciences, [d]Molecular Genetic Lab, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran 14155-6386, Iran, [e]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung 404, Taiwan, [f]Division of Endocrinology & Metabolism, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390-8857, USA

Background: Congenital adrenal hyperplasia (CAH) classical form comprises salt wasting (SW) and simple virilizing (SV) forms. This group accounts for about 75% of the affected individuals. Variation in mutation of CYP21A2 gene may cause different phenotypes.

Objectives: We reported a case of SV 21-hydroxylase deficiency that was misdiagnosed as a boy due to completely reversed external genitalia.

Methods: Allele-specific PCR for eight common mutations and dosage analysis of the *CYP21A2* gene by SALSA multiplex ligation-dependent probe amplification (MLPA) were done.

Results: The molecular analysis revealed a 30 Kb homozygous deletion of *CYP21A2* gene.

Conclusion: Genotype-phenotype correlation expected SW form of the disease rather than SV form hence, this discrepancy might be caused by other genes or modifier genes.

Keywords: Azeri family, congenital adrenal hyperplasia, simple virilizing

Congenital adrenal hyperplasia (CAH) is a group of autosomal disorders due to a deficiency of cortisol synthesis characterized by ambiguous genitalia in girls. Approximately 90 to 95% of the cases are ascribed to large deletions and mutations in a gene named *CYP21A2* encoding 21-hydroxylase enzyme [1]. This gene is located in 3' side relative to complement factor 4 (*C4*) on chromosome 6p21.3 [2]. A wide spectrum of mutations in *CYP21A2* gene brings up high variability in phenotypes. These forms are divided into three main groups of salt wasting (SW), simple virilizing (SV), and non-classic forms. There are many reports on the distribution of mutations that may be different in ethnic groups [3]. Although genotype often predicts the phenotype [4], ethnicity may have a role in genotype-phenotype correlation [5]. Due to a duplicated tandem repeated module (*CYP21A1P* pseudogene and *CYP21A2* gene) in this chromosomal region, there is a high rate of recombination events known as gene conversions [6], such that eight mutations found in the pseudogene are commonly found in all studied populations. About 75% of the *CYP21A2* mutations are due to microconversions from the *CYP21A1P* pseudogene, and 20% of alleles show large gene deletions and/or gene conversions, with a few rare and novel mutations [1].

In genetic counseling, genotypic evaluation generally helps to predict the phenotype or confirm the clinical diagnosis. However, discordance of genotype-phenotype correlation has been reported [7, 8]. Here, we report an Azeri child with a SV form who is homozygous for *CYP21A2* deletion.

Correspondence to: Mohammad Taghi Akbari, PhD, Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-331, Iran. E-mail: baharehrabbani@yahoo.com, mtakbari@modares.ac.ir

Materials and methods

A 3.9-year-old child was referred to Children's Medical Center Hospital, Tehran University of Medical Sciences, after four periods of human chorionic gonadotropin (hCG) injection for bilateral cryptorchidism and appearance of pubic hair. The patient was born as the only child of a first cousin marriage in an Azeri family living in Tehran.

The patient was misdiagnosed during infancy and was raised as a boy. Detailed clinical examinations, biochemical tests, and karyotype analysis were carried out. Following signing the informed consent, 5 mL peripheral blood was taken from the patient and all family members for DNA isolation by salting out method. Allele-specific PCR [9] was done for eight common mutations of *CYP21A2* as previously described. Dosage analysis of the *CYP21A2* gene by SALSA multiplex ligation-dependent probe amplification (MLPA) kit P050B2 (MRC-Holland, Netherlands) was also performed for gene dosage analysis. MLPA results were analyzed using GeneMarker V 1.85 (SoftGenetics). PCR amplification of *CYP21A1P* was performed with the following mutant primers: 3ms (5′-ACTACCCGGA CCTGTCCTTGGTC-3′) and 6ma (5- TCAGCTGCT TCTCCTCGTTGTGG-3′) [9].

Results

The patient had no family history of CAH. Biochemical diagnosis showed elevated 17-hydroxyprogesterone (17OHP) level of 90 ng/mL (272 nmol/L), Δ^4-androstenedione of 8 ng/mL (28 nmol/L), cortisol 10 mcg/dL (276 nmol/L), and adrenocorticotropic hormone (ACTH) of 200 pg/mL, diagnostic for classical 21-hydroxylase deficiency. Plasma renin activity (PRA) was higher than upper limit with normal serum sodium (139 mmol/L) and potassium (4.5 mmo/L) level, and karyotype was 46,XX. Pelvic sonography showed absence of testes; normal ovaries, fallopian tubes, uterus, cervix and upper vagina were apparent. Detailed examinations confirmed 46,XX disorder of sexual differentiation (DSD) with complete sex reversal of external genitalia, Prader stage 5 resembling normal male genitalia, with complete fusion of the labial folds and a penile appearance of the clitoris.

All attempts to amplify the *CYP21A2* gene using PCR primers located within the exons failed to yield the expected amplification products, whereas the presence of *CYP21A1P* pseudogene was confirmed by PCR amplification using the 3ms and 6ma primers. MLPA analysis revealed the absence of the *CYP21A2* coding probes (in exons 3, 4, 6, 8), absence of 5'*CYP21A2* gene, triplication of 5'*CYP21A1P* and 3' *CYP21A1P*, and triplication of g.656A/C>G (I2G) in intron 2 of the gene. The absence of the related *CYP21A2* probes indicated gene deletion and the triplication of pseudogene and confirmed the predicted haplotypes of the patient.

Discussion and conclusion

Detailed physical examinations and biochemical tests of this 3.9-year-old child from an Azeri family showed 46,XX DSD with male external genitalia due to a clinically SV form of CAH. Molecular analysis of the patient revealed complete deletion of both *CYP21A2* genes. MLPA analysis allowed detection of three copies of *CYP21A1P*, which results in an allele with complete gene conversion and an allele with a 30kb homozygous deletion of *CYP21A2* gene as shown in **Figure 1**. Our results demonstrated the segregation of allele b and c from parental and maternal alleles, respectively. Allele b depicted a haplotype with a complete *CYP21A2* gene conversion and allele c indicated a 30kb gene deletion. No PCR amplification of the *CYP21A2* gene could be performed using the patient's DNA, which confirmed the predicted haplotype. In contrast, the *CYP21A1P* pseudogene PCR products were readily amplified using the patient's DNA. In fact, haplotypes D (bimodule) and E (monomodule) (**Figure 1B**) were the same haplotype of the *CYP21A2* gene. Therefore, allele b and c are the same; and the genotype is functionally homozygous for the specified alleles. The *CYP21A2* gene, encoding a member of the cytochrome P450 family, is transcribed into the 21-hydroxylase protein (P450c21). P450c21 is a transmembrane protein, anchored by one of the two hydrophobic stretches of the N-terminus. This enzyme is involved in and the biosynthesis of the important adrenal steroid hormones aldosterone and cortisol, which regulate plasma volume, carbohydrate metabolism and many other functions, which P450c21 deficiency causes CAH. Patients with complete *CYP21A2* gene deletions have no enzymatic activity and are categorized as null mutations. Therefore, the phenotype for a homozygous deletion is expected to show salt wasting crises and SW CAH. However, the severity of salt wasting crises may be affected by other mechanisms of sodium homeostasis. Transcription and production of some

of the factors in the steroidogenesis pathway may influence the phenotypic variability [1]. Mineralocorticoid receptors may change the sensitivity to the steroids [10].

To our surprise, there was no sign of salt wasting in this patient, even if both copies of the *CYP21A2* gene were completely deleted. To our knowledge, this is the first example where a 46,XX CAH patient with Prader 5 genitalia and complete deletion of both *CYP21A2* alleles did not show the SW phenotype during infancy. This discrepancy may be explained by improvement of aldosterone biosynthesis in SW form [11]. Other studies have also shown that complete gene deletion of *CYP21A2* gene presented as different forms of CAH [8, 11].

Other enzymes might contribute to 21-hydroxylase activity, which may function in tissues other than the adrenal gland [1, 4, 12, 13]. Alternatively, some isozymes of multifunctional enzymes in cortisol biosynthesis pathway may at least drive critical reactions and somehow compensate the deficiency of 21-hydroxylase. Understanding these isozymes or modifiers and their functions within the steroidogenic pathways might lead to approaches to achieve novel successful therapies for CAH. Functional investigations, however, might decode this dilemma. PCR amplification of the patient's parents revealed both having at least one normal *CYP21A2* gene with no common mutation. Phenotypic variability has been described for other genetic diseases in the Iranian population [14]; however, further studies are needed to clarify this discrepancy for our patient.

In addition, ethnic differences reflecting genotypic variations cannot be ignored, given the different ethnic groups in Iran (e.g Persian, Azeri, Gilaki, Mazandarani, Kurd, Lur, Turkmen, Arab, Balooch). Previous studies of this sort have helped to explain ethnic-based differences in the phenotypes observed for mutations in the *GJB2* gene [14, 15].

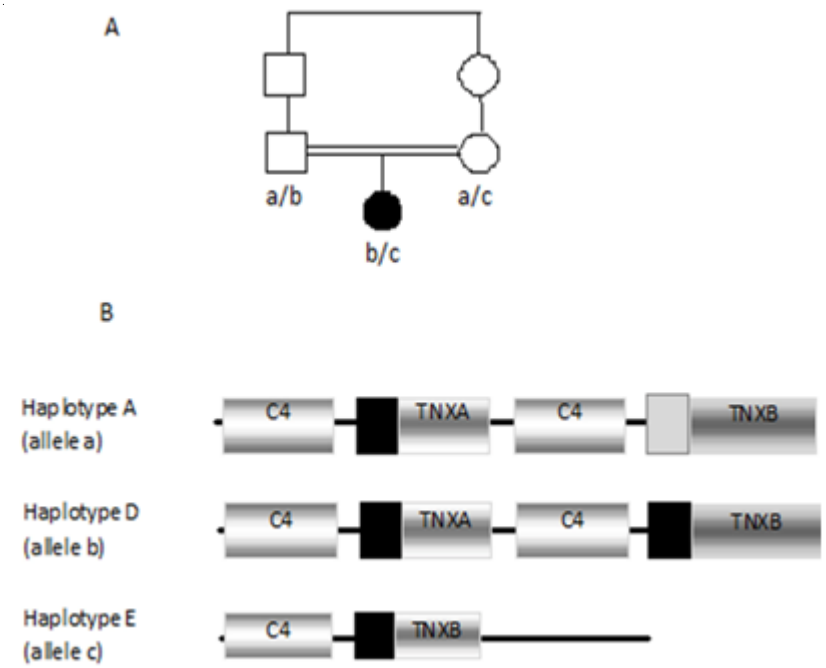

Figure 1. The family pedigree of the affected CAH child with *CYP21A2* gene lesion, the only child of a consanguineous marriage. **A:** depicts the distribution of alleles inherited by the child. **B:** demonstrates the haplotypes of the alleles of the *CYP21A2* gene: allele a shows a bimodule RCCX carrying a *CYP21A2* gene and its *CYP21A1P* showing haploype A, allele b carries haplotype D with complete *CYP21A2* gene conversion, and allele c carries haplotype E with a 30Kb gene deletion. Gray box indicate the *CYP21A2* gene; black boxes indicate the pseudogene, *CYP21A1P*. *C4* indicates complement factor 4 (serum protein) and *TNXA/B* indicates tenascin-X A/B (extracellular matrix protein). Allele b and c have the same functional effect.

Acknowledgments

We would like to appreciate the family for their kind participation. We would like to thank staff of Kawsar Institute especially Mrs Soudeh Kianfar for capillary electrophoresis. This work was supported by the Growth and Developmental Research Center and Endocrine and Metabolism Research Center, Tehran University of Medical Sciences (Grant number 7383, 2008). This work was approved by ethical committee of Growth and Development Research Center.

The authors report no conflicts of interest.

References

1. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev. 2000; 21:245-91.
2. Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. Proc Natl Acad Sci USA. 1986; 83:2841-5.
3. Wedell A, Thilen A, Ritzen EM, Stengler B, Luthman H. Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. J Clin Endocrinol Metab. 1994; 78:1145-52.
4. Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. J Clin Endocrinol Metab. 2000; 85:1059-65.
5. Wilson RC, Nimkarn S, Dumic M, Obeid J, Azar MR, Najmabadi H, et al. Ethnic-specific distribution of mutations in 716 patients with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Mol Genet Metab. 2007; 90:414-21.
6. Lee HH. Chimeric CYP21P/CYP21 and TNXA/TNXB genes in the RCCX module. Mol Genet Metab. 2005; 84:4-8.
7. Wilson RC, Mercado AB, Cheng KC, New MI. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. J Clin Endocrinol Metab. 1995; 80:2322-9.
8.]L'Allemand D, Tardy V, Gruters A, Schnabel D, Krude H, Morel Y. How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2000; 85:4562-7.
9. Wilson RC, Wei JQ, Cheng KC, Mercado AB, New MI. Rapid deoxyribonucleic acid analysis by allele-specific polymerase chain reaction for detection of mutations in the steroid 21-hydroxylase gene. J Clin Endocrinol Metab. 1995; 80:1635-40.
10. Bassett MH, White PC, Rainey WE. The regulation of aldosterone synthase expression. Mol Cell Endocrinol. 2004; 217:67-74.
11. Speiser PW, Agdere L, Ueshiba H, White PC, New MI. Aldosterone synthesis in salt-wasting congenital adrenal hyperplasia with complete absence of adrenal 21-hydroxylase. N Engl J Med. 1991; 324:145-9.
12. White PC. Aldosterone synthase deficiency and related disorders. Mol Cell Endocrinol. 2004; 217:81-7.
13. Gomes LG, Huang N, Agrawal V, Mendon□a BB, Bachega TA, Miller WL. Extraadrenal 21-Hydroxylation by CYP2C19 and CYP3A4: Effect on 21-Hydroxylase Deficiency J. Clin. Endocrinol. Metab. 2009; 94:89-95.
14. Mahdieh N, Bagherian H, Shirkavand A, Sharafi M, Zeinali S. High level of intrafamilial phenotypic variability of non-syndromic hearing loss in a Lur family due to delE120 mutation in GJB2 gene. Inter J Pediatric Otorhinolaryngol. 2010; 74:1089-91.
15. Mahdieh N, Rabbani B. Statistical study of 35delG mutation of GJB2 gene: a meta-analysis of carrier frequency. Int J Audiol. 2009; 48:363-70.