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HUMAN CYP11B2 (ALDOSTERONE SYNTHASE) MAPS TO CHROMOSOME 8q24.3

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ABSTRACT. Aldosterone synthase (AS) is encoded by the CYP11B2 gene, a candidate for familial hypertension. CYP11B2 was previously mapped to chromosome 8q but its precise localization is necessary for genetic studies of hypertension. The present study reports the genetic mapping of the human CYP11B2 gene by radiation hybrid (RH) analysis, the isolation of a bacterial artificial chromosome (BAC) containing this gene and its physical mapping by fluorescent *in situ* hybridization (FISH). The CYP11B2 locus is on the most distal segment of the long arm of chromosome 8, proximal to the microsatellite polymorphic marker D8S1704. This location, which was confirmed by FISH, is approximately 60cM telomeric to the currently listed human gene locus (chromosome 8q21-22) and corresponds to cytogenetic band 8q24.3. The BACs containing the gene and a high-resolution map of the CYP11B2 locus are useful for genetic studies of hypertension and other endocrine disorders.

There are two distinct 11β–hydroxylase (P450c11)' genes in man: CYP11B1 and CYP11B2 which code for proteins with 93% homology (1). Despite their similarity in the exonic sequences, the two genes have strikingly different promoters (2). This difference accounts for their variable expression: The product of CYP11B1, which demonstrates exclusively P450c11 activity, is regulated by adrenocorticotropin (ACTH), whereas, the product of the CYP11B2 gene, which has primarily 18-hydroxylase activity, functions as an aldosterone synthase (AS) (3). AS catalyzes the last three steps of aldosterone biosynthesis in the *zona glomerulosa*. Inactivating mutations of CYP11B2 lead to mineralocorticoid deficiency in the corticosterone methyloxidase types I and II (CMO-I and -II, respectively) syndromes (4), whereas increased activity of the enzyme causes glucocorticoid-remediable hypertension (GRA) (5) and perhaps other forms of familial hypertension (6).

Because of their sequence similarities, CYP11B1 and CYP11B2 are considered to arise from the duplication of one ancestral gene that had both P450c11 and AS activities (7). This is supported by the close genomic proximity of the two genes [approximately 40,000 basepairs (bp)] and their almost identical structure (7), which facilitate their unequal crossing-over observed in the majority of patients with GRA (5). The CYP11B1/CYP11B2 genomic locus was mapped to chromosome 8q21-22 (7-9), in a wide area covering 70 centiMorgans (cM) of genetic distance (equivalent to genomic distance of approximately 70×10^6 bp), as estimated by the currently available maps of human chromosome 8q (10).

The two genes were mapped more than 10 years ago (7). First, Chua *et al.* (8) mapped the CYP11B1 cDNA by *in situ* hybridization to a human metaphase chromosome spread. The distribution of signals was widespread on chromosome 8q, covering an area as wide as from 8q13 to 8q24; because this distribution centered on 8q21-22, the authors concluded that this was the location of the CYP11B1 gene (8). The CYP11B2 gene was mapped to 8q22, as well (7,9). However, two lines of evidence suggested that the CYP11B1 /CYP11B2 locus may be positioned further telomeric on chromosome 8. First, a hybrid cell mapping panel, that was used for regional localization of probes for this chromosome, mapped a CYP11B1 probe to 8q22-8qtel (11). Second, the mouse Cyp11b1 and Cyp11b2 genes were mapped to mouse chromosome 15 where other loci from human chromosome 8q24tel have been mapped

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(12). Conversely, genes located in the human 8q11 to 8q22 genomic area have been mapped to mouse chromosomes 3 and 4 (12).

We have recently initiated a genome-wide screen for the identification of the genetic loci involved in familial hyperaldosteronism type-II (FH-II) (13). Using a CYP11B2 probe (5) and surrounding polymorphic markers, we excluded this gene from being a candidate for FHA-II (Torpy D, Stratakis CA, *in preparation*). However, the pattern of inheritance of genotypes obtained for polymorphisms covering the long arm of chromosome 8, compared with those obtained from the intragenic probe, suggested that CYP11B2's location is further telomeric than previously thought. Thus, we sought to determine the precise genetic and physical localization of CYP11B2 by high resolution mapping methods that have not previously been used for this gene.

Materials and Methods

Radiation hybrid (RH) analysis

The 10,000 rad SHGC G3 radiation hydrid (RH) panel was utilized to determine the location of the CYP11B2 gene with regards to markers mapped to chromosome 8q (14). PCR was performed with primers from two amplicons of the CYP11B2 gene (5), one from the 5' region of the gene (primer pair ALDOXI/ALDOX2), and another, containing exon 9 of the gene (primer pair ALDOX9/ALDOX10). The RH mapping data were submitted, without prior chromosomal assignment, to the Stanford Human Genome Center (SHGC) radiation hybrid server to determine the most closely linked, previously mapped markers, as previously described (14,15. Chromosome 8 polymorphic marker D8S1704 (10) was also used for RH mapping.

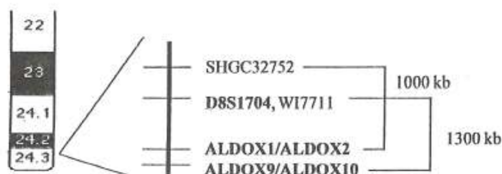


Figure 1. Ideogram of the long arm of chromosome 8, with G bands, and radiation hybrid map of markers closely linked to CYP11B2 in 8q24.3. Markers mapped by RH analysis are indicated in bold. AldoXI/AldoX2 and AldoX9/AldoX10 are two amplimers of the CYP11B2 gene (5). The previously reported position for the CYP11B2 gene was at 8q22 (7, 8, 10). Markers D8S1704 and W17711 were tightly linked and a physical distance between them could not be determined. Approximate physical distances were calculated by the SHGC RH server (14) and converted to kb using the suggested chromosome 8-specific factor of $1cR_{10,000}$=36.5kb.

Identification of a Bacterial Artificial Chromosome (BAC) containing the CYP11B2 gene

BACs 118-A-13 and 316-F-21, containing the ALDOX9/ALDOXIO and D8S1704 amplicons, respectively, were obtained from a commercially available (Research Genetics, Birmingham, AL) library, as previously described using the primer pairs mentioned above (16) (data not shown). The BACs were grown overnight in 1L medium, centrifuged and used for DNA extraction, as previously described (16). This DNA was then labelled for use in fluorescent *in situ* hybridization.

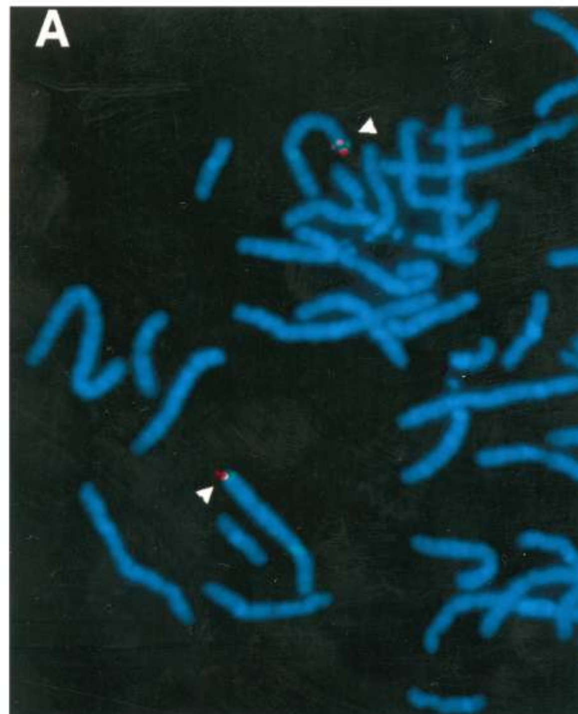
Fluorescent in situ hybridization (FISH)

FISH was performed following protocols described by Pinkel et al (17) with minor modifications (18).

Results

Radiation hybrid mapping

The ALDOXI/ALDOX2 amplicon was most closely linked to the chromosome 8qtel markers SHGC-32752, with a LOD of 7.08 and an estimated distance of 28.27 $cR_{10,000}$ [1000 kb for chromosome 8, (19)].



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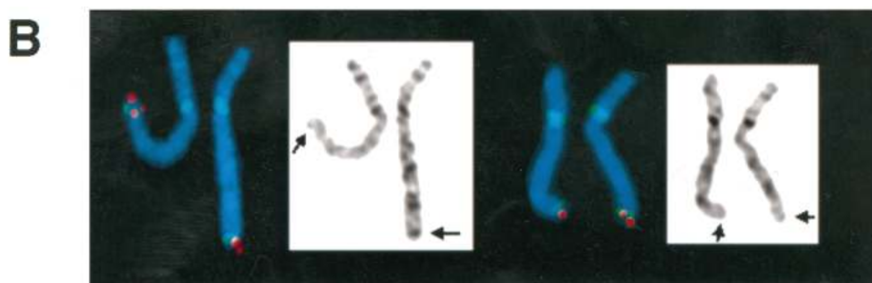

Figure 2. (A) DAPI-counterstained chromosomes in metaphase showing the location of BACs 316-F-21 (containing D8S1704, green) and 118A-13 (containing ALDOX9/ALDOXIO, red) on the distal long arm of chromosome 8. The two chromosome 8 homologues are identified by the positive α-satellite signals (green). B. Examples of chromosome 8 three color images (left) which were converted to black and white "G-band" images (right) to show the relative position of the probes to the telomeric end of the long arm of chromosome 8. The position is identified as 8q24.3, with the order cen-316-F-21 (D8SI704)-118-A-13 (ALDOX9/ALDOX10).

The ALDOX9/ALDOX10 amplicon was most closely linked to the chromosome 8qtel STS D8S1704, with a LOD score of 6.63 and an estimated distance of 35.71 $cR_{10,000}$ (1300 kb for chromosome 8). These markers are the two most telomeric framework markers on the SHGC RH map (14), and are very telomeric to the previously reported location of CYP11B2 at 8q21-22 (Figure 1). To further verify the telomeric location of the CYP11B2 gene, we checked if the linked marker, D8S1704, mapped telomerically in our hands. The analysis indicated that this marker is identical to the telomeric marker WI-7711 (LOD=1000, $CR_{10,000}$= 0) (Figure 1).

BACs containing the CYP11B2 gene and FISH analysis

Two BACs were identified (118-A-13, and 316-F-21) from a commercially available library (data not shown). These BACs can be used for further identification of the genomic structure of the CYP11B1/CYP11B2 locus. FISH was then performed on metaphase chromosomes using the BAC probes combined with the control centromeric chromosome 8 α-satellite (Figure 2). A total of 25 cells were examined. In all metaphases scored, clear signals were seen on the long arm of chromosome 8 (Fig. 2A). DAPI banding unambiguously showed the position of the signal in the region 8q24.3 (Fig 2B).

Discussion

The CYP11B2 gene codes for the main adrenal enzyme responsible for aldosterone biosynthesis (7); thus, it has long been considered a candidate gene for familial hypertension (5, 7, 20).

Several genes that had been mapped prior to the advent of contemporary mapping methods were localized in the genome by Southern blotting of a few somatic cell hybrids or by *in situ* hybridization. Most of these genes are currently reassigned in the new maps and are associated with existing markers (21).

Indeed, the CYP11B2 gene was mapped to chromosome 8q by older methods (7-9, 11). Although the originally reported locus was 8q22 (7-9), a CYP11B1 probe was mapped to 8q22-tel (11). The mouse homologues of the two genes were mapped to mouse chromosome 15, where all of the mouse homologues to human 8q24-tel genes lie (12). In our study, RH analysis provided the exact location of the CYP11B2 gene, linking it with the polymorphic D8S1704 marker. The latter is located in the 8q24-tel region (10). FISH confirmed the placement of CYP11B2 in the most telomeric region of chromosome 8, distal to the currently available RH and physical map of human 8q24 (22, 23). The latter end with framework markers D8S272 and D8S263, respectively. Since both

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these markers reside on contig WC.8.9 of human chromosome 8 at approximately 150cM genetic distance from the top of that chromosome, and D8S1704 is located 10cM further telomeric, the CYP11B2 gene should lie on the most distal contig WC.8.10 (10). The latter is very small and consists only of two anonymous markers. Additional work is needed to investigate this area of human chromosome 8, for which there is a paucity of information most likely due to repetitive sequences and other features of human telomeres (24).

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