SHORT COMMUNICATION

Mapping of the *CYP2J* Cytochrome P450 Genes to Human Chromosome 1 and Mouse Chromosome 4

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CYP2J subfamily cytochromes P450 catalyze the NADPH-dependent oxidation of arachidonic acid to several unique eicosanoids that possess numerous biological activities including modulation of ion transport, control of bronchial and vascular smooth muscle tone, and stimulation of peptide hormone secretion. We have identified sequence variants in the 3′ untranslated regions of two mouse *Cyp2j* genes (*Cyp2j5* and *Cyp2j6*) and used a PCR-based oligonucleotide hybridization assay to map both genes to the central region of chromosome 4 distal to the *Jun* oncogene. The corresponding human *CYP2J* gene (*CYP2J2*) has been assigned to human chromosome 1 on a panel of somatic hybrid cell lines and to 1p31.3–p31.2 by fluorescence *in situ* hybridization analysis. The proximity of the *Cyp2j* cluster to the *Cyp4a* cluster suggests that these genes may be part of a cassette of P450 genes involved in the oxidation of fatty acids. © 1998 Academic Press

The cytochromes P450 (P450) comprise a large gene superfamily that encodes over 480 distinct heme-thiolate proteins that catalyze the metabolism of a wide variety of xenobiotics and are responsible for the bioactivation of numerous endogenous compounds including fatty acids (1, 5, 11). The recently described CYP2J subfamily contains several members that have a wide tissue distribution and are active in the NADPH-dependent metabolism of arachidonic acid to *cis*-epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs) (8, 14–16, 21). These eicosanoid products have been shown to have potent biological effects in tissues and cells where *CYP2J* genes are constitutively expressed. For example, CYP2J isoforms are highly expressed in the airway where the EETs modulate bronchial smooth muscle tone and lung transepithelial ion transport (12, 17, 18). In the pancreas, CYP2J isoforms are highly localized to islets of Langerhans cells where the EETs mediate insulin and glucagon release (19). CYP2J isoforms are enriched in cardiac myocytes where one of the EETs protects against the functional consequences of global ischemia (15). CYP2J P450s are present at high levels in intestinal vascular endothelium where both the EETs and 19-HETE have been shown to be vasoactive (9, 13, 20). Two *CYP2J* genes have been identified in mouse (*Cyp2j5* and *Cyp2j6*) (J. Ma and D. C. Zeldin, unpublished observations) and rat (*CYP2J3* and *CYP2J4*) (15, 21), whereas only a single gene has thus far been identified in humans (*CYP2J2*) (14).

We now report the mapping of two mouse *Cyp2j* genes using PCR-based genotyping methods as recently described for other mouse genes (2–4). Compatible primers corresponding to nucleotides 1373–1399 (2J5-1F 5′-AAGAGCTTGTCTTGGAGAACAGTTGGC-3′) and 1804–1833 (2J5-1R 5′-CCATTTCCTCTGATTCTGACTCATATAAGA-3′) of the CYP2J5 cDNA (GenBank Accession No. U62294) and nucleotides 1579–1602 (2J6-1F 5′-GCGAGCTTGCCTGGGAGAACAACT-3′) and 1993–2022 (2J6-1R 5′-CAATTCAATTTAATGCACTTTGTTTGGAAG-3′) of the CYP2J6 cDNA (GenBank Accession No. U62295) were synthesized. PCR of C57BL/6J mouse genomic DNA using standard conditions (3) yielded amplification products of the expected size (~450 bp), indicating that no intron interrupts these regions of the mouse *Cyp2j* genes. These PCR products incorporate 174 nucleotides of 3′-end coding regions plus nearly the entire 3′-untranslated regions of the CYP2J5 or CYP2J6 cDNAs. PCR products amplified from C57BL/KsJ *m/m* and *Mus musculus musculus* Czech II inbred strain genomic DNAs were sequenced directly using the same PCR primers (3). Single base substitutions in the 3′-untranslated regions of *Cyp2j5* and *Cyp2j6* were identified in *M. m. musculus* Czech II PCR products. *M. m. musculus* Czech II strain-specific oligonucleotides (2J5-CzSp 5′-GAAGCAGAGCTTGAAGTT-3′, corresponding to nucleotides $\overline{1}$469–1486 of the CYP2J5 cDNA, and 2J6-CzSp 5′-TCCCTAGAGAGTGATGC-3′, corresponding to nucleotides 1740–$\overline{1756}$ of the CYP2J6 cDNA) that differed by 1–2 nucleotides (underlined) and 6–8°C in estimated melting temperature from the C57BL/KsJ *m/m* allele sequences were synthesized. PCR products of genomic DNAs from parental (C57BL/KsJ *m/m* and

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M. m. musculus Czech II) and individual intersubspecific backcross (N_2) progeny were prepared using the original primers (2J5-1F, 2J5-1R, 2J6-1F, and 2J6-1R). These products were denatured and slot-blotted onto Zeta-Probe nylon membranes (Bio-Rad Laboratories). Genotyping based upon allele-specific oligonucleotide (ASO) hybridization to the *M. m. musculus* Czech II *Cyp2j5* and *Cyp2j6* alleles was unambiguous for all 158 N_2 progeny (data not shown). Haplotype analysis of the *Cyp2j5* and *Cyp2j6* loci was performed using MapManager software. Both genes map to the same subchromosomal interval distal to *Jun* and proximal to *misty* (*m*) on mouse Chromosome 4 (10) as shown in Fig. 1. These two closely related genes may have arisen as a result of a gene duplication event, as has been inferred for other P450 gene superfamily members that map near each other (11).

Comparative mouse–human chromosomal mapping predicts that the human *CYP2J2* gene is likely to map to human chromosome 1. To identify the chromosomal localization of the *CYP2J2* gene, we performed Southern blot analysis of genomic DNA from hamster–human and mouse–human somatic cell hybrids (BIOS Laboratories, New Haven, CT) with the 1.89-kb CYP2J2 cDNA probe (GenBank Accession No. U37143) according to the manufacturer's instructions (14). The hybrid cell DNA was digested with *Eco*RI, which provided substantially different hybridization patterns between human and rodent DNA. A series of 20 human–rodent hybrids was examined, and the human *CYP2J2* gene segregated concordantly with chromosome 1 and discordantly (>15%) with all other human chromosomes. To confirm this result independently and to define the chromosomal localization of *CYP2J2* more precisely, fluorescence *in situ* hybridization (FISH) analysis was performed using the 1.89-kb CYP2J2 cDNA probe that was labeled with biotinylated dATP using the BRL BioNick labeling kit and visualized with fluorescein isothiocyanate conjugated to avidin (Vector Laboratories) (6, 7). As shown in Fig. 2, the *CYP2J2* locus mapped to human chromosome 1, short arm, region 31.2–31.3 (1p31.3–p31.2).

In those instances that have been examined thus far, genes within a given P450 subfamily have been found to be closely linked (11). Our results, which demonstrate that *Cyp2j5* and *Cyp2j6* are located within the same region of mouse chromosome 4 (*Cyp2j* cluster), support and extend these earlier studies. Although P450 genes are spread throughout the genome, comparative gene mapping shows that considerable synteny exists between mouse and human (11). The assignment of the *Cyp2j* cluster and the *CYP2J2* locus to mouse chromosome 4 and human chromosome 1p, respectively, provides further evidence to support the synteny between these mouse and human chromosomal regions (10). Interestingly, *Cyp2j5* and *Cyp2j6* map to a region of chromosome 4 that is very close to mouse *Cyp4a10* and *Cyp4a12* (*Cyp4a* cluster) (11). Similarly, the human *CYP2J2* and *CYP4B1* genes appear to be closely linked on chromosome 1, and the human orthologue of mouse *Cyp4a10* (*CYP4A11*) has also been mapped to chromosome 1 (11). Thus, it appears as though P450 genes of the *CYP2J*, *CYP4A*, and *CYP4B* subfamilies are closely linked on homologous regions of mouse chromosome 4 and human chromosome 1. Members of the CYP2J and CYP4A subfamilies

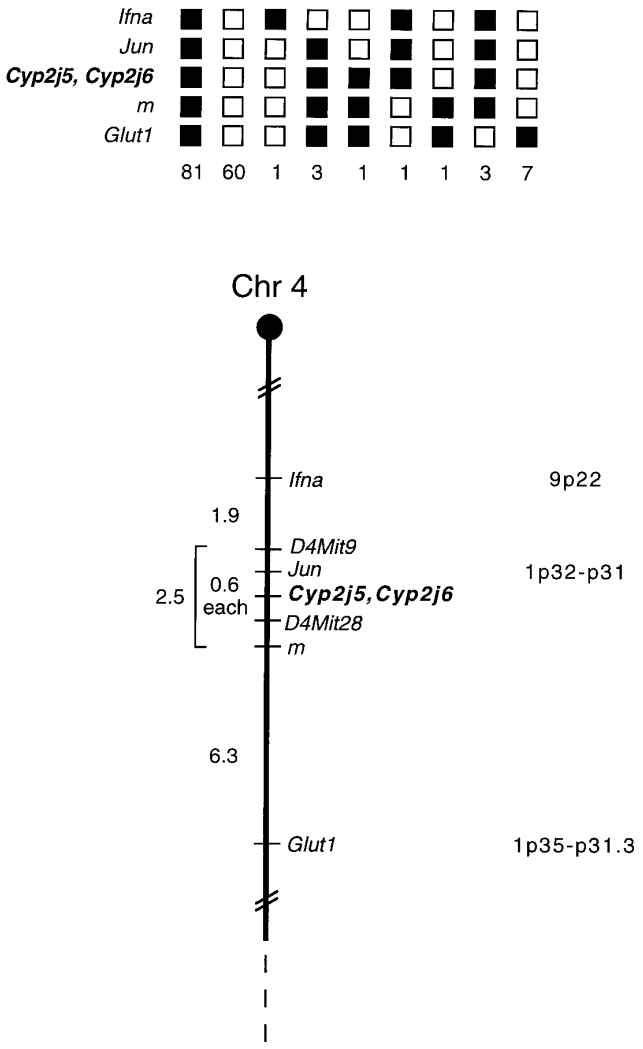

FIG. 1. Haplotype data and genetic linkage map for *Cyp2j5* and *Cyp2j6* and nearby mouse chromosome 4 loci. The segregation patterns of *Cyp2j5* and *Cyp2j6* and linked genetic loci in a C57BL/KsJ × (C57BL/KsJ × *M. m. musculus* Czech II)F_1 intersubspecific backcross consisting of 158 backcross animals are shown. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/KsJ × *M. m. musculus* Czech II)F_1 parent. Black boxes represent the presence of a *M. m. musculus* Czech II allele, and open boxes represent the presence of a C57BL/KsJ allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. These two *Cyp2j* loci map to the central region of mouse chromosome 4 as shown in the schematic linkage map of this region. The map positions of two nearby PCR-based markers (D4Mit9 and D4Mit28) are also shown to allow mapping comparisons in other extensively characterized backcrosses. Recombination distances between loci in centimorgans (1 cM = 1% recombination) are shown to the left of the chromosome, and approximate positions of homologous loci on human chromosomes are shown to the right.

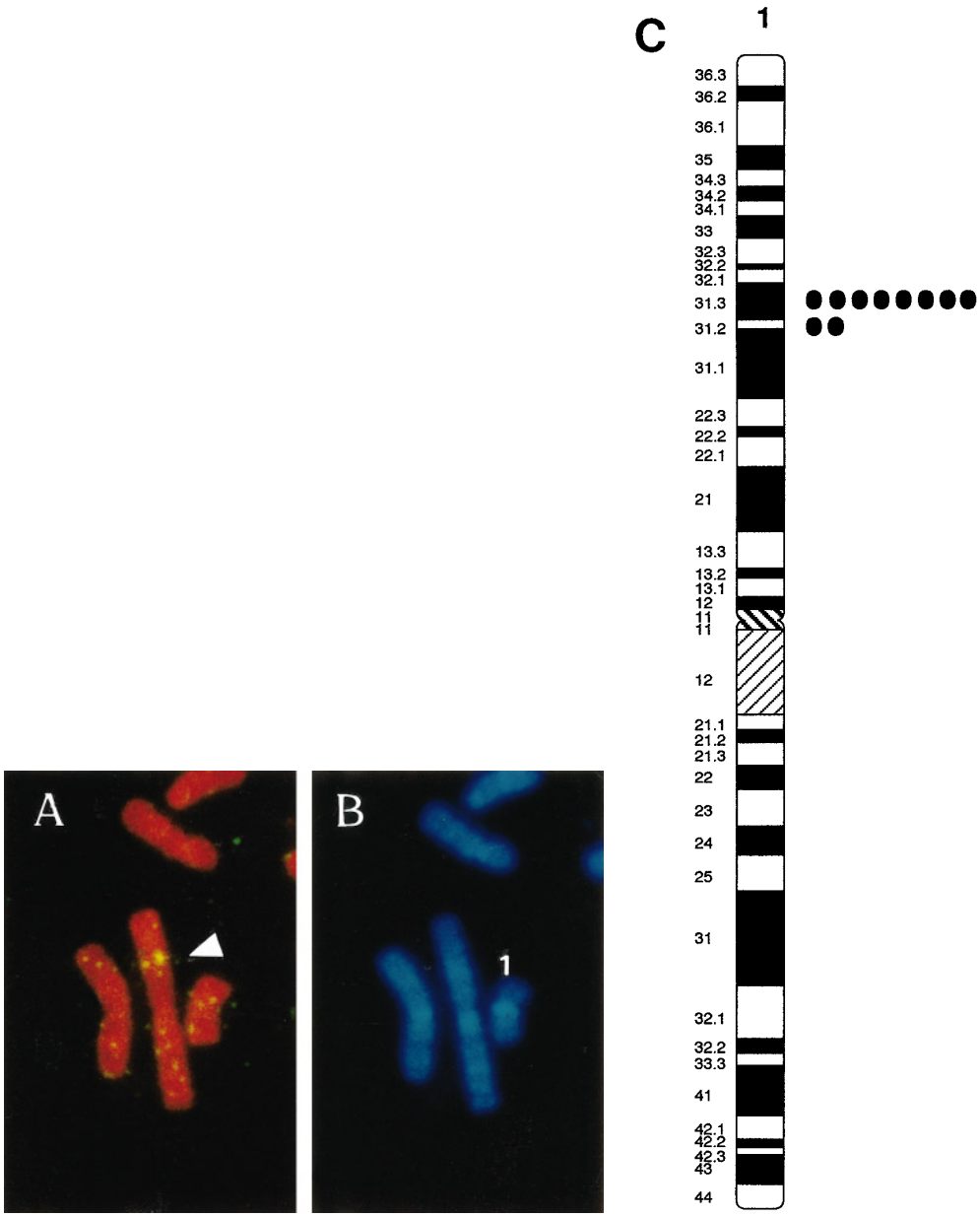

FIG. 2. Fluorescence *in situ* hybridization analysis of *CYP2J2*. The procedure for FISH detection was performed exactly as described in Refs. (6) and (7). (**A**) FISH signals on a chromosome. Under the conditions used, the hybridization efficiency was 84% for the CYP2J2 cDNA probe (i.e., among 100 examined mitotic figures, 84 showed signals on one pair of the chromosomes). (**B**) The same mitotic figure stained with DAPI to identify it as chromosome 1. (**C**) The detailed position of CYP2J2 on chromosome 1. Each dot in the diagram represents the double FISH signals detected on human chromosome 1 based on the summary from 10 photographs. There was no additional locus picked by FISH detection under the conditions used.

have been shown to catalyze the oxidation of fatty acids to form eicosanoids that possess numerous biological activities including modulation of ion transport, control of bronchial and vascular smooth muscle tone, and stimulation of peptide hormone secretion. The close proximity of the *Cyp2j* cluster to the *Cyp4a* cluster suggests that these genes may be part of a cassette of P450 genes involved in the bioactivation of fatty acids.

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