Steroid 11β-Hydroxylase Deficient Congenital Adrenal Hyperplasia with a Reversible Cardiomyopathy Caused by a Novel *CYP11B1* Mutation: Report of Three Cases

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Abstract (Click on the text to edit)

Context: Congenital adrenal hyperplasia (CAH) due to steroid 11β-hydroxylase deficiency is the second most common form of CAH, resulting from a mutation in the *CYP11B1* gene. Steroid 11β-hydroxylase deficiency results in excessive mineralcorticoids and androgen production leading to hypertension, virilization and ambiguous genitalia of genetically female infants.

Objective: The aim of the study was to identify the molecular detect causing steroid 11β-hydroxylase deficiency in two patients from a Saudi family.

Methods: Two brothers aged 21- and 10-month old presented with penile enlargement, progressive darkness of skin, hypertension, and cardiomyopathy. Their elder brother had the same family history and died at age of 30 months due to heart failure with severe dilated cardiomyopathy. All coding exons and intron-exon boundary of *CYP11B1* gene were amplified by PCR from peripheral leukocyte DNA of two patients and sequenced.

Results: A novel biallelic mutation in exon 4 of the *CYP11B1*gene was found in both patients. The mutation c.780 G>A created a premature stop codon at amino acid 260 (p.W260*), resulting in a truncated protein devoid of 11β-hydroxylase activity. Interestingly, a somatic mutation at the same codon (c.779 G>A, p.W260*) was reported in a patient with papillary thyroid cancer (COSMIC data base).Clinically, both patients were treated with hydrocortisone and anti-hypertensive medication. Nine months following treatment, cardiomyopathy disappeared with normal blood pressure and improvement in the skin pigmentation.

Conclusions: We have identified a novel nonsense mutation in the *CYP11B1*gene that causes classic steroid 11β-hydroxylase deficient CAH. Cardiomyopathy and cardiac failure can be reversed by early diagnosis and treatment.

Objectives

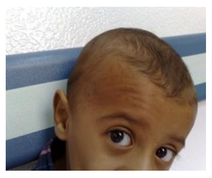
Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by inactivating mutations in genes involved in the cortisol biosynthesis. More than 90% of CAH cases are due to steroid 21-hydroxylase deficiency. Steroid 11β-hydroxylase is encoded by *CYP11B1* and its deficiency is the second most common cause of CAH. At an incidence of one in 100,000 live births, it accounts for about 5 to 8 % of cases of adrenal steroidogenic defects [1,2]**.**

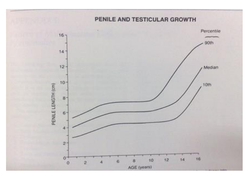
There is lack of DOC production and subsequently aldosterone, causes renal salt-wasting in most of the patients [3]

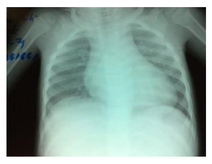
The excessive steroid precursors are shunted into the adrenal androgen synthesis pathway, resulting in virilization and ambiguous genitalia of genetically female infants [3-6]. The *CYP11B1* gene is localized on chromosome 8q21, approximately 40 kb from the paralog *CYP11B2* gene which encodes aldosterone synthase; based on HGMD database, there are 109 different mutations reported in the literature [7-10].

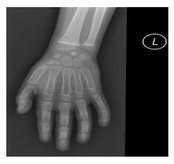
Although hypertension is common in steroid 11β-hydroxylase deficiency, cardiomyopathy due to long standing uncontrolled hypertension has less frequently been reported. Only three adults [11, 12] and two children [13, 14] have been reported in the literature. In the present study, we reported three additional cases and characterized the underlying genetic defect.

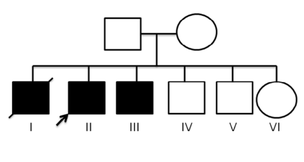
Methods











Case 1: A 30-month old boy was admitted to our Pediatric Intensive Care unit with heart failure and respiratory distress. He was found to have macropenis and excessive skin darkness. Chest x-ray showed cardiomegaly and echocardiography showed severe dilated cardiomyopathy. The patient died at third day of admission. Any lab tests?

Case 2: A 21-month old boy was admitted to the emergency department with bronchopneumonia. He had history of progressive penile enlargement and darkness of skin for the last 10 months. His older brother died 13 years ago at age of 30 months old with similar clinical presentation (Case 1). His weight was 13Kg (Z-score 1.1, 85.3th percentile), height 92 cm (Z-score 2.4, 99.2th percentile), and blood pressure 139/90 mmHg (Z-score for age-based pediatric blood pressure for systolic/diastolic pressure:4.3/3.9, 100th percentile). Z-score equal to or greater than 95th percentile indicates hypertension. Physical examination showed gum, skin and scrotal hyper-pigmentation, facial acne, and penile enlargement of 8.7 cm (above 90th percentile). Testicular size was pre-pubertal and no pubic hair (Fig. 1, A, B and C). Laboratory tests showed serum ACTH 507 pg/mL(normal:5-60pg/mL), cortisol 44 µg/dL(normal: 55– 248? µg/dL), 17α-hydroxyprogesterone (17-OHP) 67 nmol/L (normal: 0.3-2.5 nmol/L), DOC 319 ng/dL(normal:4-49 ng/dL), androstendione more than 70 nmol/L(2.4-12.6 nmol/L). His bone age was at 6-7 years of age. Chest X-ray showed mild or severe? cardiomegaly (Fig. 1, D). Echocardiography showed mild left ventricular dilatation with mild impairment of function with 44% ejection fraction (normal 60-70%). The patient was diagnosed as congenital adrenal hyperplasia steroid 11β-hydroxylase deficiency. Treatment was started with hydrocortisone (dosage?), anti-hypertensive medications(dosage?). Six months later following treatment, echocardiography showed normal left ventricular systolic function. Blood pressure became normal (?) Hydrocortisone treatment (dosage?) continued with gradual reduction of antihypertensive medications (dosage and names). The skin color was improving and acnes disappeared.

Case 3: His 10-month old younger brother was evaluated due to having similar clinical presentations as his brothers such as excessive darkening of skin and progressive penile enlargement. His weight was 12 Kg (Z-score 2.5, 99.4th percentile), height 88 cm (Z-score 6.4, 99.9th percentile) and blood pressure was 125/88 (Z-score 3.6/3.8, 100th percentile). His penile was 7.5 cm above 90th percentile with scrotal hyper-pigmentation and no pubic hair. His bone age was equivalent to 5-6 years old (Fig. 2). Laboratory tests showed serum cortisol 2.9 (3.7-19.4 ?µg/dL), androsteindlone 8.0 ng/mL (0.4-4.1 ng/mL), aldosterone 11 pg/mL (20-1100 pg/mL), 17-OHP 7.5 ng/mL (< 1.1 ng/mL), and DOC 2103 ng/L (43-160 ng/L), . Echocardiography showed mild left ventricular dilatation with 47 % ejection fraction. He was diagnosed as as congenital adrenal hyperplasia steroid 11β-hydroxylase deficiency and treated with hydrocortisone (dosage ?) and antihypertensive medications (names and dosage?). Follow-up echocardiography after 6 months treatment showed dilated left ventricle was return normal with normal blood pressure and cardiac function (60% ejection fraction). The other healthy siblings in the family (2 boys and one girl) had no signs of accelerated growth and sexual development, and their serum levels of deoxycorticosterone, 17α-hydroxyprogestrone (17-OHP), androgens, and aldosterone were within normal range, indicating that they were not affected with CAH

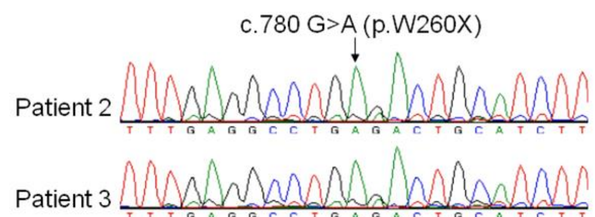
Genomic DNA isolation. Genomic DNA from peripheral blood leukocytes of patients was isolated using the Gentra Blood Kit (Qiagen Corp, CA) after informed consent was obtained from their parents. The parents and three unaffected siblings (two brothers and one sister) of the patients refused to give blood samples for genetic test, which prevented us from performing a familial genetic analysis.

DNA amplification and sequencing. Selective amplification of the *CYP11B1* gene was performed in five fragments by PCR from 100 ng of genomic DNA as described previously [11] . The resulting PCR products were directly sequenced using an automated ABI PRISM 3700 sequencer (Foster City, CA).

Results

Clinical Characteristics. The diagnosis of steroid 11β-hydroxylase deficient congenital adrenal hyperplasia was made based on their clinical and biochemical features. Both patients had characteristic features of classic steroid 11β-hydroxylase deficiency: accelerated growth, skeletal maturation, pseudoprecocious puberty, hypertension, elevated serum levels of DOC, 17-OHP, and androstendione, and low serum levels of cortisol and aldosterone. The initial presentation of the patients was cardiac complications due to hypertension, which could result in misdiagnosis and early treatment opportunities as in the first patient who died due to heart failure. Hypertention with virilized genitalia and pseudoprecocious puberty should be alerted for the diagnosis of steroid 11β-hydroxylase deficiency.

Sequence analysis of the *CYP11B1* gene. To identify the underlying genetic defects leading to steroid 11β-hydroxylase deficiency, we sequenced the entire coding region and intron-exon boundaries of the *CYP11B1* gene in both patients. A novel biallelic mutation in the *CYP11B1* gene was found in both patients. The mutation c.780 G>A created a premature stop codon at amino acid 260 (p.W260*), resulting in functional inactivation of the *CYP27B1* gene (Fig. 3). Interestingly, a somatic mutation at the same codon (c.779 G>A, p.W260*) was reported in a patient with papillary thyroid cancer (COSMIC data base). The significance of the mutation in thyroid cancer remains to be determined. . The parents and three unaffected siblings (two brothers and one sister) refused to donate blood samples for genetic test, which prevented us from performing a familial genetic



Conclusions

In the present study, we have presented three cases of classic steroid 11β-hydroxylase deficiency from non-consanguineous parents. Their diagnosis was initially missed at the local hospital and the patients developed dilated cardiomyopathy due to hypertension. This could result in misdiagnosis and delay in early treatment as demonstrated in our first patient who died due to heart failure. Routine blood pressure measurements and detailed physical examination during well baby visit can help detect underlying causes of hypertension in pediatric patients. Hypertention with virilized genitalia and pseudoprecocious puberty such as infantile acne, macropenis and darkness of skin should alert physicians for the diagnosis of steroid 11β-hydroxylase deficiency.

Hypertension is not common in patients with late-onset disease or nonclassic form of steroid 11β-hydroxylase deficiency due to partial 11β-hydroxylase activity [15].The secondary hypertension leads to dilated cardiomyopathy in our patients, which can be reversed by early diagnosis and treatment.

dilated cardiomyopathy (DCM) is the most common in children [16-19]. Apart from other types of dilated cardiomyopathy, the one described in our cases Cardiomyopathy and cardiac failure can be reversed by early diagnosis and treatment.

The nonsense mutation described in our patient has not been described in the literature. The mutated transcripts may be translated to a truncated protein or degraded via nonsense-mediated decay [21].. In either case, the 11β-hydroxylase activity is completely lost in our patients. The clinical presentation and lab data support the conclusion.

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Adrenals and HPA Axis

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