Journal of Perinatology (2013) 33, 76–78

www.nature.com/jp

PERINATAL/NEONATAL CASE PRESENTATION

Erroneous prenatal diagnosis of congenital adrenal hyperplasia owing to a duplication of the *CYP21A2* gene

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder where steroidogenesis in the adrenal cortex is impaired. The most common form is caused by 21-hydroxylase deficiency (21OHD). Classical 21OHD is characterized by glucocorticoid and mineralocorticoid deficiency and by overproduction of adrenal androgens. The diagnosis rests on biochemical and genetic analyses. In families with history of CAH, prenatal genetic diagnosis is offered. We herein present a case of an infant whose parents were identified to carry mutations on the *CYP21A2* gene. The fetal DNA analysis demonstrated that the fetus carried a paternal exon 8 (Q318X) mutation and a maternal exon 8 (R356X) mutation. The fetus was presumed to be affected with CAH, yet his clinical presentation at birth was not consistent with the diagnosis. Repeated genetic analysis identified a paternal *CYP21A2* gene duplication with Q318X mutation on one copy of *CYP21A2*. We conclude that a duplication of the *CYP21A2* gene should be suspected when clinical and hormonal findings do not support the genetic diagnosis. Furthermore, because individuals with Q318X mutation frequently have a duplication of the *CYP21A2* gene, when Q318X is detected, it is important to distinguish the severe point mutation in single gene copy alleles from the non-deficient variant in gene-duplicated alleles.
Journal of Perinatology (2013) **33,** 76–78; doi:10.1038/jp.2012.5

Keywords: congenital adrenal hyperplasia; prenatal diagnosis; adrenogenital disorder; genetic testing; 21-hydroxylase deficiency

Introduction

Congenital adrenal hyperplasia (CAH) refers to a group of enzyme deficiencies that impair normal steroidogenesis in the adrenal cortex. Each of these disorders has an autosomal recessive mode of inheritance, and the mutations lie in the *CYP21A2* gene, which is mapped to the short arm of chromosome 6 (6p21.3). The most common form is 21-hydroxylase deficiency (21OHD), accounting for more than 90% of cases. CAH owing to 21OHD is classified into classical and nonclassical forms. The classical form is characterized by glucocorticoid deficiency, mineralocorticoid deficiency (in approximately 75% of cases) leading to salt wasting, and overstimulation of the adrenal cortex leading to adrenal hyperplasia and overproduction of adrenal androgens. Females with CAH are exposed to high levels of androgens in utero, leading to masculanization of the genitalia. In the nonclassical form, females have normal genitalia, and signs and symptoms of hyperandrogenemia in males and females are apparent later in life.[1]

In families with history of CAH, it is recommended that the parents have genetic testing before the woman becomes pregnant, and if both carry mutations on the *CYP21A2* gene, prenatal genetic testing of the fetus is conducted. We herein present a case where prenatal diagnosis of CAH in a male was made by genetic analysis of the fetal DNA. The DNA analysis initially demonstrated a paternal and maternal mutation in the fetal *CYP21A2* gene. However, the child was retested postnatally when the clinical presentation and biochemical profile were not consistent with CAH, and it was demonstrated that the paternal *CYP21A2* gene was duplicated, thus negating the pathogenic mutation on the third *CYP21A2* gene inherited from the mother. This duplication rendered the fetus a heterozygote rather than an affected male with CAH.

Case

A male infant of non-consanguineous parents was born at 39 weeks gestation to a 22-year-old G1P0 female with a known history of salt-wasting CAH due to 21OHD. The mother is of Irish and German descent and the father is of German and Neapolitan descent. Prior genetic testing of the mother revealed a maternal exon 8 (R356W) mutation and a paternal intron 2 (A or C to G) mutation in the *CYP21A2* gene. Prenatal genetic testing identified the father of the fetus as a carrier for the exon 8 (Q318X) mutation in the *CYP21A2* gene. Therefore, the fetus was presumed to be at risk for CAH. The mother was treated with dexamethasone and 9α-fludrocortisone for management of her CAH; dexamethasone

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Received 1 November 2011; accepted 11 January 2012

was prescribed at a higher dose to also treat the fetus to prevent genital ambiguity, should it be an affected female. Chorionic villus sampling was performed in the first trimester, revealing 46,XY karyotype. The fetus was therefore not at risk for genital ambiguity, and the mother's glucocorticoid regimen was changed from dexamethasone to hydrocortisone (as hydrocortisone does not cross the placenta). DNA testing demonstrated that the fetus carried an exon 8 mutation (Q318X) from the father and exon 8 (R356X) mutation from the mother, which predicted the fetus to be affected with CAH.

At birth, weight of the baby was 3.3 kg, length was 51 cm, head circumference was 35.5 cm and body surface area was 0.2 m^2. Apgar scores were 9 and 9 at 1 and 5 min. The physical exam was unremarkable. Genitalia were normal for a male and without hyperpigmentation. The infant was subsequently transferred to the neonatal intensive care unit for observation and management. He had a brief episode of hypoglycemia immediately after birth, which required intravenous dextrose. The dextrose was subsequently discontinued with no further hypoglycemic episodes or electrolyte abnormalities. Treatment consisted of prednisolone 0.5 mg twice daily, 9α-fludrocortisone 0.1 mg once daily and sodium chloride 250 mg twice daily. Newborn screening and serum 17-hydroxyprogesterone level were obtained at 24 h of life, but after the infant received one dose of oral prednisolone. Serum 17-hydroxyprogesterone level was 168 ng dl^{-1} (7 to 106). Both the initial and repeated newborn screen were negative for CAH. The infant remained hospitalized for several days due to mild feeding intolerance, but the electrolyte levels remained normal. Because of a normal 17-hydroxyprogesterone level and negative newborn screening results for CAH, the diagnosis of classical CAH was questioned. However, given the documented genetic mutations consistent with classical CAH, the infant was discharged home on treatment with prednisolone, 9α-fludrocortisone and salt supplementation.

The patient returned to the outpatient clinic at 3 weeks of age and was thriving clinically. Referral for repeat genetic testing was suggested to confirm the diagnosis of 21-hydroxylase deficiency. At 3 months of age, a deceleration in linear growth was noted while the infant was treated with prednisolone 0.5 mg twice daily, but otherwise he continued to thrive clinically. Glucocorticoid treatment was changed from prednisolone to hydrocortisone 1.25 mg three times daily (12.5 mg m^{-2}). At the time, genetic testing results were equivocal.

At 5 months of age, the infant continued to do well and his linear growth improved. Laboratory testing demonstrated an undetectable 17-OH progesterone of $<$8 ng dl^{-1} (11 to 170) and a low plasma renin activity of 0.14 ng ml^{-1} h^{-1} (0.25 to 5.82). Genetic testing remained equivocal, and the decision was made to discontinue mineralocorticoid treatment and salt supplementation, and decrease the dose of hydrocortisone.

Subsequent genetic testing at Quest Nichols Institute Laboratory identified a 'pseudo' Q318X mutation in the father. Using the method of Keen-Kim *et al*[2] to detect common mutations in the *CYP21A2* gene, a pattern (haplotype) was observed that indicated that the Q318X mutation and a second copy of the CYP21A2 gene were located on a single chromosome.[2] If it is presumed that the second copy is functional, then it would effectively negate the adverse effect of the Q318X mutation. Concurrent testing of the infant detected the 'pseudo' Q318X mutation pattern observed in the father. Because the infant is a carrier of the maternal R356W mutation and a paternal duplicated *CYP21A2* gene, he is therefore a carrier of a mutation and not affected by CAH. Based on these findings, all medications were discontinued. After 1 week, repeated 17-hydroxyprogesterone was 19 ng dl^{-1} (11 to 170) and plasma renin activity was 5.15 ng ml^{-1} h^{-1} (0.25 to 5.82). The infant remains stable off all medications.

Discussion

When a female is born with ambiguous genitalia, CAH is the most common cause. The diagnosis then rests on biochemical analysis, mainly on the serum levels of 17-hydroxyprogesterone and androstenedione, 17-hydrogyprogesterone level on the newborn screen and genetic testing, which typically provides the most definitive diagnosis. In males without known family history of CAH, the diagnosis is made based on the positive newborn screen or when the baby presents with a salt-wasting crisis in early infancy. In our case, we had an apparent advantage when the analysis of the fetal DNA revealed that the fetus carried one paternal and one maternal mutation, and the baby was presumed to be affected with classical CAH, as suggested by the fetal DNA results. At birth, the clinical and hormonal findings were not consistent with CAH, however. Had the patient been a female, the absence of genital ambiguity would have alerted the physician that she was not affected with CAH. As the patient in this case was a male with normal male genitalia, the diagnosis rested on biochemical and genetic analyses. The initial genetic analysis suggested an affected fetus who inherited one mutation from each parent, and treatment of the patient was based on the prenatal genetic analysis results. However, repeated genetic analysis, which utilized the above-mentioned method, demonstrated that the paternal *CYP21A2* gene was duplicated, rendering the fetus a heterozygote rather than a patient with CAH. Hence, the genotype was consistent with the phenotype of an unaffected child.

It has been reported that the majority of the individuals carrying the Q318X mutation comprise the rare haplotype of a duplicated *CYP21A2* gene with the Q318X mutation on one of the genes. This haplotype was first characterized in 1994 by Wedell *et al*[3] in three Swedish patients. Koppens *et al*[4] also reported three patients with the same haplotype in the Netherlands. The study also looked at 365 individuals without 21OHD and found that 6, or 1.6%,

exhibited a haplotype containing two *CYP21A2* genes, making this haplotype no less common than others that carry steroid 21OHD. In a study by Parajes *et al*,[5] the *CYP21A2* gene was sequenced in 144 random individuals in Spain, and 7% of those studied were found to have a duplication of the *CYP21A2* gene, with most of these individuals carrying the Q318X mutation on one of the *CYP21A2* genes. The most comprehensive study to date was conducted by Kleinle *et al*[6] in Germany where the group looked at 38 unrelated individuals and 11 family members who were carriers of the Q318X mutation. In all, 84.2% of these individuals had a duplication of the *CYP21A2* gene, indicating that the majority of those with the Q318X mutation have the gene duplication and are therefore not carriers of the 21OHD allele. Individuals who carried the Q318X mutation on one of the duplicated *CYP21A2* genes and a pathogenic mutation on the third *CYP21A2* gene were not affected with CAH.

In conclusion, duplication of the *CYP21A2* gene should be suspected when the clinical and hormonal findings do not support the genetic diagnosis. Furthermore, because individuals with Q318X mutation frequently have a duplication of the *CYP21A2* gene, when this mutation is detected, it is important to distinguish the severe point mutation Q318X in single gene copy alleles from the non-deficient variant in gene-duplicated alleles. Making this distinction is important in genetic counseling of couples as well as in establishing the genetic diagnosis of CAH in a fetus and a newborn.

Conflict of interest

The authors declare no conflict of interest.

References

1 Nimkarn S, Lin-Su K, New MI. Steroid 21 hydroxylase deficiency congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am* 2009; **38**: 699–718.

2 Keen-Kim D, Redman JB, Alanes RU, Eachus MM, Wilson RC, New MI *et al.* Validation and clinical application of a locus-specific polymerase chain reaction and minisequencing-based assay for congenital adrenal hyperplasia (21-hydroxylase deficiency). *J Mol Diagn* 2005; **7**: 236–246.

3 Wedell A, Stengler B, Luthman H. Characterization of mutations on the rare duplicated C4/CYP21 haplotype in steroid 21-hydroxylase deficiency. *Hum Genet* 1994; **94**: 50–54.

4 Koppens PFJ, Hoogenboezem T, Degenhart HJ. Duplication of the CYP21A2 gene complicates mutation analysis of steroid 21-hydroxylase deficiency: characteristics of three unusual haplotypes. *Hum Genet* 2002; **111**: 405–410.

5 Parajes S, Quinteiro C, Dominguez F, Loidi L. High frequency of copy number variations and sequence variants atCYP21A2locus: implication for the genetic diagnosis of 21-hydroxylase deficiency. *PLos ONE* 2008; **3**: e2138.

6 Kleinle S, Lang R, Fischer GF, Vierhapper H, Waldhauser F, Födinger M *et al.* Duplications of the Functional CYP21A2 Gene Are Primarily Restricted to Q318X Alleles: Evidence for a Founder Effect. *J Clin Endocrinol Metab* 2009; **94**: 3954–3958.