DOUBLE ASO-PCR TECHNIQUE FOR SCREENING OF *CYP21A2* PSEUDOGENE-DERIVATED MUTATIONS

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder most commonly due to 21-hydroxylase deficiency. In addition to the gene, *CYP21A2,* there is an inactive pseudogene, *CYP21A1P,* which is 98% homologous. Unequal crossing over can result in deletion of the gene, whereas gene conversion transfers deleterious point mutations from the pseudogene to the active gene, causing either complete or partial deficiency of 21-hydroxylase activity. Pseudogene-derived mutations account for the majority of 21-hydroxylase gene mutations. In the present study nine pseudogene-derivated mutations were investigated by a double allele-specific PCR in 100 Brazilian families, with at least one 21-hydroxylase deficiency patient. The most frequent mutation was IVS2-13A/C>G, a mutation that creates an alternative splice site in intron 2, and it is responsible for salt-wasting phenotype. Among simple virilizing patients the most frequent mutation was I172N in exon 4. Other mutations, P30L, 8 bp deletion, *cluster* 6, V281L, F306+1nt, Q318X and R356W, were less frequently observed, corroborating with other population distributions. Around 15% of the alleles presented more than one mutation indicating that gene conversion is not always a punctual process. In addition, 15% residual alleles remained undetermined after the analysis. The segregation of mutant alleles in families is important for both treatment and genetic counseling.

Key words: CAH, *CYP21A2*, *CYP21A1P*
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