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Clinical presentation and mutational spectrum in a series of 166 patients with classical 21-hydroxylase deficiency from South China

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ABSTRACT

Classical 21-hydroxylase deficiency (21-OHD) due to mutations in the cytochrome P450 family 21 subfamily A member 2 (CYP21A2) gene is the most common type of congenital adrenal hyperplasia (CAH). In this study, we analyzed clinical and molecular data of 166 patients with classical CAH in South China. Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) method were used to detect mutations in these 99 salt wasting (SW) patients and 67 simple virilizing (SV) patients. Micro-conversion mutation IVS2-13A/C > G (I2G) was the most frequent mutation in both SW form (42.9%) and SV form (41.8%) in our large cohort, and large gene deletion or large gene conversion also commonly resulted in classical CAH. Rare mutations only account for 8.4% of all alleles, among them four novel variants p.S126X, p.C429X, c.1209_1210insT and c.840delG were responsible for the clinical presentations. CYP21A2 gene duplications linked to the mutation Q319X were found in our cohort, though these cases were rather rare. In this study, we provided detailed clinical data and mutation spectrum to confirm the common mutations in Chinese populations, especially in South China,which will contribute to further genetic consultation and prenatal diagnosis. Sanger sequencing combined with MLPA method could detect most mutation types in the CYP21A2 gene effectively.

1. Introduction

Congenital adrenal hyperplasia (CAH) is one of the most frequent autosomal recessive diseases, caused by the defect of the enzyme responsible for catalyzing the synthesis of the steroids such as cortisol and aldosterone in the adrenal cortex [1]. Among them, nearly 95% of cases are caused by 21-hydroxylase deficiency (21OHD, MIM 201910), which is characterized by the elevation of 17-hydroxyprogesterone (17-OHP) and the accumulation of adrenal androgen. According to the residual enzyme activity, 21 OHD CAH is classified into classic salt-wasting (SW) form, classic simple virilizing (SV) form and nonclassic form [2]. Due to a mild enzyme deficiency, nonclassic form has a highly variable clinical presentation [3].

21OHD CAH is caused by the mutations in the cytochrome P450 family 21 subfamily A member 2 (CYP21A2, MIM 613815) gene, which encodes the 21-hydroxylase enzyme and is located on the chromosome 6p21.3. In vitro study revealed that complete inactivation of 21-hydroxylase caused by certain mutations are linked to the SW form, and those mutations resulting in ~2% residual activity are linked to the SV form, and those resulting in 10%–75% residual activity are associated with the nonclassic form [4, 5]. Because the location of the highly homologous inactivated pseudogene (CYP21A1P) is quite close to the CYP21A2, conversions between these two genes happened frequently, which adds new complexities in detecting mutations in the CYP21A2 gene. Until now, nearly 300 different mutations have been reported worldwide according to the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Yet common point mutations resulting from the micro-conversions are the most frequent mutations [2, 4, 6], followed by large gene rearrangement (large gene deletion or gene conversion) that can lead to nine types of CYP21A2/CYP21A2 chimeras (CH1-CH9) [4, 7, 8]. Rare mutations arising independently of the pseudogene only account for < 10% of cases [9, 10]. Sanger

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Table 1
Clinical presentation, hormone level and mutation group.

Diagnosis age	SW		SV	
	M (n = 70)	F(n = 29)	M (n = 18)	F (n = 49)
	NP-8 month	NP-5 month	NP~13 year	NP~10 year 8 month
Clinical presentation				
Newborn screening	7		1	2
Adrenal insufficiency symptoms	63	12		
Ambiguous gender		17		47
Premature pubarche and/or penis enlargement and/or growth spurt			14	
Family history			3	
Hormone level				
17-OHP (< 25 ng/mL)	> 25	> 25	> 25	> 25
T (0–0.7 nmol/mL)	1.72 ~ > 52	0.7 ~ > 52	4.5–20.9	1.84 ~ > 52
ACTH (0–10.12 pmol/mL)	2.35 ~ > 278	4.22–125	8.01–160	6.32–205
DHEAS (2.17–15.2 umol/L)	0.68 ~ > 40	0.6–39	0.7–20.7	0.6–21.6
COR (118–618 nmol/L)	57.7–1636	46.5–1835	52.4–295.6	61.5–677
AND (1.0–11.5 nmol/L)	10.1 ~ > 35	26.9 ~ > 35	27 ~ > 35	10.1 ~ > 35
Mutation group				
“Null” group	16	8	1	1
“A” group	47	13	5	20
“B” group	1	2	12	21
“D” group	6	6	1	6
Mutation type				
Homozygous	19	4	5	14
Heterozygous	51	25	13	35

SW: salt-wasting forms; SV: simple virilizing forms; F: female; M: male; NP: neonatal period; 17-OHP: 17-hydroxyprogesterone; ACTH: adrenocorticotropic hormone; T: testosterone; AND: androstenedione; COR: cortisol; DHEAS: dehydroepiandrosterone sulphate.
Null group:patients with biallelic mutations resulted in completely inactive enzymes (such as gene deletions, p.G111Vfs*21, E6 cluster, p.L308Ffs*6, p.Q319*, p.R357W).
A group: patients with homozygous I2G or heterozygous I2G in trans with a null mutation.
B group: Patients with homozygous p.I173N mutation or heterozygous p.I173N mutation in trans with a mutation from group‘null’ or group A.
C group: mainly involved in the NC CAH, so not included in our study.
D group: patients with mutations with unknown influence on enzymatic activity.

sequencing combined with multiplex ligation-dependent probe amplification (MLPA) can be utilized to detect the actual mutations of CYP21A2 gene effectively [11, 12]. The 17-hydroxyprogesterone (17-OHP) test in dried blood spots on filter paper for CAH has been carried out in newborn screening centers of many countries since 1977 [13, 14], and newborn screening for CAH is also optional to the parents of newborns in Guangzhou since 2007.

The frequency of common micro-conversions of CYP21A2 gene varies greatly among different ethnic groups, and the disparity between genotype and phenotype is also reported in different races [7]. Nevertheless, there is almost no description of the mutation spectrum of the CYP21A2 gene in a large cohort in South China [12, 15–18]. In this retrospective study, the hormone level, molecular and bone age data obtained from 166 classical CAH patients due to 21OHD were analyzed, which will provide physicians with clinical experiences.

2. Materials and methods

2.1. Patients

A total of 166 Chinese patients diagnosed as classical CAH due to 21-OHD according to clinical data, hormonal level and gene analysis, were recruited in our hospital from August 2014 to May 2017. Except for 8 patients from 4 different families, all other patients came from 158 unrelated families without consanguinity. Informed consent was signed by these patients' guardians. All studies were subject to approval by the institutional review board of Guangzhou Women and Children's Medical Center.

Basal 17-hydroxyprogesterone (17OHP) was measured in all patients. Adrenocorticotropic hormone (ACTH), cortisol (COR), testosterone (T), androstenedione (AND) and dehydroepiandrosterone sulphate (DHEAS) at baseline were also determined. Based on clinical presentation and characteristic of electrolyte analysis, these classical CAH patients were diagnosed with the SW form or the SV form. For patients with ambiguous genitalia, karyotype analysis was performed to determine the gender. But most CAH patients initially diagnosed above 3 years underwent bone age assessment.

2.2. Molecular analysis of the CYP21A2 gene

Genomic DNA of all the patients and some of their parents was extracted from the peripheral blood as described in manufacturer's protocol on a DNA Isolation system (Lab-Aid 820, Zeesan). Two long PCR fragments targeting the pseudogene of the CYP21A2 gene were amplified, and the products were analyzed with a sequencer (3500xL Dx, ThermoFisher) by using 6 pairs of primers (All primers were listed in Table S1–2). DNAMAN software was utilized to analyze the sequences. MLPA-was performed to detect large deletions and gene conversions on a Genetic Analysis System (GenomeLab™ GeXP, Beckman Coulter). This method is applicable to those whose mutations could not be detected by direct sequencing and those whose parents' samples were not available. Novel variants were scanned in the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/), 1000Genomes data (http://browser.1000genomes.org/index.html) and the Exome Aggregation Consortium data (ExAC, http://exac.broadinstitute.org/) to exclude the possibility of polymorphism. Correlation between genotype and phenotype was analyzed according to the categorization of mutations described before [19, 20].

2.3. Treatment and follow up

After diagnosis, all patients were treated according to the guidelines from the Chinese Society for Pediatric Endocrinology [21]. Bone age assessment was performed according to their growth rate.