Europe PMC Funders Group
Author Manuscript
Nat Genet. Author manuscript; available in PMC 2009 April 01.

Published in final edited form as:
Nat Genet. 2008 October ; 40(10): 1156–1159. doi:10.1038/ng.218.

Identification of novel RA susceptibility loci at chromosomes 10p15, 12q13 and 22q13

Anne Barton[1,*], **Wendy Thomson**[1], **Xiayi Ke**[1], **Steve Eyre**[1], **Anne Hinks**[1], **John Bowes**[1], **Darren Plant**[1], **Laura J Gibbons**[1], **Wellcome Trust Case Control Consortium**[7], **YEAR Consortium**[8], **BIRAC Consortium**[9], **Anthony G Wilson**[2], **Deborah E Bax**[2], **Ann W Morgan**[3], **Paul Emery**[3], **Sophia Steer**[4], **Lynne Hocking**[5], **David M Reid**[5], **Paul Wordsworth**[6], **Pille Harrison**[6], and **Jane Worthington**[1]

[1]**arc**-Epidemiology Unit, Stopford Building, The University of Manchester, Manchester, UK

[2] School of Medicine & Biomedical Sciences, University of Sheffield, Sheffield S10 2JF

[3] Academic Unit of Musculoskeletal Disease, Chapel Allerton Hospital, Leeds, LS7 4SA

[4] Clinical and Academic Rheumatology, Kings College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS

[5]Bone Research Group, Department of Medicine & Therapeutics, University of Aberdeen, UK, AB25 2ZD

[6] University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre, Oxford OX3 7LD, UK

Abstract

The WTCCC study identified 49 single nucleotide polymorphisms (SNPs) putatively associated with RA at $p=1\times10^{-4}$-1×10^{-5} (Tier 3). Here, we show that 3 of these SNPs, mapping to chromosome 10p15 (rs4750316), 12q13 (rs1678542) and 22q13 (rs3218253), are also associated (trend $p = 4\times10^{-5}$, $p=4\times10^{-4}$ and $p=4\times10^{-4}$, respectively) in a validation study of 4,106 RA cases and an expanded reference group of 11,238 subjects, confirming them as true susceptibility loci in Caucasians.

Rheumatoid arthritis (RA) is a common autoimmune disease, affecting an estimated 475,000 adults in the UK, in which inflammation of synovial joints is characteristic. The WTCCC genome wide association screen of 1860 RA case and 2938 healthy control Caucasian samples confirmed association of SNPs with previously identified loci within the *HLA* region and the *PTPN22* gene (Tier1, $p<5\times10^{-7}$)1. Nine loci were associated in the next tier of significance (Tier 2, $p=1\times10^{-5}$-5×10^{-7}) and association to one, on chromosome 6q23 has been replicated and confirmed in subsequent independent studies2, 3. Forty-nine SNPs were associated at $p = 1 \times 10^{-4}$ - 1×10^{-5} (Tier 3) and we now describe investigation of these SNPs in an independent cohort of 4,106 RA samples (supplementary methods). Causal variants within this group of SNPs are likely to confer small effect sizes, by virtue of the fact that the evidence for association with them in the original WTCCC study was weaker than for those SNPs in Tiers 1 and 2. In order to maximise power to replicate true associations, genotype data from an independent cohort of 3,599 healthy controls was combined with that

[*]Corresponding author: Dr Anne Barton, Email: anne.barton@manchester.ac.uk.
[7]WTCCC (Supplementary Note online)
[8]YEAR Consortium (Supplementary note online)
[9]BIRAC Consortium (Supplementary note online)

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

from the 4 non-autoimmune disease case subjects (coronary artery disease, hypertension, type 2 diabetes and bipolar disorder) genotyped as part of the WTCCC study to create a large reference sample (total sample size of expanded reference group = 11,238)1. A Bonferroni correction of 49 was applied to account for the novel loci investigated, resulting in a p-value threshold of $P<0.001$ for claims of significance in this validation study. The validation cohort had 80% power to detect most of the effect sizes reported in the initial study at $P<0.001$(supplementary Table 1). In addition, we took the opportunity to re-evaluate the evidence for association with the Tier 2 SNPs using the expanded reference group for comparison.

Of the 49 novel Tier 3 SNPs tested, 3 showed association with RA: rs4750316 (C>G), mapping to chromosome 10p15 (OR minor allele =0.87, 95% CI 0.81-0.93, trend $P = 4 \times 10^{-5}$); rs1678542 (G>C) mapping to chromosome 12q13 (OR minor allele = 0.91, 95% CI 0.86-0.96, trend $P = 4 \times 10^{-4}$) and rs3218253 (C>T) mapping to chromosome 22 (OR minor allele = 1.11, 95% CI 1.05 - 1.17, trend $P = 4 \times 10^{-4}$) (Table 1, supplementary Tables 2 and 3). For all SNPs, the allele frequencies were similar across the control group tested in the WTCCC study and the expanded reference group tested here (rs4750316 minor allele frequency (MAF) 0.20 and 0.20; rs1678542 MAF 0.37 and 0.37; rs3218254 MAF 0.25 and 0.26, respectively) and all satisfied Hardy-Weinberg expectations. Both combined analysis and meta-analysis of the WTCCC data and the validation data provided strong statistical evidence for association between RA and all 3 SNPs (combined analysis: OR rs4750316 = 0.85 95% CI 0.80-0.90, trend $P = 1.3\times10^{-8}$; OR rs1678542 = 0.88, 95% CI 0.85-0.93, trend $P = 1.3 \times 10^{-7}$; OR rs3218253 = 1.13, 95% CI 1.07-1.18, trend $P = 1.3 \times 10^{-6}$) (Table 1 and supplementary Table 2).

RA is characterised by the presence of autoantibodies in some but not all patients. Previous work has shown that established susceptibility variants, including *HLA DRB1* and the chromosome 6q23 locus, have a stronger effect in the subgroup harbouring antibodies to citrullinated peptide antibodies (ACPA) recognised by the anti-CCP assay2, 4. Stratification analysis showed that, for all 3 loci, the direction and strength of effect size was similar in antibody positive and negative individuals although this did not always achieve statistical significance in the anti-CCP negative subgroup due to the smaller sample size (supplementary Table 4). Hence, these loci appear to be associated with RA *per se* rather than subgroups of the disease.

rs4750316 maps to an intergenic region of 10p15. Four SNPs genotyped by the HapMap Consortium (rs10752291, rs10796045, rs2146900 and rs1570527) in the same region have a pair-wise r^2 >0.96 with rs4750316 and all 5 SNPs map within 12Kb of each other in a single linkage disequilibrium (LD) block containing no known genes or transcripts (supplementary Figure 1). The closest gene lies 76Kb from rs4750316 and is the *PRKCQ* gene, which encodes an atypical member of the protein kinase C family of proteins, PKCθ. It is thought to play a key role in T cell activation and PKCθ-deficient T cells in mice show a profound defect in IL-2 production and proliferation upon CD3/CD28 stimulation (reviewed in 5). As RA is thought to be primarily a T-cell mediated autoimmune disease, investigating the role of the associated SNPs in regulation of the *PRCKQ* gene will be a priority.

rs1678542 maps to intron 15 of the *KIF5A* gene (supplementary Figure 1). The gene encodes a kinesin heavy chain and mutations of the gene have been associated with hereditary spastic paraplegia 6. Hence, it is not an obvious candidate RA susceptibility gene. *KIF5A* maps between the dynactin2 (*DCTN2*) and the phosphatidylinositol 4 phosphate 5 kinase, type II, gamma (*PIP5K2C*) genes. The latter is expressed in B cells and is thought to play a role in B-cell receptor activation as well as development and function of B cells 7. It, therefore, represents a stronger candidate for a disease characterized by the presence of

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

autoantibodies. There are now several examples where an associated variant mapping within one gene has subsequently been found to regulate a different gene but further investigation is required to determine whether this is true of the rs1678542 polymorphism 8, 9.

rs3218253 maps to the interleukin 2 receptor B (*IL2RB*) gene (supplementary Figure 1). We have previously reported borderline evidence for association with another SNP in the gene (rs743777)2. Combining the data from the validation cases, the expanded reference group and original WTCCC data, the evidence for association of the rs743777 SNP was strengthened (trend $P = 4.6 \times 10^{-8}$) (Table 2). For both SNPs, the evidence for association was even stronger under a recessive model (rs3218253 $P = 4.4 \times 10^{-8}$ and rs743777 $P = 5.2 \times 10^{-9}$, respectively). rs743777 maps 6Kb upstream of the transcription start site of *IL2RB* whilst rs3218253 maps to intron 1 but both SNPs lie within a single LD block spanning 6.8Kb and show reasonable correlation with each other ($r^2 = 0.77$). Therefore, haplotype analysis was undertaken. Haplotype frequencies were significantly different between cases and controls but the effect of the haplotype of minor alleles at each locus was not significantly increased over that of the minor allele at rs743777 alone (data not shown). Furthermore, logistic regression analysis suggested that the effect at rs3218253 could be fully accounted for by the genotype at rs743777, suggesting that the latter SNP is primarily driving the association and the association with rs3218253 is arising as a result of its LD with rs743777 (p value for rs3218253 after conditioning on rs743777 = 0.60). The *IL2RB* gene is an attractive candidate for an autoimmune disease because of the key role played by IL2 in T cell activation and regulation. The *IL2RB* gene encodes the beta unit of the IL2R, which is present in the moderate and high affinity forms of the receptor required for signal transduction from IL2 10.

Given that the evidence for association with *IL2RB* was strengthened by analysing all the available data including the expanded reference cohort, we were interested to investigate whether evidence for association with any of the other SNPs tested previously from Tier 2 was increased. In the expanded validation samples, 5 of the 9 Tier 2 SNPs showed evidence for association ($p < 0.05$) and, in a combined analysis of 6,923 RA cases and 14,425 combined reference samples, the strength of evidence for association for 4 of these 5 loci was increased over that seen in the original WTCCC study (Table 2). These included the SNP mapping to 6q23, which has previously been confirmed independently as being associated with RA susceptibility and the *IL2RB* SNP (rs743777) already discussed 2, 3. In addition, the evidence for association to SNPs mapping to the membrane metallo-endopeptidase-like 1 (*MMEL1*) and *IL2RA* genes (OR 0.90, 95% CI 0.86-0.94, $p = 8.2 \times 10^{-6}$ and OR 0.90 95% CI 0.86-0.94, $p = 6.4 \times 10^{-6}$, respectively) was enhanced. Interestingly, in analysis of an independent cohort of RA cases and controls from Europe and the United States published in this issue of Nature Genetics, evidence for association of a SNP mapping to the *MMEL1* gene region has also been reported ($P = 0.01$). Furthermore, that study also independently replicates the findings for the *PRKCQ* and *KIF5A* loci reported here ($P = 0.03$ and 0.009, respectively) suggesting that these regions harbour true RA susceptibility variants with modest but important effects11.

In conclusion, novel RA susceptibility loci, mapping close to the *PRKCQ* and *KIF5A* genes have been identified in Caucasian samples and evidence for a recessive effect at a third, the *IL2RB* gene, is compelling. In addition, evidence for association to the *MMEL1* and *IL2RA* gene regions is mounting. The challenge will now be to identify and characterise the aetiological variants of the confirmed RA susceptibility genes in order to determine how they predispose to RA12.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Arthritis Research Campaign for their support. **arc** grant reference no.: 17552. We would also like to acknowledge the NHS Research and Development Support Fund for Guy's and St Thomas' and Lewisham NHS Trusts. We acknowledge use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. We thank the YEAR consortium for allowing us access to their DNA collection. DP is supported by the European Community's Sixth Framework Programme Autocure funding. IT support was provided by Phil Steer and technical support by Paul Gilbert, Bhaneeta Lad and Stephen G Martin. Nursing support was provided by Janet Grumley, Julie Shotton and Claire Farrar.

Reference List

1. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–678. [PubMed: 17554300]
2. Thomson W, et al. Rheumatoid arthritis association at 6q23. Nat. Genet. 2007; 39:1431–1433. [PubMed: 17982455]
3. Plenge RM, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat. Genet. 2007; 39:1477–1482. [PubMed: 17982456]
4. Huizinga TW, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum. 2005; 52:3433–3438. [PubMed: 16255021]
5. Hayashi K, Altman A. Protein kinase C theta (PKCtheta): a key player in T cell life and death. Pharmacol. Res. 2007; 55:537–544. [PubMed: 17544292]
6. Fichera M, et al. Evidence of kinesin heavy chain (KIF5A) involvement in pure hereditary spastic paraplegia. Neurology. 2004; 63:1108–1110. [PubMed: 15452312]
7. Niiro H, Clark EA. Branches of the B cell antigen receptor pathway are directed by protein conduits Bam32 and Carma1. Immunity. 2003; 19:637–640. [PubMed: 14614850]
8. Moffatt MF, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature. 2007; 448:470–473. [PubMed: 17611496]
9. Wang Y, Jiang H. Identification of a distal STAT5-binding DNA region that may mediate growth hormone regulation of insulin-like growth factor-I gene expression. J. Biol. Chem. 2005; 280:10955–10963. [PubMed: 15677453]
10. Kim HP, Imbert J, Leonard WJ. Both integrated and differential regulation of components of the IL-2/IL-2 receptor system. Cytokine Growth Factor Rev. 2006; 17:349–366. [PubMed: 16911870]
11. Raychaudhuri S, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat.Genet. 2008
12. Barton A, et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. Hum. Mol. Genet. 2008

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Table 1

Replication of Tier 3 WTCCC RA susceptibility loci

SNP	Validation study [a]: n (%)								Combined analysis[b]: n (%)								Locus
	Cases (n = 4,106)			Controls (n = 11,238)			OR (95% CI)	p-trend	Cases (n = 5,968)			Controls (n = 14,176)			OR (95% CI)	p-trend	
	11	12	22	11	12	22			11	12	22	11	12	22			
rs4750316[d]	2766 (68.3)	1159 (28.6)	124 (3.1)	7283 (64.9)	3511 (31.3)	429 (3.8)	0.87 (0.81-0.93)	3.9×10^{-5}	4068 (68.9)	1659 (28.1)	180 (3.1)	9171 (64.8)	4445 (31.4)	542 (3.8)	0.85 (0.80-0.90)	1.3×10^{-8}	*PRKCQ*
rs1678542[e]	1692 (42.7)	1797 (45.3)	478 (12.0)	4487 (40.1)	5128 (45.9)	1566 (14.0)	0.91 (0.86-0.96)	0.0004	2523 (43.3)	2616 (44.9)	684 (11.7)	5661 (40.1)	6455 (45.7)	1999 (14.2)	0.88 (0.85-0.93)	1.3×10^{-7}	*KIF5A*
rs3218253[c,f]	2120 (52.1)	1587 (39.0)	360 (8.9)	6053 (54.2)	4365 (39.1)	759 (6.8)	1.11 (1.05-1.17)	0.0004	3071 (51.9)	2336 (39.5)	512 (8.7)	7667 (54.4)	5517 (39.1)	912 (6.5)	1.13 (1.07-1.18)	1.3×10^{-6}	*IL2RB*

Genotype counts are shown, with percentages in parentheses. 1 = common allele, 2 = minor allele

OR (95% CI) - Minor allele odds ratio and 95% confidence intervals; p-trend - p value under additive model

[a] The expanded reference group included 3,599 healthy controls, 1,868 bipolar disorder samples, 1,952 hypertension samples, 1,924 Type 2 diabetes samples and 1,894 Coronary heart disease samples.

[b] WTCCC cases plus validation cases were compared with WTCCC controls plus validation expanded reference group.

[c] p values under a recessive model equal to 1.6×10^{-5} and 4.4×10^{-8} in the validation study and in the combined analysis, respectively.

[d] meta-analysis of validation and WTCCC data, OR 0.85 95%CI 0.80-0.90, $p = 1.9 \times 10^{-8}$

[e] meta-analysis of validation and WTCCC data, OR 0.89 95%CI 0.85-0.93, $p = 1.7 \times 10^{-7}$

[f] meta-analysis of validation and WTCCC data, OR 1.13 95%CI 1.08-1.19, $p = 6.1 \times 10^{-7}$

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Nat Genet. Author manuscript; available in PMC 2009 April 01.

Table 2

Tier2 SNP re-analysis including WTCCC non-autoimmune disease samples as expanded control panel

SNP	Validation study [a]: n (%)								Combined analysis[b]: n (%)								Locus
	Cases (n =5,063)			Controls (n =11,487)			OR (95% CI)	p-trend	Cases (n=6,923)			Controls (n=14,425)			OR (95% CI)	p-trend	
	11	12	22	11	12	22			11	12	22	11	12	22			
rs10910099[c,f]	495 (10.6)	2020 (43.2)	2159 (46.2)	1279 (11.5)	4913 (44.2)	4922 (44.3)	0.94 (0.89-0.99)	0.02	646 (9.9)	2817 (43.3)	3042 (46.8)	1609 (11.5)	6262 (44.7)	6148 (43.8)	0.90 (0.86-0.94)	8.2×10^{-6}	In LD with rs6684865 MMEL1
rs3816587[d]	698 (17.3)	1969 (48.7)	1372 (34.0)	1977 (17.6)	5387 (48.0)	3863 (34.4)	1.03 (0.97-1.09)	NS	1086 (18.4)	2806 (47.6)	2005 (34.0)	2437 (17.2)	6853 (48.4)	4873 (34.4)	1.05 (1.01-1.10)	0.03	
rs11162922	18 (0.4)	525 (11.3)	4123 (88.4)	36 (0.3)	1331 (11.9)	9786 (87.7)	0.96 (0.86-1.06)	NS	22 (0.3)	694 (10.7)	5800 (89.0)	48 (0.3)	1729 (12.3)	12306 (87.4)	0.87 (0.80-0.95)	0.002	
rs6920220[g]	272 (5.8)	1769 (37.8)	2639 (56.4)	499 (4.4)	4005 (35.6)	6761 (60.0)	1.15 (1.09-1.22)	9.3×10^{-7}	399 (6.1)	2492 (38.1)	3646 (55.8)	628 (4.4)	5054 (35.6)	8518 (60.0)	1.18 (1.12-1.24)	2.3×10^{-11}	OLIG3-TNFAIP3
rs11761231[h]	561 (12.0)	2118 (45.3)	2001 (42.8)	1456 (12.9)	5193 (46.1)	4620 (41.0)	0.94 (0.90-0.99)	0.02	750 (11.5)	2954 (45.2)	2832 (43.3)	1891 (13.3)	6528 (46.0)	5787 (40.7)	0.91 (0.87-0.95)	1.2×10^{-5}	PODXL
rs2104286[i]	312 (6.7)	1740 (37.3)	2608 (56.0)	790 (7.0)	4464 (39.6)	6006 (53.3)	0.93 (0.88-0.98)	0.007	415 (6.4)	2442 (37.5)	3660 (56.2)	1036 (7.3)	3652 (39.8)	7506 (52.9)	0.90 (0.86-0.94)	6.4×10^{-6}	IL2RA
rs9550642	47 (1.0)	835 (17.8)	3796 (81.1)	113 (1.0)	1985 (17.6)	9162 (81.4)	1.01 (0.93-1.10)	NS	71 (1.1)	1202 (18.4)	5261 (80.5)	131 (0.9)	2444 (17.2)	11616 (81.9)	1.09 (1.02-1.17)	0.02	CRYL1
rs2837960	153 (3.3)	1332 (28.5)	3194 (68.3)	352 (3.1)	3206 (28.5)	7703 (68.4)	1.01 (0.95 - 1.08)	NS	238 (3.7)	1849 (28.4)	4415 (67.9)	412 (2.9)	4091 (28.8)	9689 (68.3)	1.04 (0.98-1.10)	NS	
rs743777[e,j]	532 (11.4)	2031 (43.4)	2117 (45.2)	1040 (9.3)	4832 (43.1)	5328 (47.6)	1.11 (1.05-1.17)	0.0001	753 (11.5)	2829 (43.3)	2946 (45.1)	1262 (8.9)	6093 (43.2)	6761 (47.9)	1.13 (1.08-1.18)	4.6×10^{-8}	IL2RB

p-trend - p value under additive model

[a] The expanded reference group included 3,599 healthy controls, 1,868 bipolar disorder samples, 1,952 hypertension samples, 1,924 Type 2 diabetes samples and 1,894 Coronary heart disease samples.

[b] WTCCC cases plus validation cases were compared with WTCCC controls plus validation expanded reference group

[c] The marker genotyped and found to be associated with RA in the WTCCC was rs6684865 but the marker genotyped in the current study, rs10910099, shows high correlation ($r^2 = 0.95$) and serves as a proxy for it.

[d] Not included in original analysis due to low genotyping success rate (<80%), now re-genotyped in 3,595 cases and 3,214 controls

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

[e] p values under a recessive model equal to 6.3×10^{-5} and 5.2×10^{-9} in the validation study and in the combined analysis, respectively.

[f] meta-analysis of validation and WTCCC data, OR 0.91 95%CI 0.87-0.95, p = 1.2×10^{-5}

[g] meta-analysis of validation and WTCCC data, OR 1.17 95%CI 1.12-1.23, p = 9.4×10^{-11}

[h] meta-analysis of validation and WTCCC data, OR 0.91 95%CI 0.87-0.95, p = 1.0×10^{-5}

[i] meta-analysis of validation and WTCCC data, OR 0.89 95%CI 0.85-0.94, p = 3.4×10^{-6}

[j] meta-analysis of validation and WTCCC data, OR 1.14 95%CI 1.09-1.19, p = 2.5×10^{-8}