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The Gene *CYP3* Encoding P450PCN1 (Nifedipine Oxidase) Is Tightly Linked to the Gene *COL1A2* Encoding Collagen Type I Alpha on 7q21-q22.1

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Summary

CYP3, the gene which encodes the hepatic cytochrome P450pcn1, the isozyme responsible for the metabolic oxidation of the calcium channel–blocking drug nifedipine, has recently been mapped to human chromosome 7 using somatic cell hybrids. Using multilocus linkage analysis in CEPH families, we examined the linkage of a cDNA probe (hPCN1) for _CYP3_ to the oncogene _MET_, the pro-alpha 2(1) collagen gene _COL1A2_, and the T-cell receptor beta-chain gene _TCRB_, together with three arbitrary loci _D7S8_, _D7S13_, and _D7S16_, defined by the anonymous DNA probes pJ3.11, pB79a, and p7C22, respectively. From 70 CEPH parents screened with a _Sty_I RFLP for hPCN1, four informative families were found each with both parental and maternal grandparents and 6–11 children per family. Tight linkage emerged between _CYP3_ and _COL1A2_, with a maximum combined lod score of 5.72 at $\theta = 0$, suggesting the most likely subchromosomal localization of _CYP3_ is 7q21.3-q22.1.

Introduction

The cytochromes P450 (P450s) are a family of ubiquitous enzymes which catalyze the mono-oxygenation of a broad array of both xenobiotic chemicals and endogenous substrates. Eight gene families each containing between 2 and 20 discrete cytochromes constitute the P450 gene superfamily in mammals (Nebert and Gonzalez 1987). The highest concentration of many P450s is found in the endoplasmic reticular membrane of hepatic parenchymal cells. These enzymes have attracted considerable interest because of the principal role which they play both in the metabolic detoxication of drugs and foreign chemicals and in chemical mutagenesis and carcinogenesis (Boobis et al. 1985).

Genetic polymorphism of P450s emerged about a decade ago through the recognition of individuals with inherited deficient metabolism of debrisoquine (Mahgoub et al. 1977) and sparteine (Eichelbaum et al. 1979), two drugs metabolized by the hepatic isozyme P450db1 (Dayer et al. 1987). About 1 in 10 persons in the United Kingdom is a recessive poor metabolizer of debrisoquine and sparteine (Evans et al. 1980, 1983), apparently owing to incorrect splicing of the P450db1 pre-mRNA (Gonzalez et al. 1988*b*). This polymorphism explains in various degrees the aberrant pharmacokinetics of and inappropriate responses to many drugs in some patients (Idle and Smith 1979, 1984; Kuper and Preisig 1983; Eichelbaum 1984), and there is also growing evidence that the P450db1 polymorphism underlies individual risk in cigarette smokers of both bronchial carcinoma (Ayesh et al. 1984; Ayesh and Idle 1985; Caporaso et al. 1987) and bladder cancer (Kaisary et al. 1987).

Nifedipine is a drug used widely in the treatment of angina pectoris and hypertension, by virtue of blockade of calcium entry into cardiac and smooth-muscle cells. Recently there have been suggestions that its metabolism is polymorphic in man (Idle and Sever 1983; Kleinbloesem et al. 1984), although the original observations have lacked confirmation (Schellens et al. 1986).

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Nifedipine is metabolized to pharmacologically inert products by the human orthologue of two highly homologous members of the rat P450III gene family (Gonzalez et al. 1986), hepatic isozyme P450pcn1, which was demonstrated conclusively by the expression of the human P450pcn1 cDNA (hPCN1) in monkey kidney fibroblasts (Cos-1 cells) (Gonzalez et al. 1988*a*). Two other human cDNAs have been isolated which show high sequence homology to rat P450pcn1, one of which shows 11 amino acid substitutions (Molowa et al. 1986) compared with hPCN1 and the other of which is virtually identical to hPCN1 (Beaune et al. 1986). By use of a panel of somatic cell hybrids, hPCN1 was localized to chromosome 7 (Gonzalez et al. 1988*a*). Interestingly, in one hybrid and its subclone which had lost the *MET* oncogene, previously mapped to 7q31 (White et al. 1985), no hybridization of hPCN1 occurred (Gonzalez et al. 1988*a*), providing the first evidence that *CYP3*, the gene encoding P450pcn1, might be linked to the *MET* gene.

In order to understand more clearly the molecular genetics of nifedipine oxidase, we have subchromosomally mapped the gene to 7q22 using Centre d'Etude Polymorphisme Humain (CEPH) pedigrees by showing tight genetic linkage between hPCN1 and a collagen gene *COL1A2* with a combined lod score of 5.72 at a recombination frequency (θ) of 0.

Material and Methods

Description of the Probe

A human P450pcn1 clone, designated hPCN1, was isolated from a human liver λgt11 library (Watson and Jackson 1985) by screening with a rat P450pcn1 cDNA (Gonzalez et al. 1986). A 1-kb 3′ *Eco*RI fragment cloned in pUC9, oligolabeled (Fleinberg and Vogelstein 1984) with ^{32}P to a specific activity of $>10^8$ cpm/μg and which detected a *Sty*I polymorphism, was used in the analysis.

Family Studies

Eleven CEPH families of the 35 screened were informative in the linkage studies with hPCN1. These CEPH families had previously been typed for several markers on 7q (table 1). For this study, DNA samples were digested with *Sty*I, fractionated on agarose gels, transferred onto nylon filters, hybridized, and washed in 0.1 × SSC at 65 C. Pairwise lod scores were calculated for hPCN1 versus selected 7q gene markers, using the LINKAGE program (Lathrop et al. 1984). Multipoint likelihoods were subsequently calculated and presented as location scores (Lathrop et al. 1984), based upon chromosome 7 map distances (Lathrop et al. 1988).

Table 1

Details of Markers Used

Locus	Probe	Location	RFLP
D7S8	pJ3.11	7q22-q32	*Msp*I
COLIA2	pNJ-3	7q21.3-q22	*Eco*RI
TCRB	pJ2	7q32 or q35	*Bgl*II
MET	p*met*H	7q31	*Taq*I
	p*met*D	7q31	*Taq*I
D7S16	p7C22	7q31.1-q31.2	*Eco*RI
D7S13	pB79a	7q22.3-q31.2	*Msp*I/*Hin*dIII

SOURCE.—Howard Hughes Medical Institute Human Gene Mapping Library; also see Spence and Tsui (in press).

Results

Figure 1 shows the RFLP for the restriction enzyme *Sty*I which was detected with the hPCN1 probe. The allele frequency in the 70 CEPH parents for the 2.1-kb fragment (A1) and the 1.65-kb fragment (A2) was .90 and .10, respectively. In 50% of the individuals with the A2 allele, an additional 2.0-kb band, which resolved

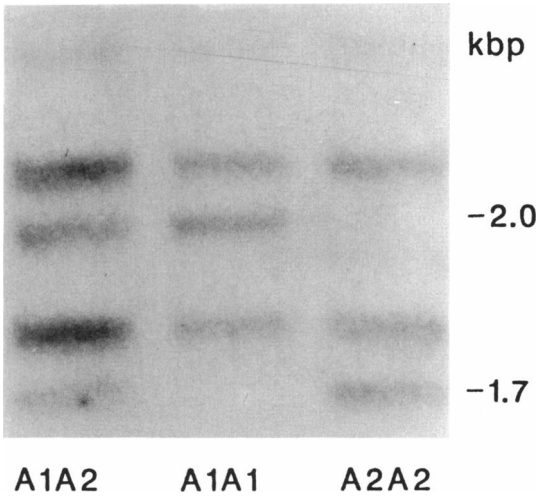

Figure 1 RFLP detected with *Sty*I. DNA from CEPH individuals was digested with *Sty*I and hybridized with the ^{32}P-labeled human cDNA 1-kb 3′ fragment of hPCN1. Lane 1, An individual heterozygous for the RFLP, with both alleles at 2.00 and 1.65 kb (A1A2); lane 2, an individual homozygous for the most frequent (0.9) *Sty*I allele at 2.1 kb (A1A1); lane 3, an individual homozygous for the rarer (0.1) allele at 1.65 kb (A2A2), with the absence of the 2.1-kb allele.

from the 2.1-kb band, was detected. In addition to this two-allele polymorphism, six invariant bands (not shown in fig. 1) were detected with *Sty*I of sizes 1.1, 1.2, 1.4, 1.8, 2.3, and 3.9 kb.

The separate male, female, and sex-combined lod scores between hPCN1 and markers on 7q are shown in table 2. Tight linkage emerged from four informative families between the *CYP3* locus and the collagen gene *COL1A2*, with a combined maximum lod score of 5.72 at $\theta = 0$. These four families each comprised parents, both sets of grandparents, and 6–11 children. The proximal marker *D7S13*, which is localized to 7q22-q31.2, showed a combined maximum lod score of 2.78 with a recombination fraction of .1. Maximum lod scores for more distal genes were 1.51 at $\theta = .1$ for *D7S16*, 1.42 at $\theta = .25$ for *MET*, 1.05 at $\theta = .25$ for *D7S8*, and 0 at $\theta = .50$ for *TCRB*.

Variation of location score with different positions of hPCN1, assuming a 2.5:1 female:male sex difference in recombination, is shown in figure 2.

Discussion

Using the genetic map for the markers *COL1A2*, *D7S13*, *D7S16*, *MET*, *D7S8*, and *TCRB* (Lathrop et al. 1988), multipoint analysis excludes hPCN1 from 7q22.3–7qter. The most likely location is 7q21.3-q22.1, as demonstrated by the peak location score depicted in figure 2. It is impossible without recombinant individuals to determine the order of *COL1A2*, *hPCN1*, and *D7S13*. Further analysis of genes centromeric to *COL1A2* in the CEPH families will enable us more precisely to localize the *CYP3* locus and to determine whether this gene is proximal or distal to *COL1A2*.

Table 2

Computed Female, Male, and Combined Lod Scores for Linkage of hPCN1 with Each Marker on 7q at Various Recombination Fractions

	Recombination Fraction									
Marker	.00	.001	.01	.05	.10	.15	.20	.25	.30	.40
COLIA2:										
Female	3.01	3.01	2.97	2.79	2.55	2.30	2.04	1.76	1.46	.79
Male	2.71	2.70	2.66	2.44	2.16	1.87	1.56	1.24	.92	.31
Combined	5.72	5.71	5.62	5.23	4.71	4.17	3.60	3.00	2.38	1.10
D7S13:										
Female	$-\infty$	−2.50	−1.51	−.83	−.50	−.29	−.15	−.07	−.02	0
Male	3.59	3.58	3.52	3.23	2.86	2.47	2.06	1.64	1.21	.40
Combined	$-\infty$	−.11	1.78	2.72	2.78	2.58	2.24	1.81	1.3	.42
D7S16:										
Female	.30	.30	.29	.26	.21	.17	.13	.10	.06	.02
Male	$-\infty$	−.29	.67	1.21	1.30	1.25	1.14	.98	.80	.37
Combined	$-\infty$	.01	.96	1.47	1.51	1.42	1.27	1.08	.86	.39
MET:										
Female	$-\infty$	− 5.39	−2.43	−.49	.20	.51	.64	.68	.65	.42
Male	$-\infty$	−14.06	−7.15	−2.58	−.90	−.11	.29	.46	.48	.22
Combined	$-\infty$	20.04	−9.24	−2.29	.06	1.01	1.37	1.42	1.27	.65
D7S8:										
Female	$-\infty$	−2.09	−.14	1.02	1.32	1.36	1.28	1.13	.93	.43
Male	$-\infty$	−6.29	−3.32	−1.35	−.61	−.26	−.06	.03	.07	.04
Combined	$-\infty$	−16.78	−7.90	−2.18	−.20	−.63	.98	1.05	.96	.47
TCRB:										
Female	$-\infty$	−7.20	−4.21	−2.16	−1.33	−.88	−.58	−.37	−.23	−.05
Male	$-\infty$	−6.89	−3.91	−1.91	−1.12	−.72	−.47	−.31	−.20	−.08
Combined	$-\infty$	−14.09	−8.12	−4.07	−2.45	−1.59	−1.05	−.68	−.43	−.14

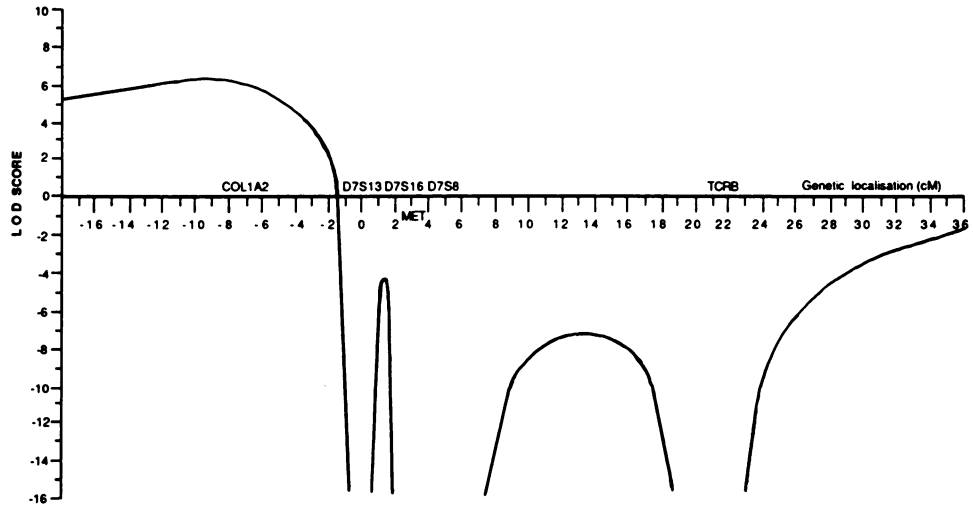

Figure 2 Variation of location score with differing positions of hPCN1. A female:male recombination sex difference and Haldane's mapping function are assumed. The male marker-to-marker recombination fractions (Lathrop et al. 1988) used were *COL1A2-D7S13* (.072), *D7S13-D7S16* (.025), *D7S16-MET* (.004), *MET-D7S8* (.022), and *D7S8-TCRB* (.174).

The functional importance of the P450s is beyond doubt, since they are responsible not only for both the metabolic detoxication and biochemical activation of chemicals to which we are exposed in the workplace and in our life-styles but also for the oxidative biotransformation of many classes of endogenous compounds (Boobis et al. 1985). The occurrence of genetic polymorphism, detectable in vivo, may go a long way to explain individual diathesis to the effects of drugs and other chemicals which are metabolized in an inappropriate manner, both quantitatively and qualitatively, in certain persons. It seems increasingly probable that these inborn errors of metabolism resemble their counterparts in intermediate metabolism. A better understanding of the molecular genetics of this pluripotential isozyme family should lead to new metabolic phenotyping strategies and a clearer perception of the interactions between host and environmental factors in disease causation.

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