MOLECULAR ANALYSIS of CYP21 GENE in PATIENTS PRESENTING with AMBIGUOUS GENITALIA

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ABSTRACT

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. The genes of the steroidogenic enzymes and the mutations involved have been described. Deficiency of the 21-hydroxylase (21-OH) enzyme is by far the most common form of CAH which arises as a result of deletions or deleterious mutations in the active gene (CYP21) located on chromosome 6p. Many different mutations of the CYP21 gene cause varying degrees of impairment of 21-OH activity that results in a spectrum of disease _expression. There is no sharp limit between the salt- wasting, the simple virilizing and the late onset forms.

The objective of our study was to determine the 21-OH deficiency mutation defects and correlate the genotype with their phenotypic _expression of the disease.

We performed mutational analysis using PCR-ASOH (Polymerase Chain Reaction - Allele Specific Oligonucleotide Hybridization) technique on six patients who presented with ambiguous genitalia (AG) and or electrolyte derangement as hyponatraemia and hyperkalaemia, suspected to have CAH. The Val281Leu and Pro30Leu mutations result in enzymes with 20-60% of normal activity and both are associated with the non-classical form of CAH. The Gln318stop mutation is categorized under the salt-wasting type.

Among the six patients, three had Val281Leu mutation, two had Pro30Leu mutation and one had Gln318stop mutation. The three patients with Val281Leu mutation had presented with adrenal crises during infancy and was classified as salt losers and treated with glucocorticoids and mineralocorticoids. These 3 patients could well be the other 40% who are categorized as salt-losers. The two patients with Pro30Leu mutations have normal male external genitalia and presented with hyponatraemia and hyperkalaemia. Only one patient required mineralocorticoid therapy that was given for about 5 months duration. Subsequently he had normal electrolytes level even without mineralocorticoid therapy. The Gln318stop mutation was identified in one patient who presented with ambiguous genitalia and adrenal crises.

Our study showed that the patients with genotype Val281Leu, Pro30Leu and Gln318stop mutations correlated with their phenotype. The mutation analysis of CYP21 gene proved to be a good complementary investigation and supportive to the diagnosis and management of our CAH patients.

Key words: ambiguous genitalia, congenital adrenal hyperplasia, CYP21 gene.

INTRODUCTION

Ambiguous genitalia (AG) are one of the clinical presentations of congenital adrenal hyperplasia (CAH). Congenital adrenal hyperplasia is an autosomal recessive disease caused by loss or severe decrease in the activity of 21-hydroxylase (21-OH). This enzyme is one of the five enzymes necessary for cortisol biosynthesis. Deficiency of the 21-OH is the most common form of CAH accounting for 90-95% of all cases of CAH[1,6].

Congenital adrenal hyperplasia presents a wide spectrum of clinical manifestations and patients are divided into 3 groups: salt wasting (SW), simple virilizing (SV) and non-classical (NC). Salt wasting patients manifest as neonatal electrolyte disturbances together with virilization of external genitalia at birth in girls and early pseudoprecocious puberty in boys, while SV patients present the same manifestation as SW patients, but without electrolyte disturbances. Non- classical patients present with late onset symptoms of androgen excess, ranging from progressive virilization and pseudoprecoclous puberty in childhood to menstrual disturbances, infertility and hirsutism in adult women[2].

It was reported that more than 90% of cases of CAH are caused by mutation of the CYP21 gene. This 21-OH (CYP21) gene is located in the HLA class III gene region on chromosome 6p21.3 and consist of 10 exons[3]. Majority of the mutations on the CYP21 gene are Val281Leu and Pro30Leu. These mutations were reported to result in 20-60% of normal enzyme activity and both are associated with the non-classical form of CAH[4,5].

AIM OF THE STUDY

To detect the presence of point mutations in Pro30Leu, Ile172Asn & Val281Leu of CYP21 gene

RESULTS

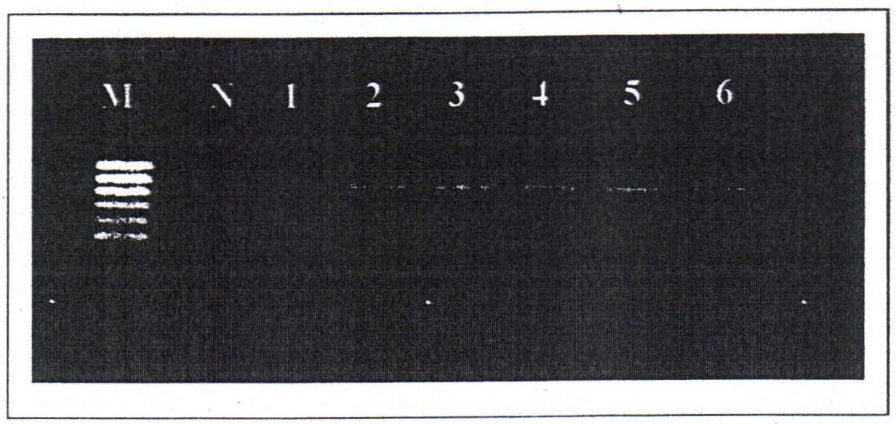

Figure 1: Presence of PCR product (873 bp) for CYP21 gene exon 1-3 using 2.0% agarose gel electrophoresis. Lane M : 100 bp DNA ladder, lane N : negative control, lane 1, 2 : normal samples, lane 3,4,5,6 : samples showing PCR amplification products for CYP21 gene.

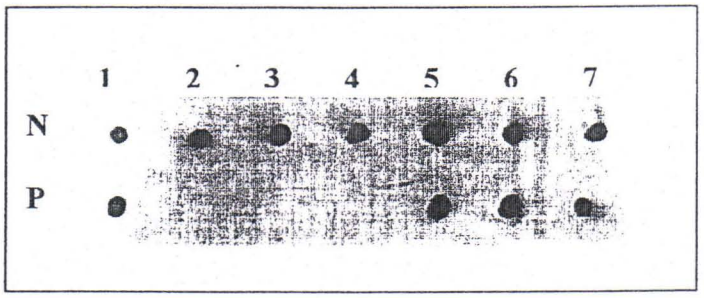

Figure 2: Dot blotting was performed using 1 µg genomic DNA. The blot was hybridized with 100 pmol/ml of the digoxigenin labeled antiphosphatase (DIG-AP) specific probe from samples of patient 1-7. ASOH of PCR product from CAH patients was performed with the probe corresponding to the site exon. The status N: normal, P: patient.

RESULTS

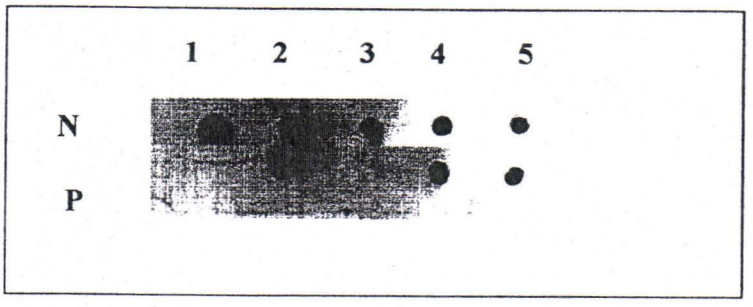

Figure 3: Pro30Leu hybridization DNA samples. Dot blotting was performed using 1 µg genomic DNA. The blot was hybridized with 100 pmol /ml of the digoxigenin labeled antiphosphatase (DIG-AP) specific probe Pro30Leu. ASOH of PCR product from CAH patients was performed with the probe (Pro30Leu) corresponding to the site exon 1. The status N: normal, P: patient.

RESULTS

Table 1

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient
	4.2	4.3	3.0	3.3	2.8	2.7
	32 days	81 days	2 months	At birth	10 weeks	6 days
	Adrenal crisis Default follow up CPP Mental Retardation	Failure to thrive Normal male external genitalia Both testes descended Sepsis, meningitis & adrenal crisis	Haematuria Salt loss Normal male external genitalia Both testes descended	Normal male external genitalia Both testes descended Hypoglycae-mia	Failure to thrive Ambiguous genitalia Phallus 2 cm Single orifice at its base No palpable gonads	Neonatal jaundice Ambiguous genitalia Prominent phallus Single orifice at its base No palpable gonads Registered as male Reassigned as female
	120	110	119	131	92	118

RESULTS

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient VI
[redacted]	4.3	4.2	6.5	6.6	7.4	8.2
[redacted]	28.8	23.0	NA	3.5	NA	NA
[redacted]	1.6	4.4	NA	NA	NA	NA
[redacted]	NA	NA	9.9 (0.07-1.7)	NA	>20 (up to 1.1)	33.2 (0.7-2.5)
[redacted]	48 (139-501)	329 (138-690)	609 (140-500)	650 (138-690)	484 (139-501)	NA

RESULTS

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient [illegible]
[illegible]	1.4	NA	4.9	13.3	NA	NA
[illegible]	46,XY Present	46,XY Present	46,XY Present	46,XY Present	46,XX Not present	46,XX Not present
[illegible]	-	-	-	-	Uterus & ovaries	Uterus & ovaries
[illegible]	Val281Leu	Val281Leu	Pro30Leu	Pro30Leu	Val281Leu	Gln318Stop

NA: Not available, CPP: central precocious puberty

DISCUSSION

Majority of the mutations in the CYP21 gene in our patients are Val281Leu and Pro30Leu. These mutations were reported to result in 20-60% of normal enzyme activity and both are associated with the non-classical form of CAH[4,7].

In our study, we found that 3/52 patients (5.7%) have Val281Leu while 2/52 patients (3.8%) have Pro30Leu respectively. No mutations were observed in Ile172Asn. Patients with Val281Leu presented with adrenal crisis during infancy and were classified as salt wasting. Two patients with Pro30Leu mutations have normal male external genitalia and presented with hyponatraemia and hyperkalaemia. We did not identify any patient with I172N mutation which is known to result in clearly reduced enzymatic activity. About 1-2% of I172N mutation is usually associated with the SV form[8,9].

Our findings showed that patients with Pro30Leu mutations were associated with non-classical form of CAH whereas Val281Leu mutations were associated with salt wasting form of CAH.

CONCLUSION

This study suggested that phenotypes are not always concordant with the genotype in patients with Val281Leu mutations diagnosed with CAH.

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