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The PTPN22*C1858T functional polymorphism is associated with susceptibility to inflammatory polyarthritis but neither this nor other variants spanning the gene is associated with disease outcome.

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Abstract

Background: The *PTPN22* gene has been widely confirmed as a susceptibility gene for rheumatoid arthritis (RA) in populations of Northern European descent. The aim of the current study was to explore the role of variants spanning the *PTPN22* gene in determining susceptibility to and outcome of inflammatory polyarthritis (IP).

Patients and methods: SNP variants spanning the gene were genotyped using the Sequenom MassArray platform and tested, firstly, for their association with susceptibility to IP. Genotype frequencies were compared between new onset IP cases (n = 843) and population controls (n = 471). Secondly, a within-cohort analysis was performed testing each variant for association with a number of clinical outcome measures reflecting disease severity including radiological erosions, physical function, measured using the Health Assessment Questionnaire (HAQ) score, and disease activity at defined time-points following disease presentation.

Results: A significant association between carriage of the *PTPN22*1858T* allele and IP (OR = 1.4 [95% CI 1.1-1.9], P = 0.02) was observed. The strength of the effect was similar in the RA subgroup (OR = 1.4 [95% CI 1.0-1.9], P = 0.05). No association between IP susceptibility and any of the other SNPs was detected. No association was detected for any of the SNPs tested, including the *PTPN22*C1858T* polymorphism, with either erosive status, Larsen score by 5 years or other markers of clinical outcome.

Conclusion: The *PTPN22*C1858T* polymorphism is associated with susceptibility to IP but we have found no evidence for association of this or other variants spanning the gene with clinical outcome measures.

Introduction

A considerable genetic contribution to rheumatoid arthritis (RA) is demonstrated by both twin and family studies (1). The strongest association to date has been found with the *HLA-DRB1* gene and, in particular, with alleles that code for a consensus amino acid sequence within the third hypervariable region of the DRB1 chain (the shared-epitope). More recently, progress has been made in identifying other RA susceptibility genes (reviewed in (2)). The most convincing association has been demonstrated with a functional single nucleotide polymorphism (SNP) mapping to the lymphoid tyrosine phosphatase, *PTPN22*, gene (*PTPN22*C1858T*) (3). This finding has been widely and consistently replicated in populations of Northern European descent (4).

The *PTPN22* gene maps to chromosome 1p13 and codes for a tyrosine phosphatase that is involved in immune regulation as a negative regulator of T cell activity. The polymorphism results in the substitution of the wild-type, arginine, with a variant, tryptophan, amino acid at codon 620 (R620W). Recent work suggests that the polymorphism may lead to a 'gain-of-function,' as the protein has been shown to be changed into a more active tyrosine phosphatase in the presence of the tryptophan variant (5). It is hypothesized that reduced T cell signalling may occur as a result and, in turn, this may enable auto-reactive T cells to escape deletion during thymic selection and thus persist in the circulation to become activated at a later stage. Although the primary association is with the *PTPN22*C1858T* polymorphism, evidence for independent association of 3 other SNPs mapping within the gene with susceptibility to RA has since been reported but this finding has not been replicated in 2 subsequent studies (6-8).

In the clinical setting, the greatest utility for genetic markers would be if they led to the identification of a marker that could predict, at the earliest stages of inflammatory polyarthritis (IP), which patients will proceed to develop more severe disease. This could help to identify a subgroup of patients that could be targeted for aggressive therapy, whilst sparing those least likely to develop severe disease from the potential side-effects associated with such drug therapy. Whilst both the shared epitope and the *PTPN22*C1858T* allele are certainly associated with RA, the question of whether these associations are primarily with susceptibility or severity has proved difficult to determine because many previous studies have investigated cross-sectional series of hospital based cases that often represent the severe end of the spectrum of disease. Ideally, such studies should be performed in inception cohorts of patients followed prospectively. In an unselected series of patients presenting to primary care with early IP and followed prospectively as part of the Norfolk Arthritis Register (NOAR), we have previously shown that the shared epitope is more strongly associated with severity markers such as radiological erosions than with susceptibility (9).

Previous studies of the *PTPN22*C1858T* SNP have revealed stronger associations in patients with a family history of disease and in patients with a younger age at onset suggesting that this polymorphism, too, may be associated more with severity than susceptibility (3;10). However, these studies were cross-sectional in nature and subject to the methodological biases outlined. Only one study has examined an inception cohort followed prospectively (11). They reported no association with remission or rates of RA joint destruction but an association between *PTPN22*1858T* risk allele and autoantibody production was noted.

We have used the opportunity afforded by the NOAR cohort to address the question of firstly, whether other variants spanning the *PTPN22* gene are associated

with susceptibility to IP and, secondly, whether any is associated with outcome of disease.

Materials and Methods

Study design

SNP variants spanning the *PTPN22* gene were tested firstly for their association with susceptibility to IP by comparing genotype and allele frequencies between new onset IP cases with population controls. Secondly, a within-cohort analysis was performed testing each variant for association with a number of clinical outcome measures reflecting disease severity including presence of radiological erosions, physical function as measured using the Health Assessment Questionnaire (HAQ) score and disease activity at defined time-points following disease presentation (12).

Study population

Cases were recruited from NOAR, a primary care based cohort of subjects with new onset IP (13). In brief, NOAR aims to recruit all new adult attenders at primary care with IP, defined as swelling of two or more joints, lasting four or more weeks, after exclusion of diagnoses other than undifferentiated IP, post-viral IP, RA or psoriatic arthritis. Subjects are assessed by trained metrologists, within two weeks of referral, using a standardized approach. Data gathered included joint counts for swelling and tenderness. Blood was taken for serum analysis (rheumatoid factor (RF), C-reactive protein (CRP) and anti-CCP testing) and DNA extraction. Subjects were followed up annually thereafter (13). The 1987 criteria for RA were applied at every visit and subjects classified as having RA if they had cumulatively satisfied the criteria by that time point (14). At baseline and annually thereafter the subjects completed the 8 domain HAQ. Radiographs of the hands and feet were obtained at 1 and / or 5 years. The criteria for requesting radiographs and the scoring process have been published previously (15). The radiographs were read by 2 readers (with arbitration by a third where necessary) using the Larsen method (16). For the purposes of this study, consecutive patients recruited between 1990 and 1994 who had a DNA sample available were selected.

Data for unmatched control individuals recruited from Norfolk general practitioners' records or from blood donors were available and have been reported previously (7). All subjects were of U.K Caucasoid origin. The study was conducted with ethical approval (LREC 2003-075, MREC 99/8/84) and all patients gave their informed consent.

Serological tests

Baseline serum samples were assayed for RF was using a latex method and a titre of $\geq$ 1:40 was regarded as positive. CRP was measured using nephelometry (www.bmglabtech.com/templates/applications). Anti-CCP testing was performed using the Axis-Shield® DIASTAT™ kit according to manufacturer's instructions and a result of >5U/ml was regarded as positive (Axis-Shield, Dundee, U.K).

Genotyping

Thirteen SNPs spanning the *PTPN22* gene were selected for investigation. These included the *PTPN22*C1858T* polymorphism, 7 tagging SNPs and 2 of 3 potentially functional polymorphisms identified by Carlton *et al.* (6). The third SNP, rs3789604 (SNP 37), was not selected for genotyping because of the strong LD

exhibited between this and another of the selected SNPs, rs3811021 (SNP 36). Genotyping was undertaken using the Sequenom® iPLEX platform™, according to the manufacturer's instructions (17) (www.sequenom.com). Samples were plated onto 2 x 384-well plates. Duplicate samples and negative controls were included to check genotyping quality. For the *PTPN22*C1858T* (rs2476601, R620W) SNP, additional samples from the cohort had been genotyped in-house previously and that data was included in the current analysis. Only SNPs that were successfully genotyped in >90% samples were included in the analysis.

Statistical analysis

Genotype and allele carriage frequencies for each SNP were compared between cases and controls using the chi-square test for trend and odds ratios (OR) with 95% confidence interval (CI), respectively. Given the linkage disequilibrium known to exist across the gene, logistic regression was undertaken conditioned on the effect of the associated SNP, rs2476601 (R620W). Estimated haplotypes were constructed in Haploview v3.2 (18) and frequencies compared between cases and controls.

For the within-cohort analysis of association with disease severity, a number of measures were assessed reflecting disease activity (CRP at baseline, swollen joint count at baseline and at 5 years following presentation), severity (presence of erosions by 1 and 5 years, Larsen score by 1 and 5 years, presence of anti-CCP antibodies and RF at baseline) and disability (HAQ score). Categorical data (erosions by 1 and 5 years, anti-CCP and RF) were analysed using the chi-square test whilst continuous variables were analysed using the Mann-Whitney test. All analyses were performed in the cohort as a whole and in the subset of patients fulfilling ACR criteria for RA by 5 years using STATA® (StataCorp LP, Texas, USA)..

For the *PTPN22*C1858T* SNP, there was a clear *a priori* hypothesis for testing for association with IP and so no correction was made for multiple testing. However, for the other SNPs tested, the prior probability of detecting association was small and a Bonferroni correction of 12 was applied to correct for number of SNPs tested.

Results

Subjects

In total, 1,098 patients were recruited to NOAR between 1990 and 1994 and a DNA sample was available from 843 of them. However, for the majority of SNPs, only 750 samples were tested. Clinical characteristics of the cases are detailed in Table 1 and are similar to those reported previously from the NOAR cohort (14). The median age at onset of IP was 53 (interquartile range (IQR) 40-65), 539 (64%) were female and 79.4% had been referred to hospital (secondary care) following onset of their arthritis. Of the 448 controls for whom data were available, 54% were female and the median age at the time of sample donation was 66 (interquartile range (IQR) 42 – 75). This data has been published previously (7). All cases and controls were of UK white Caucasian ethnicity.

Table 1 – Clinical data for subjects with IP.

Characteristic	Cases (n= 843)
RF positive: n (%)	
- baseline	202 / 773 (26%)
Erosive: n (%)	
- by 1 year	159 / 476 (33%)
- by 5 years	202 / 487 (41%)
Anti-CCP positive at baseline : n (%)	181 / 682 (27%)
ACR criteria fulfilled by 5 years: n (%)	519 / 769 (67%)
Median swollen joint count: (IQR)	
- at baseline	4 (1-10)
- at 5 years	0 (0-1)
Median HAQ score: (IQR)	
- at baseline	0.75 (0.25-1.38)
- at 5 years	0.25 (0.25-1.5)
Median CRP at baseline: (IQR)	5 (0-15)

The denominator details the number of subjects where information was available.
IQR = interquartile range

Genotyping

Genotype frequencies for one SNP (ss38346943) deviated from Hardy-Weinberg equilibrium (P = <0.01) in the control population and it was excluded from the susceptibility analysis. One variant (ss38346944) was not tested in the control samples, and hence investigation of this marker was restricted to the analysis of clinical outcome.

Association with IP susceptibility

Genotype distributions differed significantly at the *PTPN22*C1858T* polymorphism between IP cases and controls (P = 0.02) (Table 2). In addition, there was a significant association between carriage of the *PTPN22*1858T* allele and IP (OR = 1.42 [95% CI 1.06-1.90], P = 0.02). The strength of the effect was similar in the RA subgroup (OR = 1.38 [95% CI 1.01-1.89], P = 0.04) and the IP cohort as a whole. No association between IP and any of the other SNPs was detected either when analysed by genotype (Table 2) or by allele. Stratifying the cohort for the fulfilment of ACR criteria for RA by 5 years did not alter these conclusions (data not shown).

Table 2. Genotype data for SNPs spanning PTPN22 gene in patients with IP and controls

SNP (SNP number from Carlton study)	Cases				Controls				p-value (uncorrected)
	Sample size	1/1 n (%)	1 / 2 n (%)	2/2 n (%)	Sample size	1/1 n (%)	1 / 2 n (%)	2/2 n (%)	
rs1217414 (SNP 1)	736	386 (52.4)	305 (41.4)	45 (6.1)	398	221 (55.6)	161 (40.4)	16 (4.0)	0.16
rs2488458 (SNP 2)	746	425 (57.0)	278 (37.3)	43 (5.8)	387	209 (54.0)	156 (40.3)	22 (5.7)	0.45
rs12760457 (SNP 18)	731	355 (48.6)	320 (43.8)	56 (7.7)	390	182 (46.7)	179 (45.9)	29 (7.4)	0.67
rs11102685 (SNP 20)	742	614 (82.7)	119 (16.0)	9 (1.2)	403	340 (84.4)	61 (15.1)	2 (0.5)	0.35
rs12730735 (SNP 21)	736	360 (48.9)	322 (43.8)	54 (7.3)	357	169 (47.3)	160 (44.8)	28 (7.9)	0.61
rs2476601(SNP22) (PTPN22*C1858T)	832	641 (77.0)	178 (21.4)	13 (1.6)	412	342 (83.0)	66 (16.0)	4 (1.0)	0.02
rs1310182 (SNP 27)	683	227 (33.2)	323 (47.3)	133 (19.5)	351	101 (28.8)	175 (49.9)	75 (21.3)	0.17
rs1217388 (SNP 32)	736	416 (56.5)	275 (37.4)	45 (6.1)	400	208 (52.0)	168 (42.0)	24 (6.0)	0.24
ss38346942 (SNP 34)	720	694 (96.4)	26 (3.6)	0 (0.0)	386	375 (97.2)	11 (2.8)	0 (0.0)	0.50
rs1217413 (SNP 35)	732	433 (59.2)	259 (35.4)	40 (5.5)	383	240 (62.7)	126 (32.9)	17 (4.4)	0.22
rs3811021 (SNP 36)	699	446 (63.8)	215 (30.8)	38 (5.4)	386	247 (64.0)	129 (33.4)	10 (2.6)	0.41

1 = major (wild-type) allele, 2 = minor (variant) allele

When the analysis was performed using logistic regression to adjust for the genotype at the rs2476601 (R620W) SNP, another SNP showed evidence for association with susceptibility: rs1217388 (SNP 32; uncorrected p = 0.003). However, the only haplotype occurring significantly more frequently in cases than controls was the haplotype carrying the risk allele at rs2476601 (R620W) (12.4% cases, 9.3% controls, p = 0.03). When chromosomes carrying the risk allele were excluded, no evidence for association of the remaining haplotypes with RA was found. Furthermore, haplotypes carrying the risk allele at rs1217388 (SNP 32), showed no difference in frequency between cases and controls.

Association with clinical outcome measures

No association was detected for any of the SNPs tested, including the *PTPN22*C1858T* polymorphism, with either erosive status or Larsen score by 5 years (Table 3). A trend for association of the Larsen score by 5 years was noted with SNP rs1217413 (SNP35) but this was not significant when a Bonferroni correction was applied. Furthermore, no association with Larsen score at 1 year or with any other clinical outcome measure was detected (data not shown). None of the other SNPs tested demonstrated statistical evidence for association either in the IP cohort as a whole (Table 3) or in the subgroup satisfying ACR criteria for RA by 5 years (data not shown). No association was detected with other markers of outcome tested such as HAQ score at presentation or by 5 years, swollen and tender joint counts at presentation or by 5 years or CRP at baseline for any of the SNPs spanning the gene (data not shown).

Table 3. Allelic association of SNPs spanning PTPN22 gene with erosive status and Larsen score by 5 years.

SNP (SNP number from Carlton study)	Erosive status by 5 years - minor allele frequency: n (%)				**Median Larsen score at 5 years, by allele[c] (IQR)		
	Erosive n (%)	Non-erosive n (%)	OR (95%CI)	p-value[a] (uncorrected)	Allele 1	Allele 2	p-value[b] (uncorrected)
rs1217414 (SNP 1)	86 (26.9)	114 (24.4)	1.14 (0.8-1.6)	0.43	5 (0-20)	6 (0-29)	0.72
rs2488458 (SNP 2)	73 (22.3)	119 (24.9)	0.86 (0.6-1.2)	0.39	5 (0-21)	4 (0-20)	0.05
rs12760457 (SNP 18)	98 (30.4)	150 (31.8)	0.94 (0.7-1.3)	0.69	4 (0-21)	5 (0-19)	0.52
rs11102685 (SNP 20)	34 (10.3)	40 (8.4)	1.30 (0.8-2.0)	0.34	5 (0-20)	9 (0-30)	0.84
rs12730735 (SNP 21)	95 (29.5)	147 (31.1)	0.92 (0.7-1.3)	0.62	4 (0-21)	5 (0-19)	0.63
rs2476601 (SNP 22) (PTPN22*C1858T)	45 (11.1)	65 (11.6)	0.96 (0.6-1.4)	0.84	5 (0-20)	5 (0-17)	0.74
ss38346944 (SNP 23)	9 (3.0)	8 (1.9)	1.62 (0.6-4.1)	0.32	5 (0-20)	10 (2-30)	0.32
rs1310182 (SNP 27)	133 (43.2)	180 (44.6)	0.95 (0.7-1.3)	0.72	6 (0-23.5)	5 (0-22)	0.93
ss38346943 (SNP 28)	15 (4.7)	19 (4.2)	1.13 (0.6-2.2)	0.72	5 (0-21)	6 (2-20)	0.26
rs1217388 (SNP 32)	72 (22.2)	118 (24.8)	0.87 (0.6-1.2)	0.40	5 (0-21)	4 (0-18)	0.11
ss38346942 (SNP 34)	3 (1.0)	10 (2.1)	0.43 (0.1-1.5)	0.19	5 (0-20)	0 (0-7)	0.06
rs1217413 (SNP 35)	64 (19.6)	114 (24.5)	0.75 (0.5-1.0)	0.11	5 (0-22)	3 (0-17)	0.007
rs3811021 (SNP 36)	68 (22.2)	99 (21.9)	1.0 (0.7-1.4)	0.92	5 (0-20)	3 (0-20)	0.49

** Larsen score in all subjects with information available. When the analysis was restricted to patients who were erosive, the conclusions were not altered.

Uncorrected p-values are presented.
[a]Chi-squared; [b]Logistic regression by genotype

OR = odds ratio, 95%CI = 95% confidence intervals

IQR = interquartile range

[c]Allele 1 = major (wild-type) allele, allele 2 = minor (variant) allele

Association with autoantibody status

There was a significant association between the variant allele of the *PTPN22*C1858T* (rs2476601) SNP and baseline anti-CCP status in the RA subgroup (P = 0.003) (Table 4).

Table 4. Association of rs2476601 (PTPN22*C1858T) polymorphism with presence of autoantibodies in patients with IP and in the subset satisfying ACR classification criteria for RA.

Autoantibody at baseline	IP Cohort		RA subset	
	PTPN22*CC: n (%)	PTPN22*CT or PTPN22*TT: n (%)	PTPN22*CC: n (%)	PTPN22*CT or PTPN22*TT: n (%)
RF present	185 (74.0)	65 (26.0)	155 (74.5)	53 (25.5)
RF absent	442 (78.2)	123 (21.8)	231 (79.4)	60 (20.6)
Comparison: p-value**	**0.19**		**0.20**	
Anti-CCP present	109 (68.5)	50 (31.5)	102 (69.4)	45 (30.6)
Anti-CCP absent	388 (78.1)	109 (21.9)	207 (82.1)	45 (17.9)
Comparison: p-value**	**0.09**		**0.003**	

**p-values are uncorrected.

Discussion

In this unselected primary care based inception cohort of patients, we have confirmed the association between the *PTPN22*C1858T* polymorphism and susceptibility to IP but found no association with severity using a number of clinical outcome markers. We have also failed to find any association between disease susceptibility or severity and other polymorphisms spanning the gene.

The association of the *PTPN22*1858T* variant with susceptibility to RA has been widely replicated in white European populations (4). This polymorphism has also been found to be associated with susceptibility in two other inception cohorts with early disease (OR 1.3 and 1.5) (11;19). The effect size detected in the current study (OR = 1.4) is in keeping with those reports and suggests that the variant confers a modest increase in risk.

One explanation for the lower effect sizes detected in studies of early disease compared to established series of RA patients may be that the *PTPN22*C1858T* variant is associated with disease severity as well as susceptibility. An inception cohort, such as NOAR, followed prospectively is required to dissect these issues. The NOAR cohort is representative of patients with new-onset IP seen in general practice. 79.4% were referred to hospital within the 5 years of follow-up, whilst 75% satisfied ACR classification criteria for RA by 5 years. The choice of relevant outcome measures is the subject of some debate. The development of radiological erosions is generally regarded as a reliable and validated measure of severity but, the earlier use of disease-modifying (DMARD) therapy may mean that erosions are of less clinical relevance. Furthermore, the level of disability experienced by patients is of far greater importance to them than X-Ray appearances. Hence, a measure of physical function such as the HAQ score may provide a more relevant outcome measure. We have tested multiple measures of disease outcome but, despite this, we have found no association of the *PTPN22*C1858T* polymorphism with disease severity. One possibility is that the *PTPN22*C1858T* polymorphism is associated with outcome but this was masked by the effect of treatment. This could occur if patients carrying the

variant allele were more likely to receive DMARD treatment. However, this was not found to be the case (OR receiving DMARDs compared with not receiving DMARDs if carrying the *PTPN221858*T* variant = 1.36 (95% CI 0.84-2.24), p = 0.19).

Recently, Carlton *et al*. reported that 3 SNPs (rs1310182 (SNP 27), rs3811021 (SNP 36) and rs3789604 (SNP 37)) were associated with susceptibility to RA independently of the *PTPN22*C1858T* polymorphism (6). Their association with IP has not previously been determined. We did not test rs3789604 (SNP 37) in the current study but another SNP (rs3811021 (SNP 36)), which shows strong correlation with rs3789604, was examined. However, we found no association with susceptibility, either in IP as a whole or in the subgroup with RA, for any of the variants tested other than the *PTPN22*C1858T* polymorphism. Similarly, no evidence for association with severity measures was found, although it should be noted that the low minor allele frequencies for some of the SNP markers (particularly for ss38346944 (SNP 23), ss38346943 (SNP 28) and ss38346942 (SNP 34)) meant that power to test such association was limited.

Although the *PTPN22*C1858T* polymorphism was not associated with clinical markers of severity, in the subgroup of patients who fulfilled the ACR classification criteria for RA by 5 years, a statistically significant association with the presence of anti-CCP antibodies at baseline was detected, in keeping with previous reports (11;20). The presence of anti-CCP antibodies is strongly associated with adverse outcome. For example, the odds ratio for the presence of erosions by 2 years in patients who are anti-CCP antibody positive at baseline has been reported as 5.4 (95% CI 1.7-17.0) (21). Hence, although the *PTPN22*1858T* variant is associated with the presence of anti-CCP antibody, it is the autoantibody that appears to determine severity. The findings are in keeping with the hypothesis that the *PTPN22*1858T* allele, like *HLA-DRB1* shared epitope alleles, may create a permissive environment for the production of these antibodies (22).

In summary, carriage of the *PTPN22*1858T* variant allele is associated with susceptibility both to IP and RA, conferring a modest increase in risk of approximately 40%. The association appears to be solely accounted for by this SNP. Although the *PTPN22*1858T* variant allele was associated with the presence of anti-CCP auto antibodies, neither this nor other polymorphisms spanning the gene were found to be clinically useful predictors of adverse outcome.

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