Familial pericentric inversion chromosome 3 and R448C mutation of CYP11B1 gene in Turkish kindred with 11β-hydroxylase deficiency

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ABSTRACT. 11β-hydroxylase deficiency is the second most common cause of congenital adrenal hyperplasia (CAH). This isoenzyme is coded by two highly homologous genes of cytochrome P450: CYP11B1 and CYP11B2 which were mapped to the chromosomal band 8q24. The aim of this study was to perform a series of molecular and cytogenetic analyses in two families with 11β-hydroxylase deficiency of the Turkish kindred. Mutational analysis was carried out by directly sequencing the PCR products of CYP11B1 gene. We performed fluorescence in situ hybridisation (FISH) experiments with consecutive bacterial artificial chromosome (BAC) clones to map the breakpoints of the inversion of chromosome 3 which was detected during the karyotypic analysis of the propositus. Homozygous R448C mutations were detected in 2 individuals with 11β-hydroxylase deficiency. Interestingly, karyotypic change of pericentric inversion [inv(3)(p13q24)] was detected in both individuals who are cousins, one transmitted paternally and the other maternally. The breakpoint at 3p included one interesting gene PPP4R2. Here we present the data of two Turkish families’ members having 11β-hydroxylase deficiency coupled with the familial chromosomal aberration of inv(3)(p13q24). Our data suggest that codon 448, which is a mutational hot spot in CYP11B1 causing 11β-hydroxylase deficiency, is not restricted to Jews of Moroccan origin. Phenotypic variations observed in former studies in patients homozygous for R448H were stated to be due to other factors outside the CYP11B1 locus. The breakpoint in 3p might be a candidate region affecting variations in phenotypes of 11β-hydroxylase deficiency.

INTRODUCTION

Deficiency of 11β-hydroxylase enzyme accounts for 5-10% of all cases of adrenal hyperplasia (1). There are 2 cytochrom P450 isoenzymes with 11β-hydroxylase activity. These isoenzymes are coded by 2 highly homologous genes; CYP11B1 is expressed in both the zona fasciculate and the zona glomerulosa. This gene encodes 11β-hydroxylase enzyme which is necessary for 11-hydroxylation of 11-deoxycortisol to cortisol and deoxycorticosterone to corticosterone, respectively. On the other hand, CYP11B2 is only expressed in the zona glomerulosa and it encodes 11β-hydroxylase, 18 hydroxylase and 18-oxydase enzymes, which is necessary for aldosteron synthesis. These enzymes are known as aldosteron synthesizes; 11β-hydroxylase deficiency due to CYP11B1 gene mutation leads to cortisol deficiency but not through aldosteron because CYP11B2 gene encoding aldosteron synthesizes is not affected in this disorder. As a consequence, precursors, particularly 11-deoxycortisol and deoxycorticosterone, which result in hypertension, accumulate and are shunted into androgen biosynthesis. The most prominent clinical feature of 11β-hydroxylase deficiency is virilization. A female fetus that was exposed to oversecreted adrenal androgens at the critical time of sexual differentiation, would cause the external genitalia to be masculine. The internal genitalia (i.e., uterus, and fallopian tubes), which arise from the müllerian ducts, are normal, because the female fetus does not possess Sertoli’s cells of the testes, which is the source of müllerian-inhibiting factor. In 11β-hydroxylase deficiency, hypertension is a frequent finding. Untreatment of 11β-hydroxylase deficiency

Key-words: 11β-hydroxylase deficiency, CYP11B1, hot spot mutations, chromosomal abnormality.

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in girls results in progressive clitoral enlargement, advanced bone age and tall stature in early childhood with ultimate short stature caused by premature epiphyseal closure, early appearance of facial, axillary, and pubic hair and acne (1, 2).

Other reports also support that this deficit occurs relatively frequently in Israeli Jews from North African origin (3). The first missense mutation reported to be associated with 11\(\beta\)-hydroxylase deficiency is a single base substitution in exon 8 of CYP11B1 gene that changes codon 448 from CGC (Arginine) to CAC (Histidine) (R448H) (4). It was detected in six Jewish families of Moroccan origin. Other mutations at 5, 6, 7, 8 exons of CYP11B1 gene have also been identified from various ethnic groups (4, 5). CYP11B1 mutations, T318M, R374Q, R384Q, V441G, V129M, A331V, E371G, and insertion insCTG464 causing congenital adrenal hyperplasia (CAH) due to 11\(\beta\)-hydroxylase deficiency have already been confirmed by using transient transfection assays (5, 6).

We performed cytogenetic and molecular analysis of CYP11B1 in 2 Turkish families with 11\(\beta\)-hydroxylase deficiency in kindred. Sequence analysis of the CYP11B1 gene led us to identify a mutation R448C which was previously described in only one Iranian patient and Iraqi/Moroccan Jewish patient. This is a deleterious mutation, abolishing the complete enzyme activity (6, 7). As far as we know this is the first case representing both the inv(3) and 11\(\beta\)-hydroxylase deficiency inherited in the same family. That is why we have decided to investigate the genes located at breakpoints. We have localized the 3\(p\) breakpoint within a bacterial artificial chromosome (BAC) clone, RP11-286L5, which includes one RefSeq genes protein phosphates 4, regulatory subunit 2 (PPP4R2) (http://genome.ucsc.edu/).

**MATERIALS AND METHODS**

**Patients**

Two third-degree relative cousins with 11\(\beta\)-hydroxylase deficiency were studied.

**Patient 1**

In 1999 a 10-yr-old child that had been raised as male was admitted to our hospital complaining of male type hair appearance and darkness of skin (Fig. 1, V:6). The patient had 7 siblings, and he was the 6th born of non-consanguineous marriage. Of the 7 siblings, 2 had died between the ages of 2 months and 2 yr of unknown reasons. On physical examination height of the patient was 135 cm (25-50th centile) and weight 38 kg (75th centile). He had dark skin pigmentation and adult male type hair in sexual area. Phenotype of external genitalia was male (Prader stage V). Phallus size was 6.5X2.2 cm. Pubic hair was Prader stage III, and blood pressure by age was above 95th centile (135/80 mmHg). Laboratory investigations revealed a raised serum ACTH (1124 pg/ml; normal: 10-45 pg/ml), 17 hydroxy-progesterone (17-OHP) (94 ng/dl; normal <5 ng/dl) and 11-deoxycortizol (DOC) (220 ng/ml; normal <8 ng/ml) levels. X-ray graphy of left wrist by Greulich and Pyle method demonstrated that epiphysis had been fused. Pelvic ultrasound examination showed the presence of uterus and gonads, and karyotype analysis was 46, XX, inv(3)(p13q24).

In accordance with these clinical and laboratory findings, the diagnosis of CAH due to 11\(\beta\)-hydroxylase deficiency was confirmed. Hydrocortisone in dose of 20 mg/m\(^2\)/day, Captopril in dose of 1.5 mg/kg/day for hypertension were perorally begun, and total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed by surgeons, approved by the expert jury of pediatric endocrinologists, psychologists and pediatric surgeons and with the family’s consent. Pathological analysis was consistent with Müllerian structures and ovaries. The patient’s follow-up is now being monitored by our outpatient clinic and he does not attend visits regularly.

**Patient 2**

In 2004 a 5-yr-old child that had been raised as male was referred to our pediatric endocrinology outpatient department complaining of pubic hair growth. The patient was the 5th offspring of consanguineous marriage (Fig. 1, V:13). It was learned that this patient is the cousin of the patient 1 mentioned above. On physical examination the patient’s height was 133 cm (>97th centile), weight was 38 kg (above 97th centile). Bone age was consistent with 13\(\frac{3}{12}\) old. Phenotype of external genitalia was male (Prader stage V). Phallus size was 10X1.5 cm. Pubic hair was Prader stage III, and blood pressure by age was above 95th centile (135/80 mmHg).

Laboratory investigations showed high serum ACTH 320 pg/ml, 17-OHP 66 ng/dl, 11-deoxycortizol 375 mg/dl, testosterone (T) 248 ng/dl (<20 ng/dl), androstenodione (AND) 17.5 ng/dl (<5 ng/dl) levels. Pelvic ultrasound examination showed the presence of uterus and gonads. Magnetic resonance imaging (MRI) showed surreal hyperplasia and karyotype analysis was confirmed as 46, XX, inv(3)(p13q24).

As before, this patient was also diagnosed with CAH due to 11\(\beta\)-hydroxylase deficiency regarding clinical and laboratory findings. Oral hydrocortisone and Captopril therapy was started. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed by surgeons approved by the expert jury of pediatric