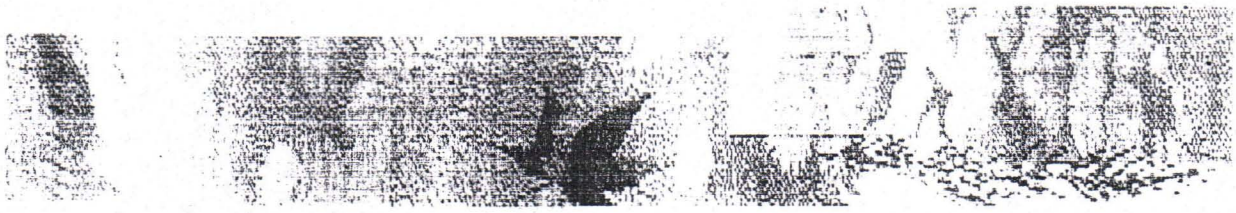
DETECTION OF POINT MUTATION (Pro30Leu) in EXON 1 OF THE 21-HYDROXYLASE GENE (CYP21) IN PATIENT with CONGENITAL ADRENAL HYPERPLASIA USING DIGOXYGENIN SYSTEM

Y.K.Muhamad[1], Fuziah MZ[2], Rus Anida A[3], M.Ros Sidek[1], S.F.Ramli[1],N.Adam[1], M.N.Isa[1]

[1]Human Genome Centre,[2]Paediatric Department, School of Medicine, USM, 16150 Kubang Kerian, Kelantan, [3]Paediatric Department,Hospital Kota Bharu, Kelantan, Malaysia



ABSTRACT

Congenital adrenal hyperplasia is an autosomal recessive disease with a wide range of clinical manifestations. Deficiency of the 21-hydroxylase is the most common form of congenital adrenal hyperplasia, accounting for over 90% of cases. The aim of the study is to detect the presence of point mutations using specific probe P30L in exon 1 of CYP21 gene. Point mutations were studied using the PCR-ASOH Allele Specific Oligonucleotide Hybridization) technique with Digoxigenin (DIG) system. The Pro30Leu(P30L) mutation is associated with electrolyte disturbances was found in 2 patients out of 30 patients. The P30L mutation might cause electrolyte imbalances during neonatal periods that subsequently normalize without hormonal replacement therapy.

Key Words: Congenital adrenal hyperplasia, ASOH (Allele Specific Oligonucleotide Hybridization).

INTRODUCTION

Deficiency of the 21- hydroxylase (21-OH) is the most common form of congenital adrenal hyperplasia (CAH), accounting for over 90% of cases. This enzyme deficiency results in a reduced ability to synthesize cortisol and aldosterone, leading to increase secretion of adrenocorticotrophin (ACTH), which causes hyperplasia of the adrenals and an increase in androgens[2]. Three different clinical phenotypes is described: which includes the salt wasting (SW), simple virilizing (SV) and the non classical (NC) form. The 21-hydroxylase gene (CYP21) is located in chromosome 6p 21.3[1]. Molecular diagnostic techniques rely on the assumption that specific mutation of CYP21 gene give rise to the same form of CAH expression. PCR–Allele Specific Oligonucleotide Hybridization (PCR-ASOH) method can be applied subsequently to identify point mutations in the amplified CYP21 gene[3], with using specific probe Pro30Leu (P30L) to detect in exon 1 of CYP21 gene. P30L mutations are associated with the simple virilizing or non-classical form of CAH.

AIM

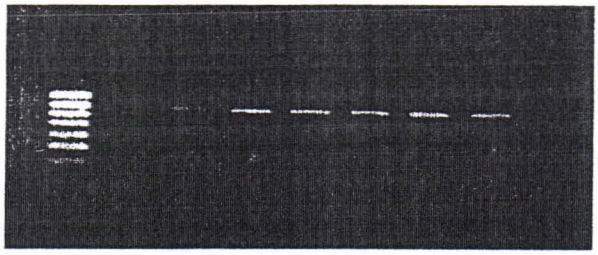
To detect the presence of point mutations using specific probe P 30 L in exon 1 of CYP21 gene.

METHODOLOGY

73 blood samples were obtained from patients and their families referred to Hospital Universiti Sains Malaysia, Kelantan. A thorough clinical examination and hormonal analyses were performed. A total of 30 samples included were suspected to have CAH based on ambiguity of the external genitalia or electrolyte imbalances. The other samples were obtained from parents and patients siblings whenever possible.

DNA extraction
(non-phenol chloroform standard procedure)

Polymerase Chain Reaction(PCR)



PCR-ASOH (Allele Specific Oligonucleotide Hybridization) technique with Digoxigenin (DIG) system.

RESULT

Presence of PCR product (873 bp) for CYP21 gene exon 1-3 using 2.0 % agarose gel electrophoresis (see figure 1).

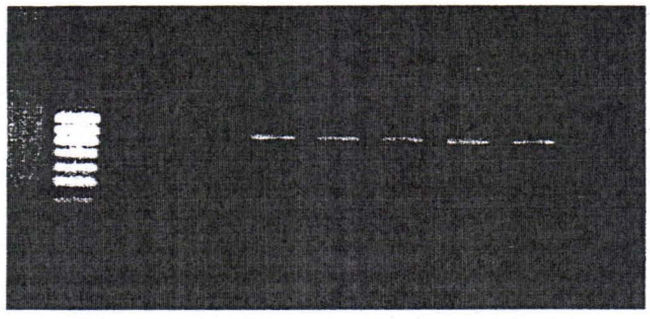


Figure 1: Lane M is a 100 bp DNA ladder, lane N is negative control,lane 1,2, are normal sample and lane 3,4,5,6 sample showed PCR amplification products for CYP21 gene.

The presence of P30L mutations, which have been established to be the cause of complete or partial 21- hydroxylase enzyme inactivation, was analyzed by PCR – ASOH using DIG system in 30 patients with ambiguous genitalia as well as in 43 family members.

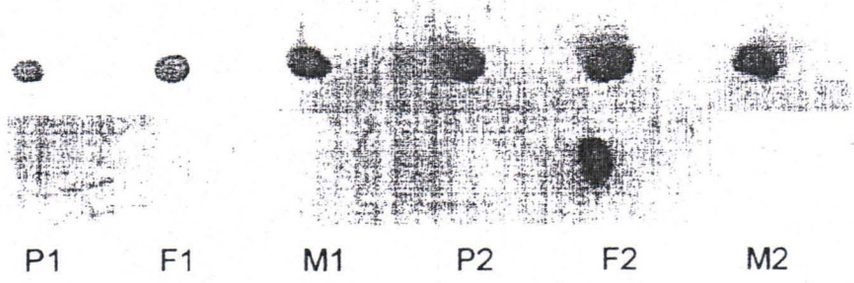


Figure 2:
Dot blotting was performed using 1µg genomic DNA. The blot was hybridized with 100 pmol/ml of the digoxigenin labeled antiphosphatase (DIG-AP) specific probe P30L. ASOH of PCR product from CAH patients or parents, with P30 L corresponding to the site exon 1 .The status P: Patient, M: Mother, F: father.

DISCUSSION

The Pro30Leu mutation is associated with electrolyte disturbances was found in 2 patients out of 30 patients. Patient I, fullterm delivered by emergency Caesarean section due to bleeding placenta praevia. Baby was admitted to the neonatal intensive care unit for transient tachypnoea of the newborn. Examination of the genitalia was normal male with both testes descended in the scrotum. Initial electrolytes showed hyponatraemia and hiperkalaemia with normal serum cortisol level. On follow-up patient was gaining weight with normal electrolytes. Patient II presented at the age of 2 months with hyponatraemia and hyperkalaemia. There was a normal male external genitalia with both testes descended. He was treated with sodium chloride and fludrocortisone. In both patients, there was no ambiguity of the external genitalia and both have salt loss. However the salt loss only require short term replacement therapy with sodium chloride and fludrocortisone in patient II and no treatment in patient I. We would like to hypothesize that P30L mutation might cause electrolyte imbalances during neonatal period that subsequently normalize without hormonal replacement therapy. From the parents' results, we observe that P30L is present in the mother whereas P30L is present in one of the fathers of both patients. We need to study more patients to be able to conclude a similar finding.

ACKNOWLEDGEMENT

This project is supported by USM short-term grant 304/PPSP/6131117

REFERENCES

1. M.Natividad Lobato et al. 1999: Mutation Analysis in-patients with congenital adrenal hyperplasia in the Spanish Population: Identification of putative novel steroid 21-hydroxylase deficiency alleles associated with the classic form of the disease. Human Hered .**49**: 169-175.

2. Hughes IA, Clark AJL9eds, 2000: Adrenal Disease in Childhood. Clinical and Molecular Aspects.Endocr Dev.Basel. Karger,vol 2. 93-111.

3. Phyllis W.Speiser et al. 1992: Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency.J. Clin. Invest. **90**: 584-595.

4. Anna Nordenstrom et al. 1999: Genotyping Is Valuable Diagnostic Complement to Neonatal Screening for Congenital Adrenal Hyperplasia due to 21- hydroxylase deficiency. J. Clin. Endocrinol Metab **84**: 1505-1509.