CYP2C19 genotype does not represent a genetic predisposition in idiopathic systemic lupus erythematosus

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Abstract

***Background*—The aetiology of systemic lupus erythematosus (SLE) is still unknown. In several cases, however, chemicals or drugs were identified as aetiological agents and associations with certain phenotypes of drug metabolising enzymes have been reported. The purpose of this study was to discover if there is an association between CYP2C19 polymorphism and susceptibility to SLE.**

***Methods*—Racemic mephenytoin (100 mg orally) was given to healthy volunteers (n=161) and SLE patients (n=37) and then S-mephenytoin and R-mephenytoin were determined in eight hour urine samples. A 10 ml blood sample was obtained from healthy volunteers (n=80) and SLE patients (n=69) for genotypic assay. Each blood sample was tested for the detection of *CYP2C19*1* and *CYP2C19*2* (formerly wt and m1 respectively) by oligonucleotide ligation assay.**

***Results*—The ratio of S/R-mephenytoin ranged from <0.1 to 1.293 in healthy subjects and from <0.1 to 1.067 in SLE patients. PM phenotype was observed in 2 of 37 patients with idiopathic SLE (5.4 %) and 6 of 161 healthy subjects (3.7 %). There were no significant differences in the frequency of PM phenotypes between the groups (Fisher's exact test, p= 0.64) or in the frequency distribution profiles of ratios of S-mephenytoin to R-mephenytoin. No significant differences in distribution of overall genotypes and in allele frequencies were observed between the two groups. No significant relation was found between clinical features and the overall genotype.**

***Conclusion*—The results of this study indicate that *CYP2C19* genotype does not represent a genetic predisposition in idiopathic SLE patients.**

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The aetiology of the systemic lupus erythematosus (SLE), a multisystem connective tissue disease, is unknown. In several cases, however, for example, drug induced lupus erythematosus, drugs were identified as aetiological agents[1] and associations with certain phenotypes of drug metabolising enzymes have been reported.[2]

One of the well described and genetically determined polymorphic drug metabolisms by the cytochromes P450 is the CYP2C19 metabolic polymorphism. The CYP2C19 poor metaboliser phenotype is present in approximately 3% of white populations[3] and up to 18% of the Asian populations.[4] This enzyme is clinically important because it is involved in the metabolism of a number of drugs, such as mephenytoin,[5] diazepam,[6] proguanil,[7] and omeprazole.[8 9] Recently several mutations have been found in the *CYP2C19* gene. The principal genetic defect is a single base mutation in exon 5 of the *CYP2C19 (CYP2C19*2)* gene (m1), which accounts for approximately 75–83% of the poor metaboliser alleles in both Japanese and white subjects.[10] Two other mutated alleles have been identified—a single base mutation (G-A) in exon 4 of the *CYP2C19 (CYP2C19*3)*, which accounts for the remaining poor metabolisers of CYP2C19 in Japanese[11] and a very rare mutation (C_{1297}-T) until now only seen in one subject.[12] The (*CYP2C19*3)* m2 mutation is found in white populations albeit with a much lower frequency.[13]

Genetic polymorphisms of drug metabolising enzymes have an important role in determining susceptibility to rheumatic diseases and adverse drug reactions. For example, CYP2D6 polymorphism has been linked with susceptibility to various diseases including SLE[14] and ankylosing spondylitis,[15] but not scleroderma[16] and rheumatoid arthritis.[17] On the other hand, SLE patients differ from normal subjects in their patterns of oxidative metabolism of certain drugs and chemicals.[18–21] Thereby differences in patterns of oxidative metabolism may be potentially important in the pathogenesis of SLE with activating drugs or environmental chemicals to toxic metabolites. Recently, May *et al* reported that CYP2C19 activity is reduced in scleroderma.[16]

Our objective was to get an insight into whether patients with SLE show impaired CYP2C19 activity. Also we ascertained whether there was an association between particular *CYP2C19* genotypes and susceptibility to SLE, and whether CYP2C19 polymorphism is linked to any specific clinical features of SLE.

Methods

The study was approved by the ethics committees of Hacettepe University Medical School and the Ministry of Health of Turkey. Seventy patients with SLE according to the American Rheumatism Association criteria[22] were recruited from rheumatology outpatients department at the Hacettepe University Hospital. All

Table 1 Comparison of genotypes in SLE patients and in controls

Genotype	*Patients (n=69)*	*Controls (n=80)*
PM	3 (4.3%) (1.2, 17.6)	1 (1.2%) (0.2, 10.9)
Heterozygous EM	11 (15.7%) (7.2, 33.1)	17 (21.2%) (10.9, 38.1)
Homozygous EM	55 (80.0%) (56.1, 91.9)	62 (77.6%) (56.3, 89.8)

95% CI are shown.

Table 2 Allele frequencies in SLE patients and in controls

Allele	*Patients (n=138)*	*Controls (n=160)*
*CYP2C19*1*	121 (87.7%) (73.8, 94.7)	141 (88.2%) (74.9, 93.9)
*CYP2C19*2*	17 (12.3%) (6.4, 23.2)	19 (11.8%) (6.43, 21.6)

95% CI are shown.

patients and control subjects gave written informed consent to participate in the study. Six of the patients were male and 64 female, mean age 38.71 (SD 10.77) years. Before the diagnosis of SLE, none of these patients had received procainamide, hydralazine, isoniazid, anticonvulsants, or other drugs known to cause the lupus syndrome. All patients were maintained with their usual medications during the study. None of the patients was taking a medication known to be metabolised by the CYP2C19 or to inhibit it, or both. One hundred and sixty one unrelated healthy subjects, 121 male and 40 female, with average age 23 (SD 2) years, served as a control population. All were students or staff members of Hacettepe University, who were born in Turkey with Turkish parents. None of the subjects were regular alcohol users and had no history of liver or kidney disease.

PHENOTYPING

After emptying the bladder, 161 healthy volunteers and 37 idiopathic SLE patients took 100 mg racemic mephenytoin tablet orally and then urine was collected over the subsequent eight hours. A 10 ml aliquot was kept frozen at −20°C. No other drugs was taken by healthy volunteers for at least one week before the study.

S- and R-mephenytoin were measured by gas chromatography.[23] Enantiomeric separation of mephenytoin was obtained using a chiral capillary column (Chirasil- Val III FSOT, 25 m × 0.25 mm, internal diameter, All-tech Associations) followed by nitrogen specific detection. The flow rates of helium, air, and hydrogen were adjusted to 2.0–2.5 ml/min, 100 ml/min, and 3.0 ml/min, respectively. Split and septum flow were adjusted to 45–50 ml/min and 2 ml/min respectively. The temperature of column, injector, and detector were adjusted to 180, 250, and 290°C. In these chromotographic conditions, the retention times for the S- mephenytoin and for R-mephenytoin were 9.94 and 10.19 minutes, respectively. S- and R-mephenytoin were extracted from urine samples by using chloroform. The reproducibility was less than 8% (coefficient of variation). The peak areas of S- and R- mephenytoin were used to calculate the S/R mephenytoin ratio. In the urine samples in which R-mephenytoin but not S-mephenytoin was detected, the S/R mephenytoin ratio was given the value of 0.1 based on the lower level of detection of the S-enantiomer. Subjects with S/R mephenytoin ratio above 0.9 were phenotyped as poor metabolisers of mephenytoin.[23]

GENOTYPING

A 10 ml blood sample was obtained from 80 healthy volunteers and 69 patients for genotypic assay and blood samples were kept frozen at −20°C.

CYP2C19 genotypes were determined by assay of leucocyte DNA extracted from blood samples. Each DNA sample was tested for the detection of *CYP2C19*1*, *CYP2C19*2* (formerly wt, m1, respectively) by oligonucleotide ligation assay as described recently.[24]

Analysis of mephenytoin and determination of CYP2C19 genotypes were performed at the Department of Clinical Biochemistry, Odense University Hospital, Denmark. Urine and blood samples were transported over dry ice and remained frozen on arrival in Odense.

STATISTICAL METHODS

Statistical analysis included 95% confidence interval (CI) of proportions, Fisher's exact test for comparison of genetic and phenotypic differences between populations and Student's *t* test for comparison of age at onset of the disease. The level of statistical significance was set at p=0.05 with two sided analysis.

Results

We initially determined whether there was an association between susceptibility to SLE and particular CYP2C19 genotypes. Subjects were assigned to three different classes of overall genotypes (poor metaboliser (PM), heterozygous extensive metaboliser (EM), and homozygous EM) on the basis of genotypic results. The frequencies of the three possible genotypes in the population with SLE and the control are shown in table 1 and the frequency of the wild-type and 2 mutant CYP2C19 alleles in table 2. There was no significant difference in the frequency of the three genotypes between controls and patients with SLE. Furthermore the frequency of the *CYP2C19*1* and *CYP2C19*2,* alleles did not seem to differ between patients and controls.

The ratio of S-mephenytoin to R-mephenytoin ranged from <0.1 to 1.293 in healthy subjects and from<0.1 to 1.067 in SLE patients. We observed PM phenotype in 2 of 37 patients with idiopathic SLE (5.4%) and 6 of 161 healthy subjects (3.7%). There were no significant differences in frequency of PM phenotypes between the groups (Fisher's exact test (two tailed) p= 0.64) or in the frequency distribution profiles of ratios of S-mephenytoin to R-mephenytoin.

Figure 1 (A) and (B) summarise the relation between the various genotypes and S/R-mephenytoin ratio for the patient group and control group. The median S/R-mephenytoin ratios for homozygous EM were similiar in patients with SLE and controls, 0.14 (range <0.1–0.59) and 0.20 (range <0.1–0.8), respectively. The median S/R-mephenytoin ratios in heterozygous EM patients with SLE and heterozygous EM controls were 0.21 (range

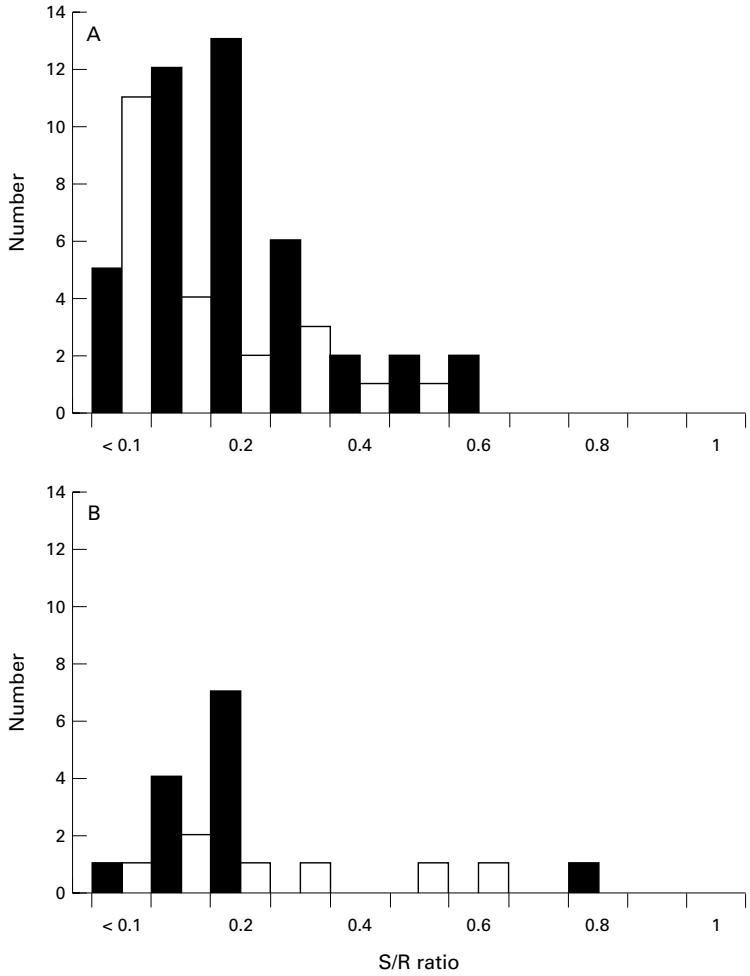

Figure 1 S/R mephenytoin ratios in (A) homozygous EM and (B) heterozygous EM subjects. (Closed bars represent healthy controls, open bars SLE patients).

0.02–0.68) and 0.24 (range 0.08–0.87), respectively. One genotypic PM in the control group and two genotypic PM in the SLE group showed urinary S/R ratios > 0.9 as expected. But unexpectedly one of the genotypic PM patients had lower urinary S/R ratios.

There were five drugs being used by 21 or more patients at the time of the study. These included aspirin (38 patients), prednisone (32 patients), hydroxychloroquine (31 patients), cyclophosphamide (23 patients), and famotidine (21 patients). These patients showed urinary S/R ratios no higher than expected. None of the patients was taking CYP2C19 substrates and/or inhibitors alone, or in combination.

Ten of the SLE patients gave a history of discoid lupus (6.0%), 10 arthritis (6.0%), 3 hypocomplementaemia (5.0%), 2 pleurisy (3.3%), 2 seizures (3.3%), 2 pericarditis (3.3%), 2 psychosis (3.3%), 2 glomerulonephritis (3.3%). At the time of the study none (0%) of the patients had a serum creatinine concentration higher than 1.5 mg/dl and 16 were positive for anti-DNA antibodies (26.6%). There was no relation between genotype and titre of anti-nuclear antibodies, or any other clinical features of SLE at the start of study, or age of onset of the disease (data not shown).

Discussion

The cause of the SLE is unknown but in several cases, for example, drug induced lupus erythematosus, drugs were identified as aetiological agents[1] and associations with certain phenotypes of drug metabolising enzymes have been reported.[2] Evidence obtained in this study suggests that CYP2C19 genetic polymorphism was not associated with the occurrence of SLE. The phenotyping status in the control group was similar to those reported in the previous studies in the Turkish population[25–27] suggesting that the control group used was representative. However, as the number of patients studied was small and the frequency of the poor metaboliser genotype in the control population is only 1.2%, the statistical power of the study is limited. Although the SLE group consists mostly of women and the control group are mostly men, previous studies showed that sex and age did not significantly affect the polymorphic oxidation of mephenytoin.[28]

Inflammatory rheumatic diseases could affect the activity of many drug metabolising enzymes. Differences in patterns of oxidative metabolism involved in conversion of xenobiotics to toxic metabolites may potentially be important in the pathogenesis of rheumatic diseases. Oxidative metabolisms of certain drugs and chemicals were impaired in SLE patients.[18–21] However, our study has clearly demonstrated that the majority of patients with SLE have normal levels of CYP2C19 activity.

Recently, May *et al* reported that CYP2C19 activity is reduced in scleroderma according to phenotyping data.[16] More recently Flockhart *et al* also reported that CYP2C19 activity is reduced in eosinophilia-myalgia syndrome, another connective tissue disease, according to phenotyping data.[29] However, only genotyping can establish whether such differences result from the disease or are because of an association between level of enzyme activity and disease susceptibility. As the genotypic data are not influenced by medications, diet, environment, or timing of urine collection, the determination of PM genotype is therefore straightforward.

In conclusion, our study has clearly demonstrated that the activity of the cytochrome P450 enzyme CYP2C19 is not impaired significantly in SLE patients nor does genetic variability at the CYP2C19 locus predispose to SLE. However, similar investigations of the other drug metabolising enzymes with differing substrate specificities and regulatory mechanisms need to be carried out.

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