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Mutational analysis of rare subtypes of congenital adrenal hyperplasia in a highly inbred population

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ABSTRACT

Context: Apart from 21 Hydroxylase deficiency, other subtypes of congenital adrenal hyperplasia (CAH) are rare. We studied the clinical features and molecular genetics of a relatively large series of patients with CYP17A1, HSD3β2 and StAR deficiencies.

Patients and methods: We studied 21 patients including 7 patients with CYP17A1, 10 patients with HSD3β2 and 4 patients with StAR deficiencies. For mutation detection, we isolated DNA from peripheral leucocytes, amplified genes of interest using polymerase chain reaction and directly sequenced the amplicons using Dideoxy Chain Termination method.

Results: Regardless of their karyotype, patients with *CYP17A1* deficiency presented with normally looking external female genitalia and were raised as females. Hypertension and hypokalemia were prominent features in 4 of 7 patients. Two missense (p.R416H, p.R239Q) and 2 non-sense (p.Y329X, p.Y329X) mutations were found in these 7 cases. In 3 unrelated families with 10 affected siblings with *HSD3β2* mutations, two non-sense mutations were found (p.Q334X, p.R335X). 46XY patients with *HSD3β2* deficiency presented with ambiguous genitalia while 46XX patients presented with normal female external genitalia. Adrenal crisis was common in patients with both karyotypes. In the 4 patients with StAR deficiency, both genetic male and female patients presented with normally looking female external genitalia and adrenal crisis. One previously reported missense mutation (p.R182H) was found in 3 unrelated patients and a novel non-sense mutation (p.Q264X) in the fourth patient.

Conclusions: These cases of rare subtypes of CAH illustrate the heterogeneous phenotypic and genetic features of these subtypes and add unique novel mutations to the previously known ones.

1. Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by defects in the steroid biosynthetic pathway leading to glucocorticoid deficiency (Speiser and White, 2003; New et al., 2014). The most common form of CAH is due to 21 hydroxylase deficiency (21-OHD), which accounts for more than 90% of cases (Speiser and White, 2003; Parsa and New, 2017). Based on the severity of the condition, 21-OHD is classified to classic (severe) and the non-classic (mild) subtypes. The classical subtype occurs with an incidence of about 1:16,000 lives birth (Therrell et al., 1998; Therrell et al., 2015), whereas the milder or non-classical form is much more common, with an estimated prevalence of 1:500 to 1:1000 live births (Parsa and New, 2017; Therrell et al., 1998; Finkielstain et al., 2012). In some ethnic groups such as Jews, Yugoslavs, Hispanics and Italians, the prevalence of non-classic 21-OHD has been reported to be much higher ranging between 1:27–1:300 newborns (Parsa and New, 2017; Speiser et al., 1985). 11β-Hydroxylase (CYP11B1) deficiency has been considered the second most common subtype of CAH. However, this is based

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on studies in certain populations, mostly from the Middle East and North Africa (Rosler et al., 1992; Khattab et al., 2017) where the prevalence of CYP11B1 deficiency seems to be much more common than other parts of the world (Turcu and Auchus, 2015). Although it is frequently quoted that CYP11B1 deficiency occurs in 5–8% of cases of CAH, this is not well supported by literature. Thrill et al. could only identify 4 cases of CYP11B1 deficiency in screening of 1.9 newborns in Texas, USA (Therrell et al., 1998). Its prevalence is much higher in Moroccan Jews and other ethnic groups from the Middle East and North Africa due to the presence of founder mutations (Rosler et al., 1992; Khattab et al., 2017). Some hospital-based data suggested that CYP11B1 deficiency is more prevalent in Saudi Arabia than in other countries accounting for about 25% (al-Jurayyan, 1995). However, these data were based on a review of all cases of ambiguous genitalia referred to a tertiary care center over many years and their diagnosis was based on clinical and biochemical findings only. Other subtypes of CAH include 3β-hydroxysteroid dehydrogenase type II (*HSD3β2*) deficiency, 17α-hydroxylase deficiency (*CYP17A1*) and the Steroidogenic Acute Regulatory (*StAR*) protein deficiency. Although these subtypes are generally rare, there is a significant variation in their prevalence between different ethnic groups. After 21-OHD, StAR deficiency seems to be the second most common subtype of CAH in Korea and Japan (Bose et al., 1996; Kim et al., 2014) while *CYP17A1* seems to be the second most common subtype in Brazil (Costa-Santos et al., 2004). This might be also due to the common occurrence of founder mutations in these populations.

The molecular genetics of 21-OHD (*CYP21A2*) and *CYP11B1* have been extensively reported in the literature (Khattab et al., 2017; Concolino et al., 2013; de Carvalho et al., 2016; Dumic et al., 2017; New et al., 2013). On the other hand, due to the rarity of other forms of CAH, less data are available on this group in general and particularly in Saudi Arabia. Consanguinity is common and genetic diseases are equally common in Saudi Arabia (el-Hazmi et al., 1995a,b; El-Mouzan et al., 2007). Although there is no formal assessment of the prevalence of CAH, these disorders are hereditary in origin and are likely to be common in Saudi Arabia where cross marriages and high paternity are prevalent (el-Hazmi et al., 1995a,b; Sabbagh et al., 2015; Bashamboo and McElreavey, 2014). The genetic and molecular bases of CAH in Saudi Arabia have rarely been studied (al Kandari et al., 2006; Chen et al., 2005,Mohamed et al., 2015). Previous reports were mostly case reports or small case series. In this study, we report our findings of the clinical features and molecular genetics of a relatively large series of Saudi patients with rare subtypes of CAH including *HSD3β2, CYP17A1* and *StAR* deficiencies.

1.1. Patients and methods

After obtaining an Institutional Review Board approval and informed consents from patients or their parents, we recruited patients diagnosed on clinical and biochemical bases with rare subtypes of CAH excluding those with *CYP21A2 and CYP11B1*. The primary aim of this study was to characterize the underlying mutations in patients with established diagnosis of one of the three subtypes of CAH, CYP17A1, HSD3B2 and StAR deficiencies. We included patients previously diagnosed with these disorders. The diagnosis was established prior to the study and was based on clinical features and confirmed by hormonal evaluation. The majority of these patients has been on follow up at the King Faisal Specialist Hospital & Research Center, the major referral center in Saudi Arabia and therefore is relatively representative of CAH in Saudi Arabia. Patients were evaluated by a pediatric or an adult endocrinologist with expertise in disorders of sex development. Patients had a full assessment including clinical evaluation, chromosomal composition (Karyotype), hormonal studies and radiological studies (pelvic and genital ultrasonography and/or MRI) as appropriate for the suspected underlying condition. Each case was reviewed carefully by the investigators for the accuracy of the diagnosis and the likely underlying genetic defect. Our inclusion criteria were as follows:

1. Patients of any age with clinically and biochemically well-defined CAH (excluding *CYP21A2 and CYP11B1*).
2. Previous work up including clinical, chromosomal, biochemical and radiological data that were adequate to define the condition (see Tables 2 and 3 and the Supplementary materials)
3. Willingness and provision of an informed consent for participation in the study.

We briefly describe the main features of these patients here but the clinical, biochemical and management details for each patient are included in the Supplementary materials and Tables 2–4.

1.2. Patients with CYP17A1 deficiency

We studied 7 patients with *CYP17A1* deficiency; two families with 5 siblings and 2 unrelated patients (Table 2). Five patients were 46 XY and two were 46 XX but they all presented with normal female external genitalia and were raised as females. Only one patient was discovered at a young age (2 years) during her management for bilateral inguinal herniae which contained undescended testes. All other cases were diagnosed at a much older age and mostly as delayed puberty with primary amenorrhea. Hypertension and hypokalemia were prominent features in 4 out of 7 cases (57%). The hormonal profile was consistent with the diagnosis of CYP17A1 deficiency with elevated serum ACTH, very low 17 OH-progesterone, androstenedione, DHEAS, testosterone, cortisol and renin (Supplementary materials).

1.3. Patients with HSD3B2 deficiency

We studied 10 patients from 3 unrelated families with 2–4 members affected per family (Table 3 and the Supplementary materials). In 46XY males, hypospadias, micropenis, chordee and penoscrotal hypospadias in addition to severe adrenal crisis were prominent features (Table 3 & the Supplementary materials). However, the severity of these features was variable even within the same family (e.g. family 1). In 46 XX females, the external genitalia were normal female type and adrenal crisis was the main presenting feature (Table 3). Biochemically, patients commonly presented with hyponatremia and hyperkalemia, elevated ACTH, low cortisol and testosterone, high 17-hydroxypregnenolne, high DHEAS and low 17-OH progesterone (Table 3 and Supplementary materials).

1.4. Patients with StAR deficiency

Four unrelated patients were diagnosed with StAR deficiency. All patients (46XY and 46XX) presented with normal female external genitalia due to severe deficiency of androgens. In addition, salt-losing adrenal crisis occurred in the first month of life with hypotension, hypernatremia and hypokalemia in all patients (Table 4 and the Supplementary materials). All adrenal steroids downstream of pregnenolone were extremely low and serum ACTH was very high (Supplementary materials).

1.5. Mutation detection

Genomic DNA was extracted from 5 cc blood collected in EDTA

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