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Low frequency of the *CYP21A2* deletion in ethnic Chinese (Taiwanese) patients with 21-hydroxylase deficiency

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Abstract

Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder which causes more than 90% of CAH cases due to defects in the steroid 21-hydroxylase gene (*CYP21A2*). The frequency of large mutations was determined in 200 ethnic Chinese (i.e., Taiwanese) CAH patients belonging to 200 families with different clinical forms of *CYP21A2* deficiency over 10 years of molecular diagnoses. For a large-gene deletion (or conversion) and the *CYP21A2* deletion identification, a PCR product covering the TNXB gene and the 5′-end of the *CYP21A2* gene with TaqI endonuclease digestion was analyzed by electrophoresis on agarose gels. For *CYP21A2* mutational analysis, secondary PCR amplification of the amplification-created restriction site method was applied. From the results of the analysis, we found that large-gene deletions (or conversions) occurred in 7.5% of the alleles including three different types of the chimeric *CYP21A1P*/*CYP21A2* genes and the haplotype of IVS2-12A/C>G in combination with the 707-714del mutation (without the P30L mutation). The *CYP21A2* deletion occurred in 2.0% of the alleles which contained three types of the chimeric *TNXA*/*TNXB* genes with two novel ones. We concluded that the *CYP21A2* deletion in the ethnic Chinese (Taiwanese) patients exhibits a low occurrence, with the haplotype of the IVS2-12A/C>G in combination with the 707-714del mutation (without the P30L mutation) being prevalent among large gene deletions or conversions.
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Keywords: Carrier frequency; Gene deletion; *CYP21A2*; CAH; Ethnic Chinese

Congenital adrenal hyperplasia (CAH, OMIM 201910) is a common autosomal recessive disorder which causes about 90–95% of CAH cases due to defects in the steroid 21-hydroxylase gene (*CYP21A2*; EC 1.14.99.10). The incidence of the disease is reported to be 1:10,000 to 1:18,000, depending on race [1,2]. The incidence in New Zealand based on hormonal newborn screening is 1:23,344 [3] and in Chinese is 1:28,000 as determined by analysis of the *CYP21A2* gene using a PCR-based method

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from 1000 healthy adults [4]. The wide range of CAH phenotypes is associated with multiple mutations known to affect 21-hydroxylase enzyme activity. Three forms of CAH are classified: the classical salt-wasting, classical simple virilizing, and non-classical forms [5,6]. The prevalence of the non-classical form is estimated to be 1:1700 in the general population [1,3].

Defective *CYP21A2* genes in CAH fall into 1 of 3 categories: (a) small-scale conversions of *CYP21A1P*, (b) spontaneous mutations, and (c) chimeric RCCX modules that include the chimeric *CYP21A1P*/*CYP21A2* and *TNXA*/*TNXB* genes [7–12]. Most (up to 15 *CYP21A1P*) of the *CYP21A2* mutations [13] identified so far are a result of small-scale conversions which account for about 70–75% of CAH cases. The most frequent mutations in ethnic Chinese are IVS2-12A/C>G, I172N, and R356W [14] which show high similar incidences worldwide in different races [1,15,16]. In addition, a mutation of V281L, the most common non-classical disease appearing in high frequency in patients in France, Austria, Italy, Spain, Turkey, Argentine, and Portugal [15–17], was not found in Taiwanese [14], Japanese [18], or Tunisian patients [19]. An increasing number of spontaneous mutations (5–10% of cases) mostly consist of nucleotide changes in the coding sequence [20].

Large-gene deletions or unequal crossover recombinations may occur anywhere within the RCCX module [21] (or C4-CYP21 repeat module) which includes *C4A* [21], *CYP21A1P*, *tenascin-XA* [22], *RP* (*RP1* and *RP2*) [21], *C4B* [21], *CYP21A2*, and *tenascin-XB* [23]. These genes are adjacent to each other and alternate with each other in the most frequent bimodular RCCX of the *RP1-C4A-CYP21A1P-TNXA-RP2-C4B-CYP21A2-TNXB* gene sequence in Caucasians [24]. The RCCX module has 3 possible forms: monomodular, bimodular, and trimodular. Chromosomes with 4 RCCX modules are very rare [24]. Because of a high degree of sequence homology and the tandem repeating order of these genes, this seems the most likely area for misalignment to occur at meiosis which would generate illegitimate genetic recombinations or unequal crossovers. It has been pointed out that the 30-kb large-gene deletions (or conversions) encompassing the RCCX module lead to the formation of *CYP21A2* hybrid genes [23,25,26] and the association of the HLA haplotype, A3;Bw47;DR7 [27], in the *CYP21A2* allele in Caucasians. Such a deletion is thought to result from unequal crossovers (or deletions) in meiosis occurring in ~20% of CAH alleles in most populations [1]. However, its frequency is dependent on the population studied [1,16,18,28]. At present, *CYP21A2* hybrid genes with a *_?_CYP21A1P-XA-RP2-C4B-_?_CYP21A2* gene array deletion exist in five types of chimeric *CYP21A1P*/*CYP21A2* genes [12]. In addition, the formation of chimeric *TNXA*/*TNXB* genes caused by a deletion of the *RP2-C4B-CYP21A2-_?_ TXNB* gene array leads to CAH and is associated with Ehler-Danlos syndrome [7,8]; these are found in three types of chimeric *TNXA*/*TNXB* genes [12]. These 2 RCCX chimeras possess the *CYP21A1P*-like gene with a 3.2-kb TaqI-produced fragment [12]. In a Caucasian population, the HLA haplotype, B62;S31;DR1 [29], with a *cis*-arrangement of the *CYP21A2* and *CYP21A1P* genes [30,31] was identified in CAH patients. Interestingly, the haplotype of IVS2-12A/C>G in combination with the 707-714del mutation (without the P30L mutation) [32] and a 3.2-kb TaqI-produced fragment caused by the deletion of *CYP21P-XA-RP2-C4B* and an intergenic recombination are most prevalent in ethnic Chinese CAH patients [33].

In this paper, we analyzed the frequency of large mutations in 200 ethnic Chinese (i.e., Taiwanese) CAH families with different clinical forms of *CYP21A2* deficiency and observed low frequencies of large-gene deletions (or conversions) and the *CYP21A2* deletion during 10 years molecular diagnoses.

Materials and methods

Patients

We have collected data on 200 CAH patients belonging to 200 families from a concerted effort among hospitals all cross Taiwan since 1994. All families requested an extensive molecular diagnosis and provided informed consent. These hospitals included the Department of Pediatrics, Mackay Memorial Hospital, Taipei; Outpatient Department of the Department of Obstetrics and Gynecology and Pediatrics Clinics of the Veterans General Hospital, Taipei; Department of Pediatrics, Changhua Christian Hospital, Changhau; Division of Genetics, Endocrinology, and Metabolism, Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung; Department of Medical Genetics, China Medical University Hospital, Taichung; Department of Pediatrics, Chang Gung Children's Hospital, Taoyuan; and Department of Pediatrics, College of Medicine, National Cheng Kung University, Tainan.

An 8.5-kb PCR product with TaqI endonuclease digestion for large-gene deletions or conversions and the CYP21A2 gene deletion analysis

To identify large-gene deletions and conversions and *CYP21A2* deletion, an 8.5-kb PCR product covering the *TNXB* gene to the 5′-end of the *CYP21* gene (Fig. 1A) was amplified and used to characterize the *CYP21A2* gene structure by TaqI endonuclease digestion. The paired primers, Tena 32F/CYP779f, were used for the 8.5-kb fragment amplification (Fig. 1B) and reaction protocol as described previously [34]. Primer Tena32F (5′-ctgtgcctggctatagcaagc-3′, nt 78,918–78,939 GenBank Accession No. AL049547), located in a non-duplicated area of exon 32 of *TNXB* gene, and primer CYP779f (5′-aggtgggctgttttcctttca-3′, nt 87,443–87,463 GenBank Accession No. AL049547 for *CYP21A2*) are located in the 5′-end of the *CYP21A1P* and *CYP21A2* genes, respectively. The 8.5-kb PCR products with 10 U in a 10-μl volume of TaqI cleavage enzymes were incubated at 65 °C for 2 h. The completely digested PCR products were analyzed by electrophoresis on 1.2% agarose gels.

A primary 3.5-kb differential PCR product of the CYP21A2 gene as a template for secondary amplification-created restriction site (ACRS) analysis for identifying the CYP21A2 mutation

Because of the high homology between the *CYP21A2* and *CYP21A1P* genes, the *CYP21A2* mutation was detected by a differential PCR amplification of the 3.5-kb PCR fragment amplified with the paired primers, BF1/21BR, as described previously [13] (Fig. 1C). The

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