

A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population

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SUMMARY A population survey of 258 unrelated white British subjects showed a polymorphism for the 4-oxidation of debrisoquine. 'Extensive metabolisers' (EM) and 'poor metabolisers' (PM) are recognisable, 8.9% of the population being PM. Nine pedigrees ascertained through PM probands show that the PM phenotype is an autosomal Mendelian recessive character. The EM phenotype is dominant and the degree of dominance has been estimated at 30%. PM subjects are more prone to hypotension during debrisoquine therapy. The alleles controlling this polymorphism appear to control the oxidation of other drugs.

The antihypertensive drug, debrisoquine, undergoes metabolic hydroxylation in man. The formation of the major metabolite, namely 4-hydroxy-debrisoquine, displays polymorphism in the population.¹ In this study, two well-resolved phenotypes were seen. The first was characterised by persons who 4-hydroxylated the majority of the drug and were thus designated extensive metabolisers (EM phenotype, about 95% of the white British population). For these subjects, the metabolic ratio (MR), defined as % of dose as debrisoquine/% of dose as 4-hydroxy-debrisoquine excreted in the 0 to 8 hour urine, was of the range 0.01 to 9. In the second phenotype (poor metabolisers, PM, about 5% of British whites), MR values greater than 20 were observed, and thus these subjects had a relative inability to carry out debrisoquine 4-hydroxylation. The few families studied suggested strongly that PM subjects were autosomal recessives.

In this paper, we describe a more extensive population study of white British subjects, together with further family data which uphold the resolution of the population into EM and PM phenotypes and confirm that the PM phenotype is an autosomal recessive trait.

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Methods

VOLUNTEER SUBJECTS

Subjects were studied in both London and Liverpool. The volunteers comprised 97 healthy staff and students from the University of Liverpool Medical School and 161 from St Mary's Hospital Medical School, part of whom were studied by Mahgoub *et al.*¹

FAMILY MEMBERS

The probands for the family studies were the poor metabolisers identified in the London and Liverpool population studies.

PHENOTYPING PROCEDURE

As described by Mahgoub *et al.*¹ a single 10 mg Declinax tablet (Roche) (equivalent to 12.8 mg debrisoquine sulphate) was ingested in the fasting state by volunteer subjects who had not been exposed to any recent drug medication. The bladder was emptied before dosing and thereafter all urine was collected up to 8 hours. Urine volume was recorded and an aliquot stored at -20°C to await analysis.

ANALYTICAL PROCEDURE

Urine was analysed for debrisoquine and 4-hydroxy-debrisoquine by electron-capture gas-chro-

matography after derivatisation with hexafluoroacetylacetone as described by Idle *et al.*² From these data the metabolic ratio (MR) was calculated from the expression:

$$\left(\frac{\% \text{ of dose as debrisoquine}}{\% \text{ of dose as 4-hydroxy-debrisoquine}} \right)$$

excreted in the 0 to 8 hour urine. The transformation \log_{10} MR was distributed in an approximately normal manner and was used for computation.

Results

The variable log MR was found to be reproducible in eight EM and nine PM subjects on whom complete repeat tests were performed on two separate occasions. Fig 1 shows a scattergram of the first estimate of log MR plotted against the second estimate of log MR. The non-parametric Spearman rank correlation gives $r_s = 0.88, p < 0.001$.

There was no association of either phenotype with age or sex. The urinary volumes of both phenotypes were within the same range and MR was not dependent on urine volume within either phenotype. There was no heterogeneity in MR values between the London and the Liverpool samples. The distribution of log MR in 258 unrelated white British subjects is shown in fig 2. Two phenotypes are apparent in the population with an antimode of 1.10. Poor metabolisers are thus defined as subjects with $\log MR > 1.10$. Twenty-three phenotypically poor metabolisers were identified, 8 of 97 subjects tested in Liverpool and 15 of 161 subjects tested in London. The PM phenotype frequency in the total sample was thus 0.0891, approximately twice that found by Mahgoub *et al.*¹ in a smaller sample.

Families of the probands derived from among the 23 PM subjects in fig 2 were phenotyped as described for the unrelated volunteers and the results are shown in the table. Metabolic ratios greater than 12.6 ($\log MR > 1.10$) are indicative of poor meta-

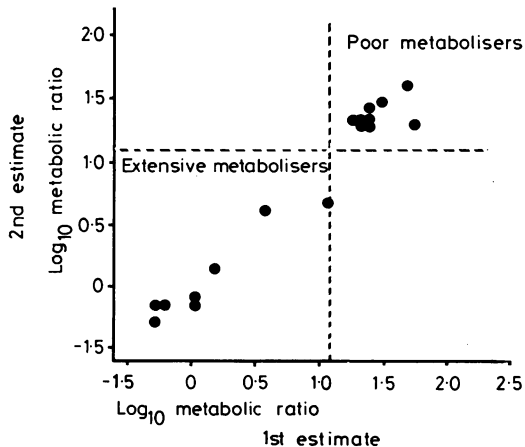


FIG 1 Repeat phenotyping tests carried out on separate occasions in unrelated people of both phenotypes

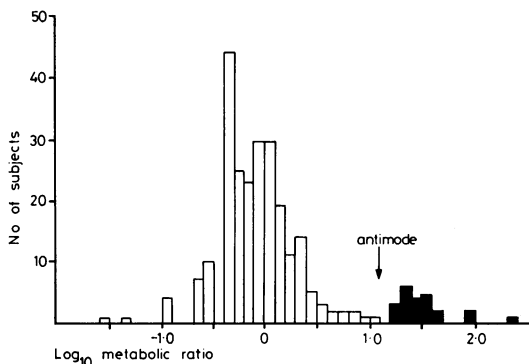


FIG 2 Frequency distribution of \log_{10} metabolic ratio in 258 unrelated white British subjects

bolisers. In pedigrees 1 to 5, all PM offspring had a PM parent (either sex), but for pedigrees 6 to 9, both parents of PM offspring were extensive metabolisers (EM). This clearly shows that PM is an

TABLE \log_{10} urinary metabolic ratios in 9 pedigrees ascertained through probands who are poor metabolisers

Pedigree no	Parents		Offspring				
	Father	Mother	1	2	3	4	5
1	1.48	-1.52	0.15 M	0.18 M	0.42 M	0.11 M	0.15 M
2	-1.29	1.78	1.78 M	0.58 M	1.95 M	—	—
3	1.32	0.23	1.43 M	-1.33 M	0.40 M	—	—
4	1.40	Not available	-1.32	1.90	—	—	—
5	2.29	0.04	-1.48 M	1.88 M	—	—	—
6	0.28	0.48	-1.41	1.32 M	0.32 M	1.84 M	—
7	0.48	0.34	-1.59	1.26	—	—	—
8	0.63	1.00	-1.30 M	—	—	—	—
9	0.72	0.04	-1.32 M	0.77 M	0.64 M	—	—

→ proband; M indicates male offspring.

autosomal recessive character. An estimate can, therefore, be made of the frequency of the allele controlling poor metabolism ($0.2986 \pm \text{SE } 0.0297$)³ and the allele controlling extensive metabolism (0.7014). Thus, the expected genotype frequencies are homozygous PM 0.0891 , heterozygous EM 0.4189 , and homozygous EM 0.4920 .

An estimate of the degree of dominance can be made from the extent of displacement of the mean observed log MR value for heterozygotes (positively identified from family pedigrees) from the mid-point between the mean log MR values for the two homozygous states. From the data given in the table and from Mahgoub *et al.*,¹ it is possible to recognise 21 heterozygotes who have a mean value of 0.27 ± 0.31 (log MR \pm SD). Accordingly, the mean value (\bar{x}) for homozygous dominants can be calculated from the expected number of homozygous (127) and heterozygous (108) subjects who comprise the extensive metabolisers (235) in fig 2 as follows:

Sum of values for 235 extensive metabolisers	Sum of values = for 127 homozygotes	Sum of values + for 108 heterozygotes
-22.58	= 127 \bar{x}	+ 108 \times 0.27

The estimated mean value (\bar{x}) for homozygous dominants is therefore $\bar{I}.5926$ (mean MR = 0.39). The observed mean value for the homozygous recessives (23 in fig 2 plus the eight non-probands in the table plus an additional one from the extended family B of Mahgoub *et al.*,¹ making 32 in all) is 1.53 (mean MR = 33.9). The mid-point between the two homozygous means is thus estimated as 0.56 . So the displacement of the observed heterozygous value from the expected mid-point value (expected for zero dominance) is 0.29 , which gives an approximate dominance of 30%.

Discussion

Two debrisoquine oxidation phenotypes were characterised, first a Mendelian autosomal recessive character (PM phenotype = 8.9% of population) with metabolic ratios greater than 12.8 (log MR >1.10), and secondly a dominant character (EM phenotype 91.2%) comprising both homozygous dominants and heterozygotes in whom a strong (about 30%) dominance effect is observed.

The calculation of dominance gives a clearer picture of the genetic structure of the white British population. There is a practical significance to this finding because if the homozygous EM genotype and the heterozygote had the same distribution they would probably be at equal risk of adverse reactions.

However, since the heterozygotes have a smaller metabolic capacity than the homozygotes it is probable that they are more prone to adverse effects of drugs undergoing C-oxidation.

The biochemical basis of the debrisoquine polymorphism is unknown, but it is possibly concerned with a hepatic mono-oxygenase enzyme which may or may not involve the haemoprotein cytochrome P450 which is considered central to the processes of drug oxidation. As encountered with most polymorphisms of drug metabolism, the natural or endogenous substrate(s) remains unknown. The clinical importance of the debrisoquine polymorphism has been stressed by Sloan *et al.*⁴ and is reiterated here. The oxidative metabolism of other drugs appears to be under the control of the same alleles as debrisoquine hydroxylation. These drugs include guanoxan and phenacetin,⁴ phenytoin,⁵ metiamide,⁶ and 4-methoxyamphetamine⁷; other drugs may be added to this list with the passage of time. In addition, hypotension is produced by a standard 20 mg therapeutic dose of debrisoquine in poor metabolisers, but not in extensive metabolisers.⁸ It would appear, therefore, that the alleles controlling debrisoquine metabolism, and the resulting phenotype of a subject, are of considerable medical importance.

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