INTRODUCTION

The steroid 21-hydroxylase deficiency compromises about 95% of cases of congenital adrenal hyperplasia (CAH) and has an over-all incidence for the classic form of about 1 in 13000 live births (1-3). Adrenal 21-hydroxylase is catalyzed by the cytochrome P450c21 encoded by the gene CYP21B (4, 5). The molecular genetics of P450c21 is unusual and often complex. Random deletions and de novo mutations almost never occur, instead, gene conversion accounts for about 85% of all mutant P450c21 alleles (6). Among conversions, all or part of the CYP21B gene is replaced by, or converted to, the sequence of the corresponding sequence of the nonfunctional CYP21A gene (3, 7, 8).

The adrenal cytochrome P450c21 catalyzes the 21-hydroxylation of progesterone to deoxycorticosterone and of 17-hydroxyprogesterone (17OHP) to 11-deoxicortisol. The 21-hydroxylase deficiency results in deficient synthesis of cortisol and aldosterone and increased synthesis of androgens (3, 8, 9). Despite the continuous spectrum of congenital adrenal hyperplasia due to steroid 21OHD, patients are classified in three different groups: 1) salt wasters (aldosterone deficiency and virilization), 2) simple virilizers (only virilization), and 3) non classical or late onset (mild signs of hyperandrogenism). The simple virilizing form of CAH (SV-CAH) results from a reduced activity by 92-98% of P450c21 due to mutations in the CYP21B gene (6, 7, 10). Recently, we demonstrated that ethnic differences may determine changes in the frequency pattern of muta-
tions in patients with the salt-wasting form of congenital hyperplasia (11). On the other side, the frequencies of mutations can also be altered when mutations in SV patients are reported together with the other forms of 21OHD.

The aim of this work was to establish the prevalence of the mutations in the CYP21B gene in a Chilean population including only patients with the SV-form of CAH and to compare these results with the frequencies of mutations found in other SV-populations of different genetic origin. The knowledge of the relative frequencies of the disease-causing mutations might be useful to delineate appropriate strategies for prenatal molecular diagnosis and genetic counseling for index cases and their relatives (12-14).

PATIENTS AND METHODS

Patients

Nineteen Chilean patients with SV-CAH (12 females and 7 males) were studied, as well as their parents, when available. These patients were unrelated and had no known consanguinity. Females presented different degrees of virilization at birth and males presented precocious puberty. All patients had high levels of 17-hydroxyprogesterone (25-300 ng/ml) and no evidence of salt-losing episodes (normokalemia and normonatremia). Informed consent was obtained from all participants according to the International Guidelines for Biomedical Research Involving Human Subjects, CIOMS, WHO, Geneva, Switzerland, 1982. The protocol was approved by the Research Commission of the School of Medicine at Catholic University of Chile.

Methods

Genomic DNA was isolated from the citrated blood of 19 unrelated SV-CAH patients and their parents as previously described (15). Genotyping was performed by allele specific-polymerase chain reaction (AS-PCR) as described by Wedell (16). A first PCR was carried out to amplify the CYP21B gene, using specific primers based on the 8 bp deletion in exon 3 present only in the pseudogene (CYP21A). By PCR reactions two fragments were obtained, one encompassing exon 1 to 3 and the other one exon 4 to 10 of the CYP21B gene. These fragments were used as template in a second round of amplification to detect the different mutations. For each mutated position, primers specific for normal and mutant alleles were used.

We studied the most frequent gene microconversions reported in Caucasian populations with the classical and non classical form of CAH: I2 splice: an A/C to G change in the second intron that produces an aberrant splice acceptor sequence, I173N: isoleucine to asparagine change at codon 173, V282L: valine to leucine change at codon 282, Q319X: glutamine to stop codon change at codon 319, R357W: arginine to tryptophan change at codon 357, F308insT: a T insertion at codon 308, Cluster E6: isoleucine-valine-methionine to asparagine-glutamine-lysine change at codons 237-238-240 respectively, P31L: proline to leucine change at codon 31, and P454S: proline to serine change at codon 454. All the samples were studied for each mutation. The complete absence of amplification for at least 5 mutations was interpreted as homozygous Del or LGC. Heterozygous CYP21B Del or LGC was inferred when the affected child appeared as homozygous for a given mutation but only one parent carried the mutation. However, the allele drop out could be a source of mis-genotyping that can not be disregarded. Positive controls for each mutation were used and kindly provided by Dr. Wedell. The sequence of all primers used and the PCR conditions were previously described (16). The parents genotypes were also analyzed to establish the segregation of the mutated allele. When the children were homozygous for a given mutation and the parents were not available, we considered one allele as an uncertain allele (17). The frequency of the different mutations was calculated taking into account the number of uncertain alleles involving the mutation (17).

RESULTS

We studied 38 chromosomes corresponding to 19 patients with SV-CAH and their parents (both parents were available for analysis in 68.4% of all cases). The mutated alleles were identified in 28 chromosomes (73.7%), 4 of them were uncertain alleles. The most frequent finding was the I173N mutation in 12/38 chromosomes (31.6%), followed by the V282L mutation in 4/38 chromosomes (10.5%) and the Del or the LGC in 3/38 (7.9%). We did not find alleles with the mutations: R357W, Cluster E6, P31L and P454S. The frequency of the mutations analyzed in this study, and the frequencies of the same mutations found in other populations, are shown in Table 1.

The complete genotype was determined in 11/19 patients (57.9%) and one allele in 6/19 patients (31.6%). The most frequent genotype found was homozygous I173N/I173N (4/19=21.1%), which could not be distinguished by this method from heterozygous for I173N/Del or /LGC because their parents were not available (uncertain alleles) and are cited in Table 2.