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Identification of a common molecular basis for combined 17α-hydroxylase/17,20-lyase deficiency in two Mennonite families

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Summary. During the course of studies to characterize mutations of the CYP17 gene that cause the 17α-hydroxylase-deficient form of congenital adrenal hyperplasia we have discovered two ostensibly unrelated Mennonite families in which affected individuals are homozygous for the same mutation. The defect is a four-base duplication in exon 8 of the CYP17 gene, which alters the reading frame encoding the C-terminal 26 amino acids of cytochrome $P450_{17\alpha}$.

Introduction

A relatively rare cause of congenital adrenal hyperplasia, 17α-hydroxylase deficiency results in impaired production of cortisol, androgens, and estrogens, as well as elevated levels of mineralocorticoids, in particular, 11-deoxycorticosterone (New et al. 1983). The elevated mineralocorticoid production results in low-renin hypertension while the reduced level or absence of sex hormones leads to sexual infantism in females and ambiguous genitalia or pseudohermaphroditism in males (Biglieri et al. 1966; Dean et al. 1984). We have recently undertaken elucidation of the molecular basis of individual cases of 17α-hydroxylase deficiency with the aim of better understanding the structure-function relationships in human $P450_{17\alpha}$ and have recently reported a Canadian patient having a 46,XY karyotype and a female phenotype (Winter et al. 1989) who was found to have a four nucleotide duplication in exon 8 (Kagimoto et al. 1988) of her CYP17 gene (Nebert et al. 1989). This mutation results in an altered reading frame encoding the C-terminal 26 amino acids of the $P450_{17\alpha}$, which renders the resultant protein inactive. In the course of investigating additional examples of 17α-hydroxylase deficiency, we have identified a second Canadian patient, ostensibly unrelated to the first, who has the same four-base duplication in exon 8 and thus the same molecular basis of her disease.

Patients and methods

The patient has a 46,XY karyotype with a female phenotype and is proband VI-1 of family A in the study by Winter et al. (1989). This family is consanguineous and of Mennonite origin now living in Canada. The previous Canadian patient having 17α-hydroxylase deficiency characterized in this laboratory (Kagimoto et al. 1988) was proband VI-4 of family B (Winter et al. 1989), also a consanguineous family of Mennonite origin. Southern analysis of DNA from the present patient (VI-1A) indicated a typical human CYP17 gene having *Eco*RI fragments of 6.9 kb and 5.7 kb (Kagimoto et al. 1988). Following the procedure described previously by this laboratory, these fragments were cloned into λgtWES, subcloned into pUC19, and the 8 exons of the CYP17 gene were sequenced by the Sanger dideoxy procedure (Sanger et al. 1977). Exactly the same mutation as found in the previous Canadian 17α-hydroxylase-deficient individual (Kagimoto et al. 1988) was identified (Fig. 1).

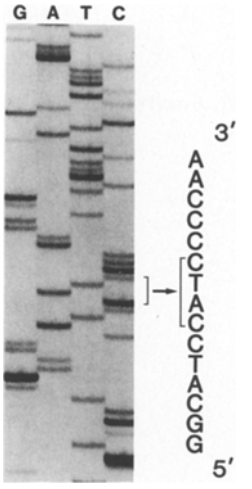

Fig. 1. Region of the sequencing ladder of exon 8 of the CYP17 gene of the patient described in this paper. The four-base duplication (CATC) is indicated and is found at the position of amino acid 480 of $P450_{17\alpha}$

Discussion

The two affected Mennonite families do not know each other, and reside more than 1,000 miles apart in Canada. In each family the pattern of inheritance was typical of an autosomal recessive disorder; both of the patients studied by gene sequencing were homozygous for the four-base duplication in exon 8 of the CYP17 gene. The Mennonites are a highly endogamous sect, which originated in the Netherlands as part of the 16th century Anabaptist reform movement. In the face of religious

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persecution they moved through the Rhineland to Prussia, and then in 1789 to the Ukraine, but in each locale intermarriage with, or proselytization of, their neighbors was restricted. The ancestors of both affected families migrated to Canada from the Ukraine early in this century. Although ties between the two families cannot be confirmed, they share several surnames. Based on the consanguinity present in both families, it seems likely that the four-base duplication arose within the Mennonite population prior to emigration to Canada, and its present expression represents a founder effect. With the development of suitable family-specific probes, detailed pedigree analysis will be possible; presumably this gene for combined 17α-hydroxylase/17,20-lyase deficiency will be found in other members of this religious sect.

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References

Biglieri EG, Herron MA, Brust N (1966) 17-Hydroxylation deficiency in man. J Clin Invest 45:1946–1954

Dean HJ, Shackleton CHL, Winter JSD (1984) Diagnosis and natural history of 17-hydroxylase deficiency in a newborn male. J Clin Endocrinol Metab 59:513–520

Kagimoto M, Winter JSD, Kagimoto K, Simpson ER, Waterman MR (1988) Structural characterization of normal and mutant human steroid 17α-hydroxylase genes: molecular basis of one example of combined 17α-hydroxylase/17,20-lyase deficiency. Mol Endocrinol 2:564–570

Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R, Waterman MR (1989) The P450 superfamily: update on listing of all genes and recommended nomenclature of the chromosomal loci. DNA 8:1–13

New MI, DuPont B, Grumbach K, Levine LS (1983) Congenital adrenal hyperplasia and related conditions. In: Stanbury JB, Wyngarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 973–1000

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Winter JSD, Couch RM, Muller J, Perry YS, Ferreira P, Baydala L, Shackleton HL (1989) Combined 17-hydroxylase and 17,20-desmolase deficiencies: evidence for synthesis of a defective cytochrome P-450$_{C17}$. J Clin Endocrinol Metab 68:309–316

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