

## No Significant Association between CYP1A2 Polymorphism and Porphyria Cutanea Tarda

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Sir,

Porphyria cutanea tarda (PCT) is a metabolic disorder related to a defective function of the haem-synthetic enzyme uroporphyrinogen decarboxylase (URO-D) in liver, resulting in a massive hepatic accumulation of uroporphyrin. Several, sometimes combined, factors may alter the activity of this enzyme such as iron overload, drugs, genetic disturbances, alcohol intake and virus infections – mainly HIV, hepatitis B and particularly hepatitis C virus. The origin of iron overload in PCT has been extensively investigated lately and a significant number of patients with PCT exhibit deleterious mutations of the HFE gene, involved in the regulation of iron metabolism and frequently mutated in genetic haemochromatosis (1). However, a large fraction of patients disclose a normal HFE genotype and other predisposing, especially genetic, factors are obviously involved. Polymorphisms in transferrin type 1 and 2 genes do not display a particular pattern in PCT, according to the rare studies conducted to date (1, 2). Conversely, it has recently been shown that the highly inducible A/A genotype of cytochrome CYP1A2, one of the isoenzymes of cytochrome P450, is increased in Danish patients with both familial and sporadic forms of PCT (3). Indeed, this enzyme seems to play a major role in catalysing oxidative reactions of chemicals involved in the triggering of experimental uroporphyrin, analogous to

PCT, in rodents (4, 5). Its activity results in the generation of reactive oxygen species which may behave as enzyme inhibitors to URO-D, a process also promoted by iron overload, and is partly regulated by gene polymorphisms, mainly a C/A polymorphism in intron 1 which appears to be associated with variations in the inducibility of CYP1A2. More precisely, the A/A genotype represents a highly inducible genotype associated with an increased CYP1A2 activity upon exposure to certain inducing agents (6–8) including ethanol in the rat (9, 10). These polymorphisms may result in differences greater than 60-fold in hepatic basal enzyme activity. Accordingly, the finding of a potential high activity of this enzyme in patients with PCT might provide a further explanation for the genetic predisposition to this condition in populations with excessive ethanol intake. However, such genetic background may be highly variable according to geographic areas, as it has been well demonstrated for HFE gene mutations, and we accordingly investigated the CYP1A2 C/A polymorphism in intron 1 in a series of PCT patients originating from southern France, using a PCR/RFLP method.

## MATERIALS AND METHODS

Forty-nine patients already diagnosed as having PCT on typical clinical and biochemical grounds were analysed for this polymorphism, along with 48 healthy volunteers used as

controls. Some of these patients had already been submitted to HFE gene analysis and transferrin receptor 1 genes status in a previous study (1). Among these 49 patients with PCT, 45 were affected with sporadic PCT whereas 4 demonstrated familial PCT with decreased URO-D activity in peripheral blood and familial cases. The male/female sex ratio was 42/7 and the mean  $\pm$  SEM age was  $52.3 \pm 9.6$  years (range 29–67). After informed consent was obtained, the intron 1 C/A genotype at nucleotide position 734 was determined on genomic DNA extracted from peripheral blood lymphocytes by a method described elsewhere (3). Briefly, a PCR-amplified fragment of 626 bp from the region of interest was submitted to digestion by the restriction enzyme *ApaI*, which cleaves the C allele into two products of 181 and 445 bp. The prevalences of the three genotypes (A/A, A/C, C/C) and the two alleles (A and C) were then calculated in PCT and control populations and compared using a  $\chi^2$  test.

## RESULTS AND DISCUSSION

The results are summarized in Table I. Subsequent statistical analysis failed to show any significant difference between patients with PCT and controls as to the prevalence of the three genotypes and of the two alleles. More specifically, *p* values were 0.553 for AC and CC genotype prevalence (prevalence strictly identical for genotype AA) and 0.650 for allelic prevalence.

Accordingly, our study does not confirm the Danish data as to the higher frequency of the highly inducible genotype AA of CYP1A2 in patients with PCT when compared to a matched control population originating from the same geographic area. This discrepancy may reflect geographical disparities as to genetic predisposing factors on PCT, since such disparities exist for HFE gene mutation patterns between patients from northern and southern European countries. Nevertheless, *p* values are far above the threshold of significance fixed at 0.05, which suggests that there was not even a tendency towards a higher frequency of the AA

genotype in our patients. An alternative explanation for these conflicting results could be a discrepancy in triggering factors according to countries, i.e. a predominance of factors requiring high CYP1A2 activity to result in URO-D inhibition in Danish patients with PCT, whereas the factors are less present, if any, in southern France. More extensive studies assessing both risk factors and genetic background for PCT in different countries could help to better understand these differences and more generally the mechanisms of this disease.

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Table I. Genotype distribution and allele prevalences for intron 1 C/A polymorphism of the CYP1A2 gene in patients with porphyria cutanea tarda (PCT) and controls from Southern France

|                       |    | PCT<br>(n = 49) | Controls<br>(n = 48) |
|-----------------------|----|-----------------|----------------------|
| Genotypes (n)         | AA | 19              | 19                   |
|                       | AC | 26              | 23                   |
|                       | CC | 3               | 6                    |
| Allele prevalence (%) | A  | 66.7            | 63.5                 |
|                       | C  | 33.3            | 36.5                 |