Deletion of the C4-CYP21 repeat module leading to the formation of a chimeric CYP21P/CYP21 gene in a 9.3-kb fragment as a cause of steroid 21-hydroxylase deficiency.

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Abstract

Gross gene deletions have been reported in 20% of alleles in patients with congenital adrenal hyperplasia (CAH) involving a 21-hydroxylase deficiency (1). This type of deletion occurs in the RCCX module, including the CYP21P, tenascin A (TNXA), RP2, C4B, CYP21, and tenascin B (TNXB) genes, as evidenced by a 30-kb deletion identified by pulse-field electrophoresis (2). Inactivation of the CYP21 gene may also occur through intergenic recombination with transfer of deleterious mutations from the neighboring CYP21P pseudogene. The frequency of gene deletions or conversions in CAH is controversial (3)(4)(5) and is dependent on the population studied. Evidence for gene deletions and/or conversions is traditionally obtained by Southern blot analysis. Multiple probes and separate restriction endonuclease digestions are used. TaqI generates 3.7-kb (functional) and 3.2-kb (pseudogene) fragments, and BglII produces 11-kb (functional) and 12-kb (pseudogene) fragments. These analyses have been used since 1984 (1)(3)(5)(6)(7)(8)(9). However, the method is indirect and time-consuming, and densitometry of fragments can be prone to error.

To identify the interchange region and improve detection of gene deletions and conversions in the RCCX module (10)(11)(12)(13), we have developed a novel Southern blot analysis that uses two restriction endonucleases, AseI and NdeI, and requires only one probe. In addition, we use a PCR product amplified with locus-specific primers covering the TNXB gene to the 5' end of CYP21P or CYP21 to directly analyze the 3.2/3.7-kb TaqI fragment and the status of the CYP21 gene.