Chromosomal assignment of human cytochrome P-450 (debrisoquine/sparteine type) to chromosome 22

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1 In order to determine on which chromosome the gene controlling human cytochrome P-450 (debrisoquine/sparteine type) is located a linkage study of polymorphic sparteine oxidation (PSO) to various polymorphic markers was carried out.

2 Positive information for linkage between PSO and the P₁ blood group was obtained with a maximal LOD-score of LOD = 3.35 for both male and female recombination fraction estimates of $\theta_m = \theta_f = 0.0$.

3 The P₁ blood group has been recently mapped to the long arm of chromosome 22. Thus it can be concluded that the gene controlling human cytochrome P-450 (debrisoquine/sparteine) is situated on the long arm of chromosome 22 in close vicinity to P₁.

Keywords polymorphism sparteine oxidation gene locus chromosome 22 linkage P₁ blood group

Introduction

Cytochrome P-450 enzymes are of paramount importance in drug detoxification as well as chemical mutagenesis, carcinogenesis and toxicity via metabolic activation. Thus genetically determined differences in the activity of cytochrome P-450 enzymes can influence individual susceptibility to adverse drug reactions and certain types of chemically induced cancer (Nebert & Gonzalez, 1985). The recent discovery of three distinct genetic polymorphisms of human oxidative drug metabolism as characterized by debrisoquine/sparteine, mephenytoin and carbocysteine has provided new insight on the genetic control and multiplicity of cytochrome P-450 enzymes in man. Debrisoquine/sparteine metabolism segregates into two phenotypes, extensive (EM) and poor metaboliser (PM). Deficient metabolism is inherited as an autosomal recessive condition (Eichelbaum, 1984; Idle & Smith, 1984; Eichelbaum *et al.*, 1982). The debrisoquine/sparteine polymorphism is of

relevance since it predisposes PMs to a variety of exaggerated and adverse drug effects (Eichelbaum, 1984; Idle & Smith, 1984) and has been implicated as a factor for the development of Parkinson's disease (Barbeau *et al.*, 1985). In contrast, EMs seem to be more susceptible to tobacco smoke induced lung cancer (Ayesh *et al.*, 1984). Thus a way of identifying individuals who are at risk of adverse drug reactions, certain diseases or chemically induced cancer associated with this polymorphism would be desirable. So far phenotype assignment involves the administration of a test drug and collection of urine and the determination of the drug and its metabolites in urine. Although phenotype assignment in healthy subject poses no problem, disease induced changes in drug disposition, metabolism and excretion might disturb correct phenotype assignment. In addition, the administration of drugs known to inhibit drug metabolism can disturb the phenotype assignment (Leemann *et*

al., 1986). Thus a way of phenotyping individuals without the administration of a probe drug would be desirable. We have searched for such a genetic marker and report here on the close linkage between polymorphic sparteine oxidation and the P₁-blood group.

Methods

The metabolism of sparteine was studied in 990 unselected subjects of German origin. The study had been approved by the Ethics Committee of the University of Bonn. The subjects were informed about the purpose of the study and gave their informed consent. After fasting overnight, they were each given a 100 mg sparteine tablet (Depasan®) orally at 07.00 h. All urine was collected for the subsequent 12 h. The samples were stored at -20° C until analysed for their content of sparteine and 2- and 5-dehydrospar-teine by FID-gas chromatography (Eichelbaum *et al.*, 1982). The metabolic ratio (MR) (urinary sparteine/urinary 2- and 5-dehydrospar-teines) was determined and used to assign phenotype. Subjects with a MR > 20 were poor metabolizers (PM) and < 20 extensive metabolizers (EM) (Eichelbaum *et al.*, 1986a). Sixty-four of our 990 subjects corresponding to 6.4% were PM. Metabolism of sparteine was then studied in 21 families of PMs identified in the study. Fifteen families informative for the segregation of polymorphic sparteine oxidation (PSO) were selected and linkage studies to known polymorphic markers GM (immunoglobulin heavy chain), Kell (blood group), Gc (group specific component), acP (acid phosphatase), Rh (rhesus blood group), Duffy (blood group), C 6 (complement component 6), Kidd (blood group), PGM₁ (phosphoglucosyltransferase 1), DHPR (quinoid dihydropyridine reductase), FGRT (formylglycinamide ribotide amidotransferase), Es-Act (esterase activator), Bf (properdin factor B), GLO (glyoxylase), MNSs (MNs blood group), Tys (sclerolytic), Tf (transferrin), Do (Dombrock blood group), PepS (Peptidase), Pi (alpha-1-antitrypsin), AK (adenylate kinase), ADA (adenosine deaminase), 6PGD (6-phosphogluconate dehydrogenase), GPT (glutamate pyruvate transaminase, soluble), EsD (esterase D), PGM₃ (Phosphoglucosyltransferase 3), C 3 (complement component 3), HLA (human leukocyte antigens), ABO (ABO blood group), P₁ (blood group) were carried out. These blood, serum and enzyme groups including P₁ as used in paternity testing at the Department of Forensic Medicine, University of Bonn, were determined with standard techniques. For HLA typing the

microcytotoxicity test with IXth workshop antisera was applied. In all families paternity was confirmed on the basis of the above polymorphic markers.

The following genetic model for PSO was assumed:

1. An autosomal locus with two alleles S (high) and s (low) enzyme activity.
2. Individuals homozygous for s/s are PMs.
3. Gene frequencies were estimated as f(s): 0.25 and f(S): 0.75 on the basis of a random sample of 990 German subjects. LOD (logarithm of the odds ratio) scores were calculated using the Family Analysis Package (FAP) (Baur *et al.*, 1984).

Results

Linkage analysis to the marker systems yielded negative or noninformative results for all polymorphisms with the exception of the P₁ blood group. On the basis of 15 families informative for PSO as well as P₁ a maximal LOD score of 3.35 was obtained for both paternal and maternal recombination fractions $\theta_m = \theta_f = 0.0$ (Figure 1).

Pedigree data can be obtained on request.

Discussion

By using various polymorphic markers we could demonstrate a close linkage between PSO and the P₁ blood group. The LOD score of 3.35 at $\theta = 0$ clearly proves linkage between P₁ and PSO (Neugebauer *et al.*, 1984). The tightness of linkage is very close since recombination is less than 2%. However, a $\theta = 0$ does not necessarily exclude possible recombination to be found in additional families. The P₁ blood group is a system different from the ABO and MN system. P₁+ denotes the presence and P₁- the absence of an antigen to the P₁ blood group. The frequency of P₁ negatives in the European population is 21.15%. The P₁ antigen is inherited as a Mendelian dominant character (Race & Sanger, 1975). There is no complete association between the deficiency allele s and the recessive allele of the P₁ blood group. Consequently P₁ cannot be used as a marker for the detection of heterozygous individuals for PSO.

P₁ has been recently mapped to the long arm of human chromosome 22 (Tippett & Kaplan, 1985; Julier *et al.*, 1985). Thus it can be concluded that the gene locus for PSO is located on chromosome 22 in close vicinity to P₁. Several human

Figure 1 Two pedigrees informative for polymorphic sparteine oxidation and P_1 . Alleles S (high) and s (low) code for the metabolizer phenotype. Homozygotes s/s are poor metabolizers by phenotype (■). P_1 is defined by the dominant allele (+) and the recessive allele (-). LOD scores are given for $\theta_m = \theta_f = 0.0$.

cytochrome P-450 isozymes including the ones involved in polymorphic debrisoquine/sparteine and mephenytoin metabolism have been isolated. These cytochrome P-450 isozymes differ in their chemical, immunological and physical properties as well as substrate specificities. Data obtained from studies with microsomes from human livers of known phenotype as well as purified cytochrome P-450 isozyme preparations suggest that impaired drug metabolism in the PM phenotype is due to either a decreased amount or altered catalytic properties of this particular cytochrome P-450 isozyme (Guengerich *et al.*, 1986; Meyer *et al.*, 1986). But so far the molecular mechanism has not been definitely elucidated. Deficient drug metabolism in PM subjects is restricted to only this form of cytochrome P-450 since the metabolism of other drugs not regulated by the debrisoquine/sparteine polymorphism is unimpaired in this phenotype (Idle & Smith, 1984; Eichelbaum *et al.*, 1986a). Thus by measuring the *in vivo* metabolism of a probe drug like sparteine or debrisoquine it is possible to pheno-

type the population for a specific cytochrome P-450 isozyme and its variant form. It is also interesting to note that this isozyme in contrast to other cytochrome P-450 forms cannot be induced by drugs known to induce human drug metabolism (Eichelbaum *et al.*, 1986b). With the exception of steroid 21-hydroxylase (Carroll *et al.*, 1985), a polycyclic aromatic hydrocarbon (PAH) inducible (Hildebrand *et al.*, 1985) and a phenobarbitone inducible (Phillips *et al.*, 1985) cytochrome P-450 the chromosomal location of the various human cytochrome P-450 isozymes is not known. Steroid 21-hydroxylase is located on the short arm of human chromosome 6. In this case it was possible through the deletion of C4 A/B to map precisely the gene for steroid 21-hydroxylase (Carroll *et al.*, 1985). Using a human cDNA clone and human-rodent hybrids a phenobarbitone inducible human cytochrome P-450 has been mapped to chromosome 19 (19 p 13.2-qter) (Phillips *et al.*, 1985) and the PAH inducible form to chromosome 15 (15q 2.2-2.4) (Hildebrand *et al.*, 1985). Since two other genetic poly-

morphisms in human oxidative drug metabolism as characterized by mephenytoin and carbocysteine do not cosegregate with the debrisoquine/sparteine polymorphism these data indicate that the genes for different cytochrome P-450s are located on different chromosomes or different regions of the same chromosome. The linkage map of human chromosome 22 also includes the locus of cytochrome b₅ reductase, myoglobin and prooncogene SiS. Whereas the precise locus for cytochrome b₅ reductase could not be determined, linkage of the latter two loci with P₁ was demonstrated (Julier *et al.*, 1985; Tippet & Kaplan, 1985). Although there is no complete association between the deficiency allele for PSO and the recessive allele of P₁ the existence

of this linkage group may facilitate the search for other markers or cDNA probes which will allow in the near future a more precise mapping of the defect and help to elucidate the mechanism responsible for defective drug oxidation at the genomic level.

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References

- Ayesh, R., Idle, J. R., Ritchie, J. C., Crothers, M. J. & Hetzel, M. R. (1984). Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. *Nature*, **312**, 169–170.
- Barbeau, A., Roy, M., Paris, S., Cloutier, T., Plasse, L. & Poirier, J. (1985). Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. *Lancet*, **i**, 1213–1216.
- Baur, M. P., Neugebauer, M., Sigmund, M. & Willems, J. (1984). FAP—Family analysis program. *Cytogen. Cell Gen.*, **37**, 416–423.
- Carroll, M. C., Belt, K. T., Palsdottir, A. & Yu, Y. (1985). Molecular genetics of the fourth component of human complement and steroid 21-hydroxylase. *Immun. Rev.*, **87**, 39–60.
- Eichelbaum, M. (1984). Polymorphic drug oxidation in humans. *Fed. Proc.*, **43**, 98–102.
- Eichelbaum, M., Bertilsson, L., Säwe, J. & Zekorn, C. (1982). Polymorphic oxidation of sparteine and debrisoquine: Related pharmacogenetic entities. *Clin. Pharmac. Ther.*, **31**, 184–187.
- Eichelbaum, M., Mineshita, S., Ohnhaus, E. E. & Zekorn, C. (1986b). The influence of enzyme induction on polymorphic sparteine oxidation. *Br. J. clin. Pharmac.*, **22**, 49–53.
- Eichelbaum, M., Reetz, K.-P., Schmidt, E. K. & Zekorn, C. (1986a). The genetic polymorphism of sparteine metabolism. *Xenobiotica*, **16**, 465–481.
- Guengerich, F. P., Distlerath, L. M., Reilly, P. E. B., Wolff, T., Shimada, T., Umbenhauer, D. R. & Martin, M. V. (1986). Human-liver cytochromes P-450 involved in polymorphisms of drug oxidation. *Xenobiotica*, **16**, 367–378.
- Hildebrand, C. E., Gonzalez, F. J., McBride, O. W. & Nebert, D. W. (1985). Assignment of the human 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible cytochrome P₁-450 gene to chromosome 15. *Nucleic Acid Res.*, **13**, 2009–2016.
- Idle, J. & Smith, R. L. (1984). The debrisoquine hydroxylation gene: A gene of multiple consequence. In: *Proceedings of the Second World Conference on Clinical Pharmacology & Therapeutics. Am. Soc. Pharmac. exp. Ther.*, 148–164.
- Julier, C., Reghis, A., Szajnert, M. F., Kaplan, J. C., Lathrop, G. M. & Lalovel, J. M. (1985). A preliminary linkage map of human chromosome 22. *Cytogen. Cell Gen.*, **40**, 665.
- Leemann, T., Dayer, P. & Meyer, U. A. (1986). Single-dose quinidine treatment inhibits metoprolol oxidation in extensive metabolizers. *Eur. J. clin. Pharmac.*, **29**, 739–741.
- Meyer, U. A., Gut, J., Kronbach, T., Skoda, C., Meier, U. T., Catin, T. & Dayer, P. (1986). The molecular mechanisms of two common polymorphisms of drug oxidation—evidence for functional changes in cytochrome P-450 isozymes catalysing bufuralol and mephenytoin oxidation. *Xenobiotica*, **16**, 449–464.
- Nebert, D. W. & Gonzalez, F. J. (1985). Cytochrome P-450 gene expression and regulation. *Trends Pharmac. Sci.*, April, 160–164.
- Neugebauer, M., Willems, J. & Baur, M. P. (1984). Analysis of multilocus pedigree data by computer. In *Histocompatibility testing*, eds Albert, E. J., Baur, M. P. & Mayr, W., pp. 52–58. Berlin, Heidelberg, New York, Tokyo: Springer Verlag.
- Phillips, I. R., Shephard, E. A., Povey, S., Davis, M. B., Kelsey, G., Monteiro, M., West, L. F. & Cowell, J. (1985). A cytochrome P-450 gene family mapped to human chromosome 19. *Ann. Hum. Genet.*, **49**, 267–274.
- Race, R. R. & Sanger, R. (1975). *Blood groups in man*, pp. 139–177. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.
- Tippet, P. & Kaplan, J.-C. (1985). Report of the committee on the genetic constitution of chromosomes 20, 21 and 22. *Cytogen. Cell Genet.*, **40**, 268–295.

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