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Rheumatoid arthritis association at 6q23

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Abstract

The Wellcome Trust Case Control Consortium (WTCCC) identified nine single SNPs putatively associated with rheumatoid arthritis at $P = 1 \times 10^{-5}$ - 5×10^{-7} in a genome-wide association screen. One, rs6920220, was unequivocally replicated (trend $P = 1.1 \times 10^{-8}$) in a validation study, as described here. This SNP maps to 6q23, between the genes oligodendrocyte lineage transcription factor 3 (*OLIG3*) and tumor necrosis factor-α-induced protein 3 (*TNFAIP3*).

The WTCCC genome-wide association screen (GWA) of 1,860 rheumatoid arthritis cases and 2,938 healthy controls confirmed association with SNPs within the HLA region and the *PTPN22* gene ($P < 1 \times 10^{-7}$; ref. 1). Nine other loci showed strong evidence for association ($P = 1 \times 10^{-5}$ - 5×10^{-7}). SNPs at these loci were genotyped in an independent cohort of 5,063 rheumatoid arthritis cases and 3,849 healthy controls (Supplementary Methods and Supplementary Table 1 online), using the Sequenom iPlex platform (http://

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AUTHOR CONTRIBUTIONS

W.T., A.B., X.K., S.E. and J.W. participated in the conception, design and coordination of the study, data analysis and writing of the manuscript. D.S., S.H., I.N.B., A.G.W., I.M., A.M., P.E., YEAR Consortium, A.C., S.S., L.H., D.M.R., P.W., P.H. and D.S. recruited study subjects and extracted phenotype data. S.E., A.H. and J.B. performed the genotyping. X.K. undertook the statistical analysis. R.D. contributed to preparation of the manuscript. WTCCC provided the initial data for rheumatoid arthritis cases and controls. Members of the WTCCC and YEAR consortium are listed in the Supplementary Note.

[9]For full lists of members of these consortia, see Supplementary Note online.

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www.sequenom.com), to establish whether they are genuinely associated with the disease. In this cohort, we had 80% power to detect most of the effect sizes reported in the initial study at $P < 0.05$ (Supplementary Table 2 online). We selected ten SNPs for genotyping; these included the known rheumatoid arthritis susceptibility variant rs2476601, mapping to the *PTPN22* gene, and nine previously unknown SNPs identified by the WTCCC study1. A Bonferroni correction of 9 was applied to account for the previously unknown loci investigated, resulting in a *P* value threshold of $P < 0.006$ for claims of significance in this validation study. We regarded SNPs validated at *P* values between 0.05 and 0.006 as suggestive evidence.

We detected strong association with the rheumatoid arthritis-causing SNP in the *PTPN22* gene (rs2476601), as expected (odds ratio (OR) = 1.53, 95% CI = 1.39-1.68, trend $P = 2.0 \times 10^{-18}$). This was not a completely independent replication, as association of rheumatoid arthritis with this locus has been reported in previous studies using some of the same samples included in the current study2-4. However, it confirmed the suitability of this cohort for validation studies. Of the nine newly identified SNPs tested, rs6920220 (G > A) showed association with rheumatoid arthritis in this cohort (OR for minor allele = 1.23, 95% CI = 1.15-1.33, trend $P = 1.1 \times 10^{-8}$) (Table 1). For this SNP, the allele frequencies were similar across control groups tested in the WTCCC study and the healthy controls tested here (minor allele frequency (MAF) 0.22 and 0.21, respectively). We therefore undertook a combined analysis of the WTCCC data and the validation data, and we obtained strong statistical evidence for association between this SNP and rheumatoid arthritis (OR = 1.22, 95% CI = 1.15-1.29, trend $P = 3.6 \times 10^{-12}$) (Table 1).

The validation samples came from six different centers in the UK, raising the possibility that the results were affected by population substructure and heterogeneity. The WTCCC study paid particular attention to the potential effect of population structure on disease association studies and found only 13 loci exhibiting notable geographical variation, none of which overlapped with loci in the current study. In addition, a stratified analysis by center revealed that the association of rs6920220 with rheumatoid arthritis was independently observed in 4 of the 5 centers tested (one center had no controls, and therefore association could not be statistically tested for this center; Supplementary Table 3 online). No heterogeneity was detected among the samples from the different centers or among the samples in the WTCCC study, and combined evidence from the different centers (using a Cochran-Mantel-Haenszel test) attained a significance level virtually the same as that from the combined samples (Supplementary Table 3). This study, therefore, provided convincing evidence for the association of rs6920220 with rheumatoid arthritis.

We did not detect evidence for interaction with *HLA-DRB1* or *PTPN22* susceptibility markers, suggesting that the rs6920220 variant confers an additional effect independent of the two known susceptibility genes. Together, *HLA-DRB1* (shared epitope), *PTPN22* (rs2476601) and rs6920220 account for 8.5% of the total variance in rheumatoid arthritis susceptibility.

Rheumatoid arthritis is a phenotypically heterogeneous disease with a female gender bias and variation in a number of features, such as the presence of autoantibodies (in particular, rheumatoid factor and antibodies to cyclic citrullinated peptides (CCP)) and joint erosions. Analysis of the WTCCC study indicated a significant association for SNP rs6920220 in anti-CCP-positive (OR = 1.38, 95% CI = 1.22-1.55, trend $P = 1.7 \times 10^{-7}$) but not in anti-CCP-negative individuals (OR = 1.03, 95% CI = 0.82-1.29). The same pattern was seen in rheumatoid factor-positive and negative subgroups (Table 2). However, it should be noted that the anti-CCP-negative subgroup was small. In the combined WTCCC and validation cohorts, reasonable numbers in both subgroups were attained, and stronger associations were

again observed in the antibody-positive subjects (Table 2). Indeed, the difference between the anti-CCP-positive and negative subgroups was statistically significant (trend $P = 0.003$). This lends support to the mounting evidence that the clinical phenotype of rheumatoid arthritis may actually comprise at least two genetically distinct subsets, one of which is characterized by the presence of antibody to CCP and is associated with exposure to cigarette smoking5,6.

The rs6920220 SNP maps to an intergenic region of 6q23. Four SNPs genotyped by the HapMap Consortium (rs6933404, rs2327832, rs6927172 and rs17264332) in the same region have a pairwise $r^2 = 1$ with rs6920220, and all five SNPs map to a single linkage-disequilibrium block spanning 60 kb (Supplementary Fig. 1 online). The block contains no known genes or transcripts but does encompass part of the *PTPN11* pseudogene. The latter, notably, is a pseudogene for a locus that was highlighted in a combined analysis of autoimmune diseases (rheumatoid arthritis, type 1 diabetes and inflammatory bowel disease) in the WTCCC study. A recent report has suggested that up to 20% of pseudogenes may be transcribed, but none have been reported to be functional in humans to date7. The block lies between the genes *OLIG3* and *TNFAIP3* (also known as *A20*). *OLIG3* appears to be important in development and differentiation of neuronal cells8, and therefore it is not an obvious candidate for association with rheumatoid arthritis susceptibility. The *TNFAIP3* gene acts as a negative regulator of the transcription factor NF-κB in response to tumor necrosis factor-α and toll-like receptor-induced signals but not in response to IL-1β-induced activation9,10. Mice deficient for the TNFAIP3 protein developed multiorgan inflammation, including inflammation of joints11.

Of the remaining eight SNPs, we found nominal statistical evidence for association of rs743777 mapping to the *IL2RB* gene and observed the most significant effect under a recessive model ($P = 0.005$), in line with the findings from the WTCCC study1 (Supplementary Table 4 online). In the combined cohort analysis, this SNP showed modest evidence for association (OR = 1.13, 95% CI = 1.07-1.19; trend $P = 5.3 \times 10^{-6}$), suggesting the effect, if any, is weak. Larger studies or meta-analysis of additional cohorts may be required to confirm this association. The WTCCC study observed that rs11761231, located close to *PODXL*, had a sex-differentiated effect in rheumatoid arthritis1. The present study provided only borderline evidence (trend $P = 0.042$) to support this observation (Supplementary Table 4). Although a stronger association was observed in females (trend $P = 0.025$) than in the combined male and female samples (and no significant association with rheumatoid arthritis was observed in males alone), a two-degree-of-freedom sex-differentiated test that combined trend tests in males and females did not reach a significance level of $P < 0.05$. For the other six SNPS, one (rs3816587) failed genotyping quality control measures, and no evidence for association was detected for any of the others in the validation dataset (Supplementary Table 4). Notably, rs2104286, which maps to *IL2RA*, a gene already associated with a number of other autoimmune diseases (type 1 diabetes, multiple sclerosis and Graves' disease) and known to play a role in T cell regulation, was not associated with rheumatoid arthritis in this dataset12,13.

In conclusion, a previously unknown locus has emerged for rheumatoid arthritis, and the challenge will now be to characterize the etiological variant and determine its role in susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Replication of rs6920220 association with rheumatoid arthritis

Summary statistics						
	WTCCC study		**Validation study**		**Combined samples**	
	Cases, *n* (%)	**Controls, *n* (%)**	**Cases, *n* (%)**	**Controls, *n* (%)**	**Cases, *n* (%)**	**Controls, *n* (%)**
Allele						
G	2,737 (73.7)	4,563 (77.7)	7,274 (75.3)	5,840 (79.0)	9,979 (74.9)	10,403 (78.5)
A	977 (26.3)	1,307 (22.3)	2,370 (24.7)	1,550 (21.0)	3,347 (25.1)	2,857 (21.5)
Genotype						
GG	1,007 (54.2)	1,757 (59.9)	2,713 (56.5)	2,287 (61.9)	3,311 (55.4)	3,137 (60.6)
AG	723 (38.9)	1,049 (35.7)	1,816 (37.8)	1,266 (34.3)	2,291 (38.3)	1,836 (35.5)
AA	127 (6.8)	129 (4.4)	277 (5.8)	142 (3.8)	372 (6.2)	204 (3.9)

Association analysis						
	WTCCC study		**Validation study**		**Combined samples**	
Test	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Allelic (A allele)	1.25 (1.13-1.37)	6.1×10^{-6}	1.23 (1.15-1.33)	1.6×10^{-8}	1.22 (1.15-1.29)	5.9×10^{-12}
Trend		5.0×10^{-6}		1.1×10^{-8}		3.6×10^{-12}
Genotypic		1.6×10^{-5}		5.8×10^{-8}		1.4×10^{-11}
GG	1.00 (reference)		1.00 (reference)		1.00 (reference)	
AG	1.20 (1.06-1.36)	0.003	1.21 (1.11-1.33)	4.1×10^{-5}	1.19 (1.11-1.28)	1.6×10^{-6}
AA	1.72 (1.33-2.22)	3.1×10^{-5}	1.65 (1.33-2.03)	2.8×10^{-6}	1.62 (1.38-1.90)	2.7×10^{-9}

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Table 2

Association of rs6920220 after stratification of rheumatoid arthritis cases according to subphenotypes (anti-CCP and rheumatoid factor)

Anti-CCP

	WTCCC study			Validation study			Combined samples		
	Positive vs. control	**Negative vs. control**	**Positive vs. negative**	**Positive vs. control**	**Negative vs. control**	**Postive vs. negative**	**Positive vs. control**	**Negative vs. control**	**Positive vs. negative**
Sample size	881 vs. 2,935	224 vs. 2,935	881 vs. 224	1,634 vs. 3,694	815 vs. 3,694	1,634 vs. 815	2,515 vs. 6,629	1,039 vs. 6,629	2,515 vs. 1,039
Allelic OR (vs. controls)	1.38 (1.22-1.55)	1.03 (0.82-1.29)	1.34 (1.05-1.71)	1.31 (1.19-1.45)	1.15 (1.01-1.30)	1.14 (0.99-1.31)	1.32 (1.23-1.43)	1.10 (0.99-1.23)	1.20 (1.06-1.35)
P value for allelic test	2.0×10^{-7}	n.s.	0.020	3.9×10^{-8}	0.037	n.s.	2.9×10^{-13}	n.s.	0.003
P value for trend test	1.7×10^{-7}	n.s.	0.019	2.9×10^{-8}	0.034	n.s.	1.8×10^{-13}	n.s.	0.003
P value for genotypic test	1.6×10^{-7}	n.a.	n.a.	1.6×10^{-7}	n.s.	n.s.	3.7×10^{-13}	n.s.	0.002

Rheumatoid factor

	WTCCC study			Validation study			Combined samples		
	Positive vs. control	**Negative vs. control**	**Positive vs. negative**	**Positive vs. control**	**Negative vs. control**	**Positive vs. negative**	**Positive vs. control**	**Negative vs. control**	**Positive vs. negative**
Sample size	1,307 vs. 2,935	251 vs. 2,935	1,307 vs. 251	3,234 vs. 3,694	1,255 vs. 3,694	3,234 vs. 1,255	4,541 vs. 6,629	1,506 vs. 6,629	4,541 vs. 1,506
Allelic OR (vs. controls)	1.32 (1.19-1.47)	1.00 (0.81-1.25)	1.32 (1.05-1.65)	1.28 (1.19-1.39)	1.12 (1.00-1.25)	1.15 (1.03-1.28)	1.28 (1.20-1.36)	1.08 (0.98-1.18)	1.19 (1.08-1.31)
P value for allelic test	2.6×10^{-7}	n.s.	0.017	7.0×10^{-10}	0.042	0.014	1.4×10^{-14}	n.s.	5.0×10^{-5}
P value for trend test	2.0×10^{-7}	n.s.	0.017	4.6×10^{-10}	0.039	0.013	7.6×10^{-15}	n.s.	4.5×10^{-5}
P value for genotypic test	5.4×10^{-7}	n.s.	0.047	2.3×10^{-9}	n.s.	0.038	2.3×10^{-14}	n.s.	0.0014

n.a., no test was carried out due to limited numbers in a cell of the 2 × 3 genotype table. n.s., not significant (*P* value threshold at 0.05).