Short Report

High carrier frequency of 21-hydroxylase deficiency in Cyprus


Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is a common autosomal recessive disorder caused by mutations in the CYP21A2 gene. The carrier frequency of CYP21A2 mutations has been estimated to be 1:25 to 1:10 on the basis of newborn screening. The main objective of this study was to determine the carrier frequency in the Cypriot population of mutations in the CYP21A2 gene. Three hundred unrelated subjects (150 males and 150 females) from the general population of Cyprus were screened for mutations in the CYP21A2 gene and its promoter. The CYP21A2 genotype analysis identified six different mutants and revealed a carrier frequency of 9.83% with the mild p.Val281Leu being the most frequent (4.3%), followed by p.Qln318stop (2.5%), p.Pro453Ser (1.33%), p.Val304Met (0.83%), p.Pro482Ser (0.67%) and p.Met283Val (0.17%). The notable high CYP21A2 carrier frequency of the Cypriot population is one of the highest reported so far by genotype analysis. Knowledge of the mutational spectrum of CYP21A2 will enable to optimize mutation detection strategy for genetic diagnosis of 21-OHD not only in Cyprus, but also the greater Mediterranean region.

Conflict of interest
The authors declare no conflict of interest.

The most common form of Congenital adrenal hyperplasia (CAH) is due to 21-hydroxylase deficiency (21-OHD) that results from molecular defects in the CYP21A2 gene and the severe or moderate deficiency leads to the classic or non-classic (NC) CAH form, respectively. The incidence of the classic form is 1:10,000 to 1:15,000 depending on the population studied, while the NC-CAH occurs in a frequency of 1:500 to 1:100 live births with a high frequency especially between Ashkenazi Jews and in Mediterranean populations, such as Hispanics and Italians (1–4).

Clinical observations and functional studies have shown that large deletions, conversions, and some point mutations found on both chromosomes – p.Arg356Trp, p.Qln318stop – and the cluster p.Ile235Asn/p.Val236Glu/p.Met238Lys result in complete inactivation of the enzymatic activity and are associated with the severe salt wasting phenotype (5). Mutations that reduce the enzyme activity to 2%, for example p.Ile172Asn, are associated with the severe simple virilizing form (6, 7); whereas, defects such as p.Pro30Leu, p.Val281Leu and p.Pro453Ser that reduce the activity to 10–75% are linked to the mild NC-CAH phenotype (7). Several studies in the Mediterranean region identified p.Val281Leu, p.Qln318stop, IVS2-13A/C > G (c.655A/C > G) and Del8bpE3 (c.707_714delGAGACTAC) mutations as the most prevalent (8–12).

So far, the carrier incidence of CYP21A2 mutations in the general population has been estimated to be
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1:25 to 1:10 on the basis of newborn screening. Alternatively, two studies have determined CAH carrier frequency by CYP21A2 genotyping. One of the studies was performed in randomly chosen New Zealand neonates where they observed a frequency of 4.8% (1 in 21) (13) and the other one in a middle European population in which they revealed a higher carrier frequency of 9.5% (14).

These population studies however, do not represent the true carrier frequency in the Mediterranean region. Thus, this study aimed to establish the frequency and association of identified mutations in the CYP21A2 gene in the general population of Cyprus.

Materials and methods

Subjects

Three hundred adult individuals (150 males and 150 females) of Cypriot origin were recruited from the cohort of healthy individuals seeking biochemical evaluation prior to their marriage from the National Unit for Thalassemia at the Makarios Hospital in Nicosia, Cyprus. The numbers for both groups were calculated based on the inheritance recessive model of the disease. Informed consent was obtained from all individuals. Bioethics approval was received from the Cyprus National Ethics Committee.

Amplification of the CYP21A2 gene

Molecular analysis was performed according to a cascade strategy as previously described (12, 15).

MLPA analysis

DNA from all individuals were examined with the multiplex ligation-dependent probe amplification (MLPA) technique (MRC Holland, Amsterdam, Netherlands) (12, 16, 17).

Results and discussion

A cohort of 300 unrelated asymptomatic individuals (150 females and 150 males) were screened by DNA sequencing analysis and MLPA for mutations in the CYP21A2 gene and its promoter. The CYP21A2 genotypic analysis detected 32 male and 27 female mutated alleles in a total of 600 alleles. This accounts for a carrier frequency of 9.83% (approximately 1:10) (Table 1). The most frequent mutation among the tested subjects of this study was the mild p.Val281Leu (4.3%) followed by p.Qln318stop (2.5%), p.Pro453Ser (1.33%), p.Val304Met (0.83%), p.Pro482Ser (0.67%) and p.Met283Val (0.17%). The concurrent screening of the CYP21A2 promoter region in all samples did not reveal any unusual variants. Further genetic analysis of the 300 subjects by MLPA analysis did not detect any large gene deletions and/or conversions. In both the male and the female cohort, the overall frequency of p.Val281Leu was proven to be the highest. This explains the high incidence of p.Val281Leu in the pool of Cypriot NC-CAH patients which were reported in previous studies by our group (12, 16, 18).

In this study, the detected CYP21A2 true carrier frequency of the Cypriot population is the highest reported so far by genotypic analysis. A similar high CYP21A2 carrier frequency of 9.5% was also reported in a study that examined 200 Middle European individuals of Austrian and Yugoslav origin (14). The majority of defects detected in the Middle European population by Baumgartner-Parzer et al. (2005) were single nucleotide substitutions (6.5%) with the rest (3%) being the severe gene deletions/conversions. It is interesting to note that the so-called non classic mild mutations were the majority (5/6) with the exception of the severe causing p.Qln318stop. Additionally, no deletion or conversion of the CYP21A2 gene was identified in the Cypriot allelic pool. Interestingly, the allelic frequency of p.Val281Leu in the Cypriot

<table>
<thead>
<tr>
<th>Known CYP21A2 mutations</th>
<th>Number of mutated alleles</th>
<th>Percentage of alleles</th>
<th>Number of mutated male alleles</th>
<th>Number of mutated female alleles</th>
<th>Reported frequencies by Baumgartner-Parzer et al. (14) and Fitness et al. (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Val281Leu</td>
<td>26</td>
<td>4.33</td>
<td>11 (3.67%)</td>
<td>15 (5.0%)</td>
<td>2% Yugoslavs (14), 1.5% Austrian (14), 0.99% New Zealanders (13)</td>
</tr>
<tr>
<td>p.Qln318stop</td>
<td>15</td>
<td>2.50</td>
<td>7 (2.33%)</td>
<td>8 (2.67%)</td>
<td>0.5% Yugoslavs (14), 0.66% New Zealanders (13)</td>
</tr>
<tr>
<td>p.Pro453Ser</td>
<td>8</td>
<td>1.33</td>
<td>6 (2.0%)</td>
<td>2 (0.67%)</td>
<td></td>
</tr>
<tr>
<td>p.Val304Met</td>
<td>5</td>
<td>0.83</td>
<td>4 (1.33%)</td>
<td>1 (0.33%)</td>
<td></td>
</tr>
<tr>
<td>p.Pro482Ser</td>
<td>4</td>
<td>0.67</td>
<td>3 (1.0%)</td>
<td>1 (0.33%)</td>
<td></td>
</tr>
<tr>
<td>p.Met283Val</td>
<td>1</td>
<td>0.17</td>
<td>1 (0.33%)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>9.83</td>
<td>32 (5.33%)</td>
<td>27 (4.5%)</td>
<td></td>
</tr>
</tbody>
</table>
The allelic frequency of the severe p.Qln318stop detected in this study was one of the highest reported so far and accounts for 25.4% of the Cypriot CYP21A2 mutant alleles. The detected p.Qln318stop allelic frequency of this study is even higher than the 19% and 12.5% reported (22, 25) in Arab populations. Since none of the 300 subjects tested in this study were identified with any of the five severe [p.Pro30Leu, p.F306insT+p.Val281Leu, p.Ile172Asn, Del8bpE3 (c.707_714delGAGACTAC) and IVS2-13A/C->G (c.655A/C->G)] mutations previously identified in 11 Cypriot patients with severe CAH (12), an estimate of their prevalence was attempted by using the binomial distribution approach. The point estimate of the prevalence was found to be 0% with a 95% exact confidence interval (CI) between 0% and 1.22% (Table 2).

The fact that no other severe mutations apart from the p.Qln318stop were detected in the sample of 300 Cypriots who underwent genotypic analysis for CYP21A2 explains why the severe classical form of CAH is considered rare in the Cypriot population (12). The incidence of the severe classical CAH in the island is 1:30,000, which is much less than expected compared with other Mediterranean countries (26, 27). On the contrary, the incidence of the mild form of the disease (NC-CAH) in Cyprus is quite frequent and is estimated to be 1:400. This estimation is based on the detected approximate 1:10 allelic CYP21A2 carrier rate of this study.

In conclusion, this study demonstrates the frequency of the underlying genetic defects in the Cypriot population to be one of highest ever reported. This result can be considered as a small indication of the carrier frequency of CAH that exists within the Mediterranean region. Knowing the genetic defect is of immense help in the management and genetic counseling of the disease.
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References