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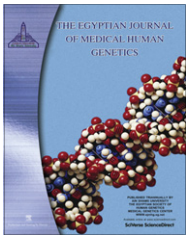

ORIGINAL ARTICLE

Cytogenetic and molecular study in intersex

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KEYWORDS

Developmental Sex Disorders;
SRY gene;
CYP21 gene;
Genetic counseling;
Cortisol;
17-Hydroxyprogesterone

Abstract "Disorders of Sex Development" (DSD) is the state of a person whose sex chromosomes, genitalia and/or secondary sex characteristics are determined to be neither exclusively male nor female. The aim of this work was the genetic study of patients with ambiguous genitalia and genetic counseling of these cases. This study was conducted in the period from November 2007 to February 2009. Cases were obtained from the Genetics and Endocrine Units, Pediatric department, Faculty of Medicine, Menoufiya University, Egypt. Thirteen cases with ambiguous genitalia were studied, 10 genetic female and 3 genetic male patients. Their ages ranged from the first week to eight years. All cases were subjected to the following: Detailed history, thorough clinical examination, routine investigations, hormonal, imaging studies, cytogenetic study and molecular study. Sequencing for both the SRY and the CYP21 genes and genetic counseling were performed. Study revealed that among our patients, cases number 2 and 11 were mainly presented as salt losing crisis. Cases 5 and 13 were presented with hirsutism of the external genitalia and upper and lower limbs. Eight cases (61.5%) had a positive consanguinity and Cases 3 and 4 were sisters. Hormonal study revealed that (adrenocorticotropic hormone) ACTH, was within the normal range with a mean 83.14 pg/ml ± 53.6 pg/ml for all patients. Serum Cortisol and 17-OHP (17-hydroxyprogesterone) were elevated in all patients. Karyotype using G-banding showed that there were no apparent anomalies in the sex chromosomes. We found four cases (30.8%) with different mutations in the SRY gene at codon Q57R and S143C. As for the CYP21 gene, we found a variety of deletions in size and site within the structure of the gene in 85.4% of cases leading to alteration in the function of the CYP21 gene which ultimately lead to congenital adrenal hyperplasia (CAH) in these cases.

1. Introduction

Genital anomalies are estimated to occur in 1 in 4,500 births. The most common cause of ambiguous genitalia in the newborn is congenital adrenal hyperplasia (CAH) – 1 per 15,000 live births. CAH appears to be more common in those of European Jewish, Hispanic, Slavic and Italian descent.

The ability to diagnose Developmental Sex Disorders (DSD) has advanced rapidly in recent years. In most cases today, clinicians can promptly make an accurate diagnosis

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and counsel parents on therapeutic options [1]. The ultimate goal in any management strategy is to provide a framework that will allow the affected child to develop into a well-adjusted psychosocially stable individual who identifies with and is happy in the assigned sex [2]. Factors that influence sex assignment include diagnosis, genotype, genital appearance, surgical options, need for lifelong replacement therapy, potential for fertility, views of the family, and sometimes circumstances relating to cultural practices [2–4].

The aim of this work was the genetic study (both cytogenetics and molecular genetics) of DSD cases with special prominence of Karyotyping and DNA extraction, PCR, gel electrophoresis, SSCP and automated DNA sequencing for both the SRY and the CYP21 genes and genetic counseling of DSD cases and determination of the causes and/or risk factors in each case.

2. Patients and methods

2.1. Patients

The study was conducted on 13 children with ambiguous genitalia. They were selected from Menoufiya university hospitals, Genetics and Endocrine Unit, Pediatric department, Egypt, during the period from 2007–2009. They were 10 genetic females and 3 genetic males. Their ages ranged from the first week up to eight years.

2.2. Methods

All studied cases were subjected to the following: A detailed history with stress on maternal exposure to androgens, virilization symptoms in the mother or the use of phenytoin and the presence of similar conditions in the family and family pedigree.

A thorough clinical examination including general examination of all systems, body hair distribution (hirsutism) and skin examination (signs of dehydration).

External genitalia examination with stress on: Phallus: size, degree of differentiation, length, ventral curvature, appearance of the foreskin and location of the urethral meatus. We also used Prader scoring system for this [5]. The scrotum or labia: were examined for separation and/or fusion between the two halves of the labioscrotal tissue. Scrotal rugea or labioscrotal folds with increased pigmentation were noted. Tanner staging was established and it included examination of the breast in females, the external genitalia in males and pubic hair in both.

Gonadal examination with stress on its site: (within the labioscrotal tissue or outside it), fully descended or not. Also anthropometric measurements were obtained.

Routine investigations included complete blood picture, serum electrolytes and glucose levels which were closely monitored in cases with salt losing congenital adrenal hyperplasia (CAH) especially sodium (Na) and potassium (K).

Hormonal investigations included serum levels of ACTH (Adrenocorticotropic hormone), Cortisol and 17-OHP (17-hydroxyprogesterone) [6,7] .

Imaging studies including genitogram and abdomino-pelvic ultrasound, CT and/or MRI were needed to determine the presence and/or absence of internal genital structures (such as undescended testes).

Cytogenetic study included chromosomal karyotype using G-banding [8].

Molecular study: focused mainly on the SRY gene and the CYP21 gene. The SRY gene is the main regulator of sexual differentiation, while the CYP21 gene is the main gene affected in CAH, the commonest form of Developmental Sex Disorders (DSD). The following steps were applied:

a. DNA extraction [9].
b. PCR (Polymerase Chain Reaction) [10].
c. Gel electrophoresis [11].
d. SSCP (Single-strand conformational polymorphism) [12].
e. Automated DNA sequencing [13].

And lastly genetic counseling was done to all families in order to prevent, avoid or ameliorate the disorder [14].

3. Results

The results of this study were illustrated in Tables 1–5 and Figs. 1–4.

Generally, cases number 2 and 11 were mainly presented as salt losing crisis in the form of severe, recurrent vomiting, diarrhea and dehydration.

Cases 5 and 13 were presented with hirsutism of the external genitalia and upper and lower limbs. While cases number 1, 3, 4, 6 to 10 and 12 were mainly presented as ambiguous genitalia. Case number 7 was presented by clitoromegaly with no palpable gonads (as shown in Fig. 1) and case number 9 was presented with micropenis with bilateral undescended testes (as shown in Fig. 2).

We must note that the patient number 13 was not subjected to the molecular study because the patient died before we could obtain a blood sample.

Demographic data of studied patients (Table 1) showed that eight cases (61.54%) of the 13 (cases number 1–7 and 11) had positive consanguinity. Cases 3 and 4 were sisters. Most of the cases had irrelevant maternal history.

Anthropometric measurements (Table 2) showed that 4 patients were less than the 5th centile (2 females, 2 males: cases 2, 8, 11 and 12), 6 patients were within the normal range (5 females, 1 male: cases 3–6, 9 and 10) and 1 female (case number 1) was above the 95th centile as regards weight, height and head circumference.

Table 3 showed that Tanner staging in most cases were in the 1st stage.

Case 1 was a female with a Tanner staging of B1, P3, and A1, while cases 5 and 13 were females with tanner staging of B1, P3 and A2. Also this table showed that clitoromegaly was the most common presentation in our patients (77%).

As regards laboratory investigations (Table 4), hormonal assay showed that adrenocorticotropic hormone (ACTH) was within the normal range with a mean 83.14 pg/ml ± 53.6 pg/ml for all the patients. Serum Cortisol and 17-OHP were elevated in all the patients:

- Cortisol mean levels (±SD) were *A.M* sample: 59.8 μg/dl (±62.45 μg/dl) and

  P.M sample: 40.58 μg/dl (±11.8 μg /dl).