Relationship between genotype for the cytochrome P450 CYP2D6 and susceptibility to ankylosing spondylitis and rheumatoid arthritis

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Abstract

Objectives—To determine whether particular genotypes for the cytochrome P450 enzyme CYP2D6, a polymorphic enzyme, are associated with susceptibility to ankylosing spondylitis (AS) and rheumatoid arthritis (RA), or linked with any specific clinical or familial features of the two conditions.

Methods—CYP2D6 genotypes were determined in 54 patients with AS, 53 patients with RA, and 662 healthy controls. Leucocyte DNA was analysed for the presence of mutations by restriction fragment length polymorphism analysis with the restriction enzyme XbaI and by two separate polymerase chain reaction assays.

Results—On the basis of odds ratio (OR), individuals with two inactive CYP2D6 alleles were more susceptible to AS than controls (OR 2.71, 95% confidence interval (CI) 1.04 to 7.08), with a stronger effect for the CYP2D6B allele (OR 4.11, 95% CI 1.54 to 11.0). No significant differences in the distribution of overall genotypes and allele frequencies were observed between RA and controls. No significant relationships were found between the skeletal, extra-skeletal or familial features of AS or RA (iritis, psoriasis, inflammatory enteropathy and rheumatoid nodules, keratoconjunctivitis sicca, pleuritis, rheumatoid and antinuclear factors) and the overall genotype.

Conclusions—Our findings suggest a modest association between homozygosity for inactive CYP2D6 alleles, particularly CYP2D6B alleles, and susceptibility to AS. However, our results fail to demonstrate a genetic link between CYP2D6 genotype and RA.

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genotyping methods allow the identification of more than 95% of poor metabolisers.³ Polymorphisms in the gene encoding this enzyme have been linked with susceptibility to various diseases including certain cancers⁴ and systemic lupus erythematosus,⁵ but not scleroderma.⁶ They are also linked to adverse drug reactions of at least 30 different therapeutically important drugs including antiarrhythmics, neuroleptics, antihypertensives and analgesics including codeine and dextro-propoxyphene, both used widely in rheumatology.² Ankylosing spondylitis (AS) is strongly linked genetically with the HLA-B27 antigen.⁷ Rheumatoid arthritis (RA) is associated with several subtypes of HLA-DR4, which share a similar sequence of the DR β chain.⁸ However, in both rheumatic diseases there are likely to be additional predisposing genes besides HLA-B27 and -DR4 subtypes, which may or may not be HLA linked. Such additional genetic factors and gender may influence disease expression and severity.

In view of the reported association of CYP2D6 genotype with the other chronic inflammatory disease, systemic lupus erythematosus, it seemed appropriate to study the genotype in AS and RA to determine whether this might form a susceptibility marker when compared with a control population, and whether it is linked with any specific clinical features of the conditions.

Patients and methods

Ethics committee approval was obtained from the appropriate ethics committees. All patients and controls gave consent to participate in the study.

Fifty four patients with ankylosing spondylitis (AS) according to the modified New York criteria⁹ were recruited from the rheumatology outpatients department at the General Infirmary at Leeds, UK. There were 47 men, and seven women, mean age 47 (SD 10.8) years and mean duration of AS 20 (10.9) years. From the same department, 53 patients with rheumatoid arthritis (RA) fulfilling the modified American Rheumatism Association criteria¹⁰ were studied: 42 were women and 11 were men, mean age 59 (12.1) years and mean duration of RA 11 (10.7) years. All had RA for at least six months and disease activity considered sufficient to warrant the use of a conventional disease modifying antirheumatic drug. The control group comprised 662 healthy volunteers recruited from the staff of a

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Genetic factors have an important role in determining susceptibility to rheumatic diseases and adverse drug reactions. Cytochrome P450 enzymes are responsible for the majority of oxidative (phase I) drug metabolism.¹ The cytochrome P450 enzyme CYP2D6 is polymorphic with 5–10% of Europeans lacking this activity (described as poor metabolisers) compared with extensive metabolisers.² The genetic basis of this deficiency is now well understood, and

British pharmaceutical company and the staff and students of the University of Newcastle-upon-Tyne, UK: 285 were women, 377 were men, and the mean age was 35.2 (9.9) years.

For all patients one physician (CB) completed a questionnaire about the skeletal and extraskelatal features and age of onset of the rheumatic diseases, and about the family history of AS, psoriasis, inflammatory enteropathy, and RA.

CYP2D6 genotypes were determined by assay of leucocyte DNA extracted from EDTA blood samples transported from Leeds to Newcastle. Samples were analysed for the presence of mutations in the CYP2D6 gene cluster, by restriction fragment length polymorphism analysis with XbaI and a CYP2D6 cDNA probe (CYP2D6D, CYP2D6E alleles) and polymerase chain reactions specific for CYP2D6A and CYP2D6B alleles, respectively.³ It has been demonstrated that a combination of these analyses detected about 95% of phenotypic poor metaboliser subjects as genotypic poor metabolisers. The four different mutant alleles (CYP2D6A, CYP2D6B, CYP2D6D, CYP2D6E) analysed are associated with lack of CYP2D6 activity, and an individual homozygous for one or heterozygous for two of these alleles shows a poor metaboliser metabolic phenotype.

Statistical analyses included 95% confidence interval (CI) of proportions, χ^2 tests for comparison of genetic differences between populations and Student's *t* test for comparison of age at onset of the disease. Odds ratios (OR) with 95% CI were calculated using the computer program CIA.¹¹ The level of statistical significance was set at $p = 0.05$ with two sided analysis.

Results

Table 1 shows the prevalence of the three separate genotypes in AS, RA, and controls and the odds ratio for development of the two diseases with the homozygous wild-type genotype as baseline. The value of 2.71 calculated for the homozygous mutant genotype and susceptibility to AS is significant on the basis of the 95% confidence interval. Table 2 shows the prevalence of the different alleles in the three populations studied. There was a tendency for more CYP2D6B alleles in the AS group, and when odds ratios for AS development for the presence of one or two CYP2D6B alleles with no CYP2D6B alleles as baseline were calculated (table 3), a significantly increased risk of AS development (4.11 with 95% CI 1.54 to 11.0) was observed for individuals with two CYP2D6B alleles.

Twenty one of the AS patients described peripheral arthritis (37%), 20 iritis (37%), seven psoriasis (13%), and one inflammatory enteropathy (2%). Ten patients gave a family history of AS (18%), seven a family history of psoriasis (13%), and one a family history of inflammatory enteropathy (2%). Twenty five of 29 were genotyped HLA-B27 positive (86%). Relationships were sought between all the various features and the three genotype classes. None emerged, though there was a trend suggesting that AS patients with homozygous mutant genotypes may have had an earlier onset of disease (23 (7.3) years) than those with heterozygous (25 (9.8) years) and homozygous wild-type genotypes (29 (9.0) years). These findings lend support to a possible relationship between gradation of genetic make up and disease onset of AS.

Twenty six of the RA patients gave a history of rheumatoid nodules (49%), 23 keratoconjunctivitis sicca (42%), one pleuritis (2%), and none vasculitis (0%). Fourteen patients reported one or more relatives of first or second grade affected by RA (26%). At the time of the study, 29 patients had a rheumatoid factor titre greater than 16 (55%), 15 were antinuclear factor positive (28%) and 40 of 47 had radiographic changes typical of RA (85%). No associations were found between all those features and the three genotype classes. In addition, age of onset of RA was similar in patients with a homozygous mutant (53 (12.3) years), heterozygous (45 (13.0) years), and homozygous wild-type genotype (49 (13.4) years).

Discussion

This study has demonstrated that there appears to be a modest association between

Table 1 Risk of AS and RA development in relation to genotype

Genotype	Frequency (No (%) [95% CI])	Odds ratio [95% CI] v controls
Wild-type/wild-type		
AS	26 (48.2) [35 to 61]	1
RA	35 (66.0) [53 to 79]	1
Controls	364 (55.0) [51 to 59]	
Wild-type/mutant†		
AS	22 (40.7) [28 to 54]	0.62 [0.34 to 1.12]
RA	14 (26.4) [15 to 38]	0.55 [0.29 to 1.03]
Controls	267 (40.3) [37 to 44]	
Mutant/mutant		
AS	6 (11.2) [3 to 19]	2.71 [1.04 to 7.08]
RA	4 (7.6) [0 to 15]	1.34 [0.45 to 4.02]
Controls	31 (4.7) [3 to 6]	

†Not positive for CYP2D6A, CYP2D6B, CYP2D6D, CYP2D6E alleles.

‡Positive for CYP2D6A, CYP2D6B, CYP2D6D or CYP2D6E allele.

Table 2 Distribution of alleles in ankylosing spondylitis (AS), rheumatoid arthritis (RA) and healthy controls

Allele	AS (n = 108)	RA (n = 106)	Controls (n = 1324)
Wild-type	74 (68.5) [60 to 77]	84 (79.3) [72 to 87]	995 (75.2) [73 to 77]
CYP2D6A	3 (2.8) [0 to 6]	1 (0.9) [0 to 3]	19 (1.4) [1 to 2]
CYP2D6B	29 (26.9) [18 to 35]	20 (18.9) [11 to 26]	257 (19.4) [17 to 22]
CYP2D6D	1 (0.9) [0 to 3]	1 (0.9) [0 to 3]	51 (3.9) [3 to 5]
CYP2D6E	1 (0.9) [0 to 3]	0	2 (0.1) [0 to 0.4]

Values are number (%) [95% CI].

Table 3 Risk for possession of CYP2D6B allele in AS and RA

Alleles	(No (%) [95% CI])	Odds ratio [95% CI] v controls
No B allele		
AS	31 (57.4) [44 to 71]	1
RA	36 (67.9) [55 to 80]	1
Controls	425 (64.2) [62 to 66]	
One B allele		
AS	17 (31.5) [19 to 44]	1.07 [0.58 to 1.98]
RA	14 (26.4) [16 to 38]	0.76 [0.40 to 1.44]
Controls	217 (32.8) [29 to 36]	
Two B alleles		
AS	6 (11.1) [3 to 19]	4.11 [1.54 to 11.0]
RA	3 (5.7) [0 to 12]	1.77 [0.50 to 6.24]
Controls	20 (3.0) [2 to 4]	

susceptibility to AS and CYP2D6 genotype, and that this effect is greatest for the CYP2D6B allele. However, no association between RA and CYP2D6 genotype was detected. The number of patients studied was small and because the frequency of the poor metaboliser genotype in the control population is only 5%, the statistical power is limited. We estimate that to achieve 60% statistical power with the sample size of the AS or RA group, the odds ratios would have to lie between 4 and 5. The allele frequencies for the CYP2D6B allele in the control group were similar to those reported for other British studies,^{12 13} suggesting that the control group used was appropriate. It can be assumed that, because recruitment was from special clinics, patients with severe disease have been investigated. This is reflected by the high proportion of patients with different extraskelatal features and the need for disease modifying anti-rheumatoid drugs. Differences from a control population should thus be detected easily.

The biological basis of the apparent association between CYP2D6 genotype and susceptibility to AS is unclear. Associations between CYP2D6 genotype and diseases such as lung cancer, bladder cancer, and Parkinson's disease have been demonstrated,⁴ but these associations are probably related to metabolism of environmental toxins by CYP2D6. However, the finding of a stronger association for the CYP2D6B alleles than for the other mutant alleles might be suggestive of linkage disequilibrium with another gene with a more direct role in the disease in the region of CYP2D6 on chromosome 22.

The finding of an apparent increase in the frequency of the poor metaboliser phenotype among AS patients suggests that the incidence of certain adverse drug reactions in individuals suffering from this disease may be greater than normal. We have previously demonstrated in a study of RA patients that the widely used analgesic dextropropoxyphene affects CYP2D6 phenotype.¹⁴ However, this drug is believed to be a CYP2D6 inhibitor rather than a substrate, therefore its use in poor metabolisers may not necessarily present problems. The other most commonly used CYP2D6 drug substrate likely to be used in the treatment of AS is codeine. Poor metabolisers

are unable to activate this drug to morphine, and it has been demonstrated to be an inefficient analgesic among a group of poor metabolisers.¹⁵

Although requiring confirmation in a larger patient group, this study suggests that, in addition to playing a part in susceptibility to systemic lupus erythematosus,⁵ CYP2D6 genotype may also play a modest role as a susceptibility factor in AS.

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