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## Genetic variants at *CD28*, *PRDM1*, and *CD2/CD58* are associated with rheumatoid arthritis risk

Soumya Raychaudhuri<sup>1,3,††</sup>, Brian P. Thomson<sup>2</sup>, Elaine F. Remmers<sup>4</sup>, Stephen Eyre<sup>5</sup>, Anne Hinks<sup>5</sup>, Candace Guiducci<sup>2</sup>, Joseph J. Catanese<sup>6</sup>, Gang Xie<sup>7</sup>, Eli A. Stahl<sup>1</sup>, Robert Chen<sup>1</sup>, Lars Alfredsson<sup>8</sup>, Christopher I. Amos<sup>9</sup>, Kristin G. Ardlie<sup>2</sup>, BIRAC Consortium<sup>†</sup>, Anne Barton<sup>5</sup>, John Bowes<sup>5</sup>, Noel P. Burtt<sup>2</sup>, Monica Chang<sup>6</sup>, Jonathan Coblyn<sup>1</sup>, Karen H. Costenbader<sup>1</sup>, Lindsey A. Criswell<sup>10</sup>, J. Bart A. Crusius<sup>11</sup>, Jing Cui<sup>1</sup>, Phillip L. De Jager<sup>2,12</sup>, Bo Ding<sup>8</sup>, Paul Emery<sup>13</sup>, Edward Flynn<sup>5</sup>, Pille Harrison<sup>14</sup>, Lynne J. Hocking<sup>15</sup>, Tom W. J. Huizinga<sup>16</sup>, Daniel L. Kastner<sup>4</sup>, Xiayi Ke<sup>5</sup>, Fina A. S. Kurreeman<sup>1,16</sup>, Annette T. Lee<sup>17</sup>, Xiangdong Liu<sup>7</sup>, Yonghong Li<sup>6</sup>, Paul Martin<sup>5</sup>, Ann W. Morgan<sup>13</sup>, Leonid Padyukov<sup>18</sup>, David M. Reid<sup>15</sup>, Mark Seielstad<sup>19</sup>, Michael F. Seldin<sup>20</sup>, Nancy A. Shadick<sup>1</sup>, Sophia Steer<sup>21</sup>, Paul P. Tak<sup>22</sup>, Wendy Thomson<sup>5</sup>, Annette H. M. van der Helm-van Mil<sup>16</sup>, Irene E. van der Horst-Bruinsma<sup>23</sup>, Michael E. Weinblatt<sup>1</sup>, Anthony G. Wilson<sup>24</sup>, Gert Jan Wolbink<sup>25,26</sup>, Paul Wordsworth<sup>14</sup>, YEAR Consortium<sup>†</sup>, David Altshuler<sup>2,3</sup>, Elizabeth W. Karlson<sup>1</sup>, Rene E. M. Toes<sup>16</sup>, Niek de Vries<sup>22</sup>, Ann B. Begovich<sup>6,27</sup>, Katherine A. Siminovitch<sup>7</sup>, Jane Worthington<sup>5</sup>, Lars Klareskog<sup>18</sup>, Peter K. Gregersen<sup>17</sup>, Mark J. Daly<sup>2,3</sup>, and Robert M. Plenge<sup>1,2,††</sup>

<sup>1</sup>Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, 02115, USA. <sup>2</sup>Broad Institute, Cambridge, Massachusetts, 02142 USA <sup>3</sup>Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, 02114, USA. <sup>4</sup>Genetics and Genomics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, US National Institutes of Health, Bethesda, Maryland 20892, USA. <sup>5</sup>Arthritis Research Campaign (arc)–Epidemiology Unit, Stopford Building, The University of Manchester, Manchester M13 9PT, United Kingdom. <sup>6</sup>Celera, Alameda, California 94502, USA. <sup>7</sup>Dept of Medicine, University of Toronto, Mount Sinai Hospital and University Health Network, Toronto, Ontario M5G 1X5, Canada. <sup>8</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden. <sup>9</sup>University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, USA. <sup>10</sup>Rosalind Russell Medical Research Center for Arthritis, Department of Medicine, University of California, San Francisco, California 94143, USA. <sup>11</sup>Laboratory of Immunogenetics, Department of Pathology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands. <sup>12</sup>Department of Neurology, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115, USA. <sup>13</sup>NIHR-Leeds Musculoskeletal Biomedical Research Unit, Leeds

URLs Gene Relationships Across Implicated Loci (www.broad.mit.edu/mpg/grail/ Illumina Genotype Control Database (www.illumina.com)

<sup>&</sup>lt;sup>††</sup>Corresponding authors .

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<sup>&</sup>lt;sup>†</sup>Supplementary Note Online

Institute of Molecular Medicine, University of Leeds, LS9 7TF, United Kingdom. <sup>14</sup>University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre, Oxford OX3 7LD, United Kingdom. <sup>15</sup>Musculoskeletal and Genetics Section, Division of Applied Medicine, University of Aberdeen, AB25 2ZD, United Kingdom. <sup>16</sup>Department of Rheumatology, Leiden University Medical Centre, 2333 ZA Leiden, The Netherlands. <sup>17</sup>The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, New York 11030, USA. <sup>18</sup>Rheumatology Unit, Department of Medicine, Karolinska Institutet at Karolinska University Hospital Solna, Stockholm 171 76, Sweden. <sup>19</sup>Genome Institute of Singapore, Singapore 138672. <sup>20</sup>Rowe Program in Genetics, University of California at Davis, Davis, California 95616, USA. <sup>21</sup>Clinical and Academic Rheumatology, Kings College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS, United Kingdom. <sup>22</sup>Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam 1105AZ, The Netherlands. <sup>23</sup>Department of Rheumatology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands. <sup>24</sup>School of Medicine & Biomedical Sciences, Sheffield University, Sheffield S10 2JF, United Kingdom. <sup>25</sup>Jan van Breemen Institute, 1056 AB Amsterdam, The Netherlands. <sup>26</sup>Sanguin Research Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, 1006 AD Amsterdam, The Netherlands. <sup>27</sup>Roche Diagnostics, Pleasanton, CA 94588 USA.

### Abstract

To discover novel RA risk loci, we systematically examined 370 SNPs from 179 independent loci with p<0.001 in a published meta-analysis of RA GWAS of 3,393 cases and 12,462 controls<sup>1</sup>. We used *GRAIL*<sup>2</sup>, a computational method that applies statistical text mining to PubMed abstracts, to score these 179 loci for functional relationships to genes in 16 established RA disease loci<sup>1,3-11</sup>. We identified 22 loci with a significant degree of functional connectivity. We genotyped 22 representative SNPs in an independent set of 7,957 cases and 11,958 matched controls. Three validate convincingly: *CD2/CD58* (rs11586238,  $p=1\times10^{-6}$  replication,  $p=1\times10^{-9}$  overall), and *CD28* (rs1980422,  $p=5\times10^{-6}$  replication,  $p=1\times10^{-9}$  overall), *PRDM1* (rs548234,  $p=1\times10^{-5}$  replication,  $p=2\times10^{-8}$  overall). An additional four replicate (p<0.0023): *TAGAP* (rs394581, p=0.0002 replication,  $p=4\times10^{-7}$  overall), *PTPRC* (rs10919563, p=0.0003 replication,  $p=7\times10^{-7}$  overall), *TRAF6/RAG1* (rs540386, p=0.0008 replication,  $p=4\times10^{-6}$  overall), and *FCGR2A* (rs12746613, p=0.0022 replication,  $p=2\times10^{-5}$  overall). Many of these loci are also associated to other immunologic diseases.

Rheumatoid arthritis is a chronic autoimmune disease characterized by an inflammatory polyarthritis<sup>12</sup>. Genetic studies have now identified multiple risk alleles for autoantibody positive RA within the *MHC* region, a *PTPN22* missense allele, and risk alleles in 14 other loci (see Table 1)<sup>1,3-11</sup>. Most RA risk loci contain multiple genes, and currently the causal genes are unknown. However, most contain at least one plausible biological candidate gene involved in immune regulation, and these genes suggest an important set of processes involved in RA pathogenesis. For example, risk alleles highlight genes involved in T-cell activation by antigen presenting cells (class II *MHC* region, *PTPN22, STAT4*, and *CTLA4*), the *NF-кB* signaling pathway (*CD40, TRAF1, TNFSF14*, and *TNFAIP3*, and the recent report of *REL*<sup>13</sup>), citrullination (*PADI4*), natural killer cells (*CD244*), and chemotaxis (*CCL21*).

Based on these observations, we hypothesized that as yet undiscovered autoantibody positive RA risk loci might also contain genes with functions similar to those of genes in known risk loci. Therefore, known RA risk loci can be used to prioritize SNPs for replication from GWAS, especially SNPs with modest statistical support, in independent samples (Figure 1).

To objectively quantify the degree of functional similarity between genes within candidate loci (identified from GWAS) and genes within validated RA risk loci, we used a published functional genomics method **GRAU** (Gene Relationships Across Implicated Loci)<sup>2</sup>

loci (identified from GWAS) and genes within validated RA risk loci, we used a published functional genomics method, *GRAIL* (Gene Relationships Across Implicated Loci)<sup>2</sup>. GRAIL quantifies functional similarity between genes by applying established statistical text mining methods<sup>14</sup> to text from a reference database of 250,000 published scientific abstracts about human and model organism genes. For each locus, GRAIL identifies the gene with the greatest number of observed relationships. GRAIL estimates the statistical significance of the number of observed relationships with a null model where relationships between genes near SNPs occur by random chance. This significance score,  $p_{text}$ , represents the output *GRAIL* score. GRAIL is already able to effectively identify functional interconnectivity between genes within the previously known RA loci (Figure 2); it might also be able to establish connections between these 16 loci and as yet undiscovered RA risk loci.

Since GRAIL might demonstrate variable performance across different phenotypes, we wanted to carefully quantify its predictive ability in RA before using it to prioritize SNPs for replication. To estimate GRAIL's ability to distinguish true RA loci from spurious associations, we examined 12 RA risk loci discovered since 2006 (see Table 1, Supplementary Table 1 online). The current GRAIL implementation is based on PubMed abstracts published prior to December 2006. As these 12 risk loci were subsequently discovered, they constitute a representative set to evaluate GRAIL's performance. In a leave-one-out analysis, we used GRAIL to score each of these loci for functional relationships to the other 15 validated RA risk loci. A total of 10 of the 12 loci obtain GRAIL scores of  $p_{\text{text}} < 0.01$ . This analysis suggests that at that  $p_{\text{text}}$  threshold, GRAIL has an ~83% true positive rate (or sensitivity). To assess the false positive rate of this same  $p_{\text{text}}$ threshold, we modeled spurious loci by sampling 10,000 random SNPs from the Affymetrix 500K array; we scored these SNPs against all 16 RA loci. Of the random SNPs, 5.4% scored  $p_{\text{text}} < 0.01$ ; this corresponds to a specificity of ~95%. Assessment of true and false positive rates at different cutoffs revealed an area under the curve (or C statistic) of 0.97 (see Supplementary Figure 1). We note that if a large number of candidate SNPs are screened in a study, this might still result in a large number of false positives.

Next, we attempted to identify novel RA risk loci from a set of SNPs with modest evidence of association from our recent GWAS meta-analysis of 3,392 cases and 12,462 controls<sup>1</sup>. In our original study, we genotyped SNPs with  $p<10^{-4}$  in the meta-analysis, and found that 6 out of 31 replicated in our independent samples. However, many RA risk alleles have modest effects (e.g., OR <1.2) and will be missed at that significance threshold. We therefore expected that some SNPs at p<0.001 may be risk alleles. After excluding SNPs that were known validated RA risk loci, we identified a total of 370 SNPs from 179 independent regions that obtained p<0.001 (see **Methods** and Supplementary Note online). The total number of SNPs observed at this threshold is consistent with the approximate number of SNPs expected by chance, suggesting that the majority represent spurious associations and should not be reproducible in an independent case-control study.

For each of the 179 candidate loci we selected the single SNP with the strongest evidence from the GWAS meta-analysis, and then scored it against the 16 validated RA risk loci with GRAIL. If all 179 SNPs were spurious, then ~10 should score  $p_{text}<0.01$  based on the estimated 5.4% false positive rate. However 22 of the 179 (12.3%) scored  $p_{text}<0.01$  (see Figure 3A, Supplementary Table 2 online). This represented a significant enrichment compared to random sets of 179 SNPs ( $p=3.3 \times 10^{-4}$  by simulation). We therefore expected that of this select subset of 22 SNPs, as many as half might represent true RA risk alleles.

In order to identify which of these 22 SNPs represent true RA risk loci, we genotyped them in an independent validation study of 7,957 cases and 11,958 controls from 11 collections

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from Europe and North America (see Supplementary Table 3). All cases met 1987 American College of Rheumatology classification criteria<sup>15</sup> or were diagnosed by a board-certified rheumatologist, and were seropositive for disease-specific autoantibodies (anti-cyclic citrullinated peptide [CCP] antibody or rheumatoid factor [RF]). All individuals were self-described white and of European ancestry. We assessed association with a Cochran-Mantel-Haenszel (CMH) stratified association statistic<sup>16</sup>. For each SNP we calculated a *z*-score, where a *z*>0 indicates the same allele confers risk in both the replication and the meta-analysis samples. To interpret statistical significance, we used a Bonferroni-corrected one-tailed *p*-value of 0.0023 (=0.05/22, *z*>2.83). Additionally, we calculated the overall association *p*-value across all samples (GWAS meta-analysis plus replication).

Strikingly, of the 22 SNPs examined, 19 (86%) obtained z>0 (see Figure 3B). If these SNPs represented spurious associations then only about half should have z>0; the probability of such a positive skew by chance alone is  $p_{skew}$ =0.0005 – suggesting the likelihood of a large number of true RA risk loci within this set of 22 SNPs.

Of the 22 SNPs selected by GRAIL, 13 obtained nominal levels of association at p<0.05 (corresponding to z>1.65); whereas no more than 2 might be expected by chance alone. More compellingly, 7 SNPs achieved a Bonferroni-corrected level of significance in replication (p<0.0023, z>2.83).

When we aggregated both GWAS meta-analysis and replication genotype data (see Table 2, Supplementary Table 4 online), we observed the strongest associations at rs11586238 on *1p13.1* near the *CD2* and *CD58* genes  $(1.4 \times 10^{-6} \text{ replication}, 1.0 \times 10^{-9} \text{ overall})$ , at rs1980422 on 2q33.2 near *CD28* ( $4.7 \times 10^{-6}$  replication,  $1.3 \times 10^{-9}$  overall), and at rs548234 on *6q21* near *PRDM1* ( $1.2 \times 10^{-5}$  replication,  $2.1 \times 10^{-8}$  overall). Based on conservative estimates of genome-wide significance these represent confirmed RA risk alleles.

Four additional loci replicated; however, aggregate analysis of GWAS meta-analysis and replication genotype data did not exceed a conservative estimate of significance. We observed evidence of association at rs394581 on *6q25.3* near *TAGAP* ( $1.5 \times 10^{-4}$  replication,  $3.8 \times 10^{-7}$  overall), rs10919563 on *1q31.3* within a *PTPRC* intron ( $2.6 \times 10^{-4}$  replication,  $6.7 \times 10^{-7}$  overall), rs540386 on *11p12* within a *TRAF6* intron ( $8.3 \times 10^{-4}$  replication,  $3.9 \times 10^{-6}$  overall), and rs12746613 on *1q23.3* near *FCGR2A* ( $2.2 \times 10^{-3}$  replication,  $1.5 \times 10^{-5}$  overall). These SNP associations likely represent true RA loci, but additional genotyping will be necessary for definitive confirmation.

Interestingly, many of the SNPs picked by GRAIL that validated in independent genotyping were not those with strongest evidence of association in the initial GWAS meta-analysis (see Figure 3A). That is, prioritization based purely on meta-analysis *p*-values might have missed many of these associations. For example, rs12746613 (*FCGR2A*) was ranked 163<sup>rd</sup> of 179 and rs540386 (*RAG1/TRAF6*) was ranked 110<sup>th</sup>. Of the 5 SNPs that we genotyped with the most significant GRAIL  $p_{text}$  scores, 3 replicated and 1 demonstrated nominal evidence of association; only rs2614394 (*IRAK4*) demonstrated no evidence of association.

Many of these seven alleles further implicate genomic regions already associated with autoimmune diseases (see Table 3). At this point none of these RA risk alleles correspond perfectly to any previously established autoimmune allele; but in some cases fine mapping of the region in multiple diseases could clarify the relationship between them. The rs12746613 *FCGR2A* SNP is 13 kb away from a missense SNP in *FCGR2A* that has been associated with systemic lupus erythematosus<sup>17,18</sup>; they are in the same LD block ( $r^2$ =0.19, D'=1.0). The rs394581 SNP is located in the 5' untranslated region of *TAGAP* and is 17 kb away from a SNP associated with celiac disease and with type I diabetes<sup>19,20</sup>; they are in partial LD ( $r^2$ =0.32, D'=.73). The rs10919563 *PTPRC* SNP is 35 kb away from a rare (~1%

allele frequency) non-synonymous SNP that alters splicing of  $PTPRC^{21}$ ; there have been inconsistent reports that it is associated with multiple sclerosis<sup>22-24</sup>. We also note that the rs7234029, a *PTPN2* intronic SNP, is 41 kb away from a SNP associated with both type I diabetes and celiac disease<sup>20</sup>; they are in the same LD block (r<sup>2</sup>=0.14, D'=1.0). The rs548234 SNP is located 10 kb downstream from the *PRDM1* transcript and is 133 kb away from a SNP previously associated with Crohn's disease<sup>25</sup>. The rs11586238 SNP is 50 kb upstream of the *CD2* start site, but is also near multiple other key immunological genes including *CD58*, and *IGSF2*. It is 159 kb away from a multiple sclerosis associated SNP within a *CD58* intron<sup>26,27</sup>.

The rs1980422 SNP is located about 10 kb away from the 3' UTR of *CD28* and is 129 kb away from a known RA and type I diabetes risk allele in the *CTLA4* region (rs3087243)<sup>11</sup>. There is minimal LD between these two SNPs ( $r^2$ =0.04, D'=0.40); conditional analysis confirmed that the two SNPs independently confer RA risk (see Supplementary Table 5 online).

These SNP associations continue to clarify critical biological processes involved in RA pathogenesis, including T-cell activation, NF- $\kappa B$  signaling, and B-cell activation and differentiation. The *CD2* protein is a co-stimulatory molecule on the surface of natural killer cells and T-cells; *CD2* signaling is mediated by binding *PTPRC* directly<sup>28</sup>. Association to *CD28*, contributes additional evidence of the role of T-cell activation in disease pathogenesis. *TRAF6* is involved in downstream *NF*- $\kappa B$  activation; it binds *CD40* directly and is a key component of B-cell activation<sup>29</sup>. Our study has also implicated novel processes represented by *PRDM1* (*BLIMP-1*), a transcription factor that regulates terminal differentiation of B-cells into immunoglobulin secreting plasma cells<sup>30</sup>. Functional studies and re-sequencing will be required to confirm that these genes are indeed the truly causal genes.

We examined all 7 replicated RA SNPs along with known RA risk alleles for epistatic interactions (see Supplementary Note online). Despite the functional relationships between these genes, we found no evidence of significant interactions.

Population stratification could result in spurious associations. However, we were careful for each collection either to use (1) epidemiologically matched samples or (2) ancestry informative markers to match cases and controls. We further note that our seven replicated SNP associations demonstrate consistent effects across all 14 collections without evidence of heterogeneity (p>0.05 by Breslow-Day test of Heterogeneity, see Table 2).

In this study we demonstrate the utility of functional information to prioritize SNPs for replication. We did not pre-define pathways, but rather we used GRAIL to look for genes that had relationships to other validated RA genes. We note that GRAIL is limited in its ability to identify disease genes in entirely novel pathways (i.e., pathways not suggested by the 16 previously known RA risk loci). Arguably, those loci could point to truly novel pathogenic mechanisms. Additionally, successful application of GRAIL is contingent on the scientific literature's comprehensive description of relevant gene relationships. The general application of GRAIL to other diseases will depend critically on the completeness of the validated loci list and the documentation about relevant processes in the literature. Despite these limitations, our study has identified at least three novel RA risk loci, with strong evidence for additional risk loci.

### METHODS

### Evaluating GRAIL for its ability to identify RA loci

GRAIL is a method that leverages statistical text mining principles to assess whether putative disease loci harbor genes with functional relationships to genes in other associated disease loci<sup>2</sup>. Two genes are considered similar if the words used to describe them in PubMed abstracts suggest similar functionality. The implementation of GRAIL used here leverages a text database of 250,000 abstracts published prior to December 2006.

To test the ability of GRAIL to distinguish RA risk loci from spurious associations we defined a set of true positive loci that were discovered since December 2006; these loci would not be described in the GRAIL text database. We also approximated a set of spurious associations by randomly selecting 10,000 SNPs from the Affymetrix 500K genotyping array. We tested both SNP sets for relationships to known associated RA loci with GRAIL. Validated SNPs were tested against the other 15 independent loci; spurious SNPs were tested against all 16 loci. The sensitivity was defined as the proportion of true positive associations that GRAIL assigned a  $p_{\text{text}}$ <0.01 score; the specificity was defined as the proportion of spurious associations that GRAIL assigned a  $p_{\text{text}}$ <0.01 score.

### Selecting Nominally Associated SNPs for Follow-up

In order to identify SNPs for follow-up, we examined the results of a recently published meta-analysis of three GWAS studies (see Supplementary Table 3)<sup>1</sup>. We examined 336,721 SNPs outside the *MHC* region that passed strict quality control criteria. We identified those SNPs that were nominally associated with RA (p<0.001). We grouped SNPs into independent loci; two SNPs were placed in the same locus if there was evidence of LD ( $r^2$ >0.1 in CEU HapMap). We removed all loci that overlapped with validated RA risk regions (see Table 1). We also removed loci with p<10<sup>-4</sup> that were genotyped in most available patient collections and had failed to validate in a previous study<sup>1</sup>. From the remaining set of independent loci, we selected the single SNP that demonstrated the greatest evidence of association in the published meta-analysis.

### **Testing SNPs with GRAIL**

We tested 179 candidate SNPs for relationships to genes within the 16 independent loci known to be associated with RA using GRAIL. SNPs that obtained compelling GRAIL scores ( $p_{text}<0.01$ ) were selected for follow-up investigation. To assess the degree of enrichment among high scoring SNPs we sampled 100,000 random sets of 179 SNPs and tested these SNP sets with GRAIL. We calculate the proportion of sets with as many or more GRAIL hits to calculate a permutation based *p*-value. We note that the version of GRAIL that we used is a previous implementation that differs slightly from the published implementation<sup>2</sup> - results are not substantially affected when we do the same experiment with the current version of GRAIL (see Supplementary Figure 2).

### **Patient Collections**

Patient collections are described in detail in Supplementary Table 3 and in the Supplementary Note. Each collection consisted only of individuals that were self-described white and of European descent, and all cases either met 1987 ACR classification criteria or were diagnosed by board certified rheumatologists. Informed consent was obtained from each patient, and the Institutional Review Board at each collecting site approved the study.

All cases were autoantibody positive (CCP and/or RF). For most of the collections, matched control samples were collected along with case samples as part of the same study. For some of the collections - where control samples were unavailable - we matched these case

collections to shared controls. We used a total of eleven separate patient collections for replication genotyping: (1) CCP positive cases from the Brigham Rheumatoid Arthritis Sequential Study (BRASS)<sup>31</sup> and controls from three separate studies on multiple sclerosis<sup>32</sup>, age-related macular degeneration<sup>33</sup>, and myocardial infarction<sup>34</sup>; (2) CCP positive cases from the Toronto area (CANADA)<sup>13</sup> and controls recruited from the same site along with additional controls taken from a disease study of lung cancer<sup>35</sup>; (3) CCP positive cases and controls from Halifax and Toronto (CANADA-II)<sup>13</sup>; (4) CCP positive cases from Sweden and epidemiologically matched controls (EIRA-II)<sup>36</sup>; (5) CCP positive Dutch cases and controls collected from the greater Amsterdam region (GENRA)<sup>37,38</sup>; (6) North American RF positive cases and controls matched on gender, age, and grandparental country of origin from the Genomics Collaborative Initiative (GCI)<sup>4</sup>; (7) CCP or RF positive Dutch cases and controls from Leiden University Medical Center (LUMC) <sup>39,40</sup>; (8) CCP positive cases drawn from North American clinics and controls from the New York Cancer Project (together this collection is called NARAC-II)<sup>13,36</sup>; (9) CCP positive cases drawn from North American clinics (NARAC-III)<sup>13</sup> and publicly available controls taken from a Parkinson's study<sup>41</sup> and study 66 and 67 of the Illumina Genotype Control Database; (10) CCP or RF positive cases identified by chart review from the Nurses Health Study (NHS) and matched controls based on age, gender, menopausal status, and hormone use<sup>42</sup>; and (11) CCP or RF positive cases recruited at multiple sites in the United Kingdom by the United Kingdom Rheumatoid Arthritis Genetics (UKRAG) collaboration<sup>6</sup>. We used available SNP data from this and previous studies to identify genetically identical samples from the same country; we assumed these represented duplicated individuals and we removed them.

### Genotyping

Detailed description of genotyping is provided in the Supplementary Note. All GWAS metaanalysis genotyping was previously described. We genotyped replication samples at the Broad Institute using a single Sequenom iPlex Pool (for EIRA-II and GENRA collections) and Affymetrix 6.0 (BRASS), the National Institutes of Health using a single Sequenom iPlex Pool (NARAC-II), Analytic Genetics Technology Centre in Toronto using a single Sequenom iPlex Pool (CANADA-II), Epidemiology Unit at The University of Manchester using a single Sequenom iPlex Pool (UKRAG), Celera using kinetic PCR<sup>43</sup> (GCI and LUMC), at the Nurses Health Study in Boston using the BioTrove multiplex SNP genotyping assay (NHS), at the Feinstein Institute using the Illumina 317K array (NARAC-III); and at Illumina using the Illumina 370K array (CANADA). For NARAC-III we additionally obtained publicly available shared controls genotyped on a similar platform from two separate studies. In the cases where whole genome data were available we either extracted data for the 22 SNPs (BRASS) or used imputation to estimate genotypes for them (CANADA and NARAC-III).

For each collection we applied stringent quality control criteria. We required that each SNP pass the following criteria for each collection separately: (1) genotype missing rate < 10%, (2) minor allele frequency > 1%, and (3) Hardy-Weinberg equilibrium with  $p>10^{-3}$ . We then excluded individuals with data missing for > 10% of SNPs passing quality control.

### Population stratification

For each replication collection we corrected for possible population stratification by either (1) using only epidemiologically matched samples when cases and controls were drawn from the same population, or (2) matching at least one control for each case based on ancestry informative markers (see Supplementary Note for details). Since the cases in the NHS, GCI, LUMC, EIRA-II, CANADA-II, UKRAG, and GENRA collections were well matched to controls, we did not pursue further strategies to correct for population stratification. For the BRASS, NARAC-II, CANADA, and NARAC-III, we matched cases

and controls with ancestry informative markers and placed them each into a single stratum. For the BRASS cases and shared controls, GWAS data on Affymetrix 6.0 (unpublished data) was available; we used 681,637 SNPs passing strict quality control as ancestry informative markers. For NARAC-II cases and NYCP shared controls, case and controls were matched using genotype data on 760 ancestry informative markers. For the NARAC-III cases and NYCP shared controls, case and controls were matched using genotype data on 760 ancestry informative markers. For the NARAC-III cases and shared controls, we used available Illumina 317K GWAS data for 269,771 SNPs passing stringent quality control criteria. For the CANADA cases and controls, we used available Illumina 317K GWAS data for 269,771 SNPs passing stringent quality control collection, we used these SNPs to define the top 10 principal components and to remove genetically-distinct outliers (sigma threshold = 6 with five iterations) with the software program EIGENSTRAT<sup>44</sup>. We eliminated vectors that correlated with known structural variants on chromosomes 8 and 17, demonstrated minimal variation, or did not stratify cases and controls. After mapping cases and controls in the space of eigenvectors, we matched cases to controls that were nearest in Euclidean distance as described elsewhere<sup>1</sup>.

### Analysis of Genetic Data

For each SNP we conducted three statistical tests. First, we conducted a one-sided CMH statistical test across eleven strata to assess if RA association was reproducible in the replication collections in the same direction as the GWAS meta-analysis. We set our significance threshold, after correcting for 22 hypothesis tests, to be p<0.0023 (=0.05/22). Second, we conducted a 573 strata joint analysis across all meta-analysis strata and substrata and replication strata; the eleven replication collections were each placed into their own strata, while the meta-analysis samples were partitioned into 562 strata to be consistent with the approach taken in the original analysis to correct for stratification<sup>1,36</sup>. Third, we calculated a Breslow-Day test of heterogeneity of odds ratios. We performed all analyses in MATLAB.

### **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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### Figure 1. Using Gene Relationships Across Implicated Loci (GRAIL) to prioritize candidate RA SNPs

We select a set of candidate SNPs to pursue in an independent genotyping experiment by starting with all SNPs that obtain p<0.001 in an independent GWAS meta-analysis. Then for each candidate SNP, GRAIL identifies the genomic region in LD, and identifies overlapping genes. It then checks to see how many other loci, already known to be associated with disease, contain functionally related genes. SNPs representing those candidate loci with significantly related genes are forwarded for genotyping in large numbers of independent case-control samples.





We place the known RA associated SNP along the outer ring; the internal ring represents the genes near each SNP (as listed in Table 1) with a box. We illustrate the literature-based functional connectivity between these genes with lines drawn between them - the redder and thicker the lines are the stronger the connectivity between the genes is. RA SNPs implicate a small number of highly connected genes – those genes are indicated by labeled boxes.



### Figure 3.

A. GRAIL identifies 22 SNPs among the 179 candidate SNPs with p<0.001 in a GWAS meta-analysis. We plot a histogram of the 179 SNPs as a function of their GWAS meta-analysis *p*-value. Gray bars represent the 157 SNPs that were not selected, while colored bars represent the 22 SNPs that were selected; purple indicating SNPs that replicated convincingly in follow-up genotyping (p<0.0023), orange indicating nominally associated SNPs in follow-up genotyping (p<0.05), and yellow indicating genotyped SNPs without any independent evidence of association. 3B. Enrichment of SNPs with z-scores >2 in replication samples. For each of the 22 SNPs tested, we calculated a one-sided CMH *z*-score statistic from our two-staged replication data. A *z*-score of 0 corresponds to a p=0.5; a *z*-score of 1.65 corresponds to a p=0.05; and a *z*-score of 2.83 corresponds to p=0.0023. For a random collection of unassociated SNPs, this histogram should approximate a normal distribution (dotted line).

### Table 1 Validated RA loci used in functional analyses

We list each of the 16 established RA loci (column 1), and representative SNPs (column 2). Also we list all of the genes in LD with the SNP (column 3); for each SNP the gene in bolded font is the one that GRAIL selected as the most functionally connected gene when that locus was scored against the 15 other validated risk loci.

Validated RA Locus	Representative Allele (SNPs)	Genes within Associated Regions
† 1p13.2	rs2476601	PTPN22 AP4B1 RSBN1 BCL2L15 DCLRE1B MAG13 PHTF1
† 1p36.13	rs2240340	PADI3 PADI4
1p36.32	rs3890745	PANK4 MMEL1 PLCH2 HES5 TNFRSF14
1q23.3	rs6682654	LY9 <b>CD244</b>
† 2q33.2	rs3087243	ICOS CTLA4
2q32.3	rs7574865	STAT1 GLS STAT4
4q27	rs6822844	IL2 IL21 ADAD1 KIAA1109
6q23.3	rs10499194, rs6920220	OLIG3 TNFAIP3
<sup>†</sup> 6p21.32 (MHC class II)	rs6457620, DRB1*0401, *0101	<b>HLA-DRA</b> HLA-DQB1 BTNL2 HLA-DQA1 HLA-DRB5 HLA- DRB1
7q21.2	rs42041	PEXI FAM133B GATAD1 CDK6
9q33.2	rs3761847	PHF19 CEP110 <b>TRAF1</b> RAB14 C5
9p13.3	rs2812378	CCL21
10p15.1	rs4750316	RBM17 PFKFB3 <b>PRKCQ</b>
12q13.3	rs1678542	DTX3 METTLI AVIL DDIT3 XRCC6BP1 MBD6 GLI1 CYP27B1 KIF5A GEFT CTDSP2 MARS CDK4 AGAP DCTN2 TSPAN31 FAM119B MARCH9 TSFM <b>B4GALNT1</b> OS9 PIP4K2C ARHGAP9 SLC26A10
20q13.12	rs4810485	SLC12A5 NCOA5 <b>CD40</b>
22q12.3	rs3218253	IL2RB

 $^{\dagger}$ Loci discovered prior to December 2006.

# Table 2

# SNPs tested for RA susceptibility

reported based on stratified two-tailed CMH statistic across all fourteen patient collections. The final column lists the Breslow-Day Test for heterogeneity The first 6 columns list SNP characteristics. The next four columns list GWA meta-analysis results including allele frequencies, a two-tailed *p*-value for significance is reported based on stratified one-tailed CMH statistic. The next three columns summarize joint (overall) analysis results. Significance is SNP association, and an odds ratio (OR) with respect to the minor allele. The next four columns list similar results for replication genotyping; of odds ratios across all fourteen collections (3 from the meta-analysis and 11 from the replication study).

Joint	Breslow- Day	d		0.29	0.81	0.66	0.63	0.64	0.08	0.25		0.61	0.524	0.74	0.21	0.07	0.23		0.11
	OR			1.13	1.13	1.11	0.91	0.88	0.89	1.12		1.10	0.94	0.94	1.08	1.07	1.08		1.08
	d			1.0E-09	1.3E-09	2.1E-08	3.8E-07	6.7E-07	3.9E-06	1.5E-05		4.4E-05	8.9E-05	1.5E-04	4.3E-05	1.9E-04	5.3E-04		9.5E-04
lication	Allele	Case		0.254	0.255	0.343	0.270	0.117	0.130	0.130		0.172	0.461	0.401	0.401	0.362	0.184		0.155
Rep	Minor 4	Control		0.228	0.237	0.323	0.286	0.132	0.145	0.124		0.164	0.474	0.414	0.393	0.354	0.172		0.155
	OR			1.12	1.11	1.10	0.92	06.0	0.91	1.10		1.06	0.96	0.96	1.05	1.04	1.05		1.04
	d			1.4E-06	4.7E-06	1.2E-05	1.5E-04	2.6E-04	8.3E-04	0.0022		0.013	0.015	0.021	0.041	0.042	0.048		0.077
	Allele	Case		0.260	0.255	0.351	0.269	0.108	0.130	0.133		0.179	0.457	0.385	0.409	0.388	0.195		0.161
Analysis	Minor /	Control		0.237	0.230	0.328	0.302	0.128	0.142	0.120		0.158	0.489	0.421	0.378	0.359	0.178		0.142
Meta-A	OR			1.14	1.16	1.12	0.89	0.84	0.86	1.16		1.16	06.0	06.0	1.13	1.12	1.14		1.16
	d			2.0E-04	4.2E-05	3.4E-04	5.6E-04	3.8E-04	6.1E-04	9.1E-04		1.9E-04	4.4E-04	4.4E-04	4.6E-05	3.5E-04	6.0E-04		4.0E-04
	ele	Minor		ß	С	С	С	А	Т	Т		Ð	А	G	Т	Τ	A		Т
	IIV	Major		C	Т	Т	Т	ß	С	С		Y	ß	А	С	С	IJ		A
	Gene(s)			CD2, IGSF2, CD58	CD28	PRDMI	TAGAP	PTPRC	RAGI, TRAF6	FCGR2A	₀<0.05)	PTPN2	PLCL2	CD247	ICAMI, ICAM3	NHLH2	TNIP2		CD3G
	Pos (HG18)		ted <i>p</i> <0.0023)	117,064,661	204,318,641	106,674,727	159,402,509	196,967,065	36,481,869	159,733,666	(Uncorrected)	12,867,060	17,048,001	165,688,387	10,270,793	116,149,950	2,664,183		117,735,474
	Chr		ci (Uncorreci	Ip13.1	2q33.2	6q21	6q25.3	1q31.3	11p12	1q23.3	ociated Loci	18p11.21	3p24.3	1q24.2	19p13.2	Ip13.I	4 <i>p</i> 16.3	d to replicate	11q23.3
SNP	Ð		Replicated Lot	*rs11586238	*rs1980422	*rs548234	$*_{18394581}$	*rs10919563	rs540386	*rs12746613	Nominally Ass	* <sub>rs</sub> 7234029	rs4535211	rs1773560	rs892188	rs4272626	rs231707	Loci that faile	rs2276418

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<b>ChrPos</b> ( <b>HG18</b> ) <b>Gene(s)</b> $\overline{\mathbf{AIIE}}$ <b>pMinPinpMinpMinDayBreslow</b> $(1, 1, 2, 1, 2, 2)$ $(1, 2, 1, 2)$ $(1, 1, 2, 1, 2)$ $(1, 1, 2, 1, 2)$ $(1, 2, 1, 2)$ <								Meta-/	Analysis				Repl	lication			Joint
13 $143$ $143$ $143$ $110$		Chr	Pos (HG18)	Gene(s)	All	ele	þ	OR	Minor 4	Allele	d	OR	Minor A	Allele	d	OR	Breslow- Day
<i>j</i> (3) <i>I</i> (AMI, <i>L</i> (AM3)         T         G         1.0E-04         1.15         0.224         0.245         0.09         1.03         0.239         6.9E-04         1.07         0.00 <i>j</i> (3)         149,676,235 <i>RARES2</i> G         A         9.1E-04         1.12         0.239         0.242         1.02         0.260         0.266         4.4E-03         1.06         0.045 <i>j</i> 21.1         149,676,235 <i>RARES2</i> G         4.7E-04         1.12         0.230         0.231         0.207         0.260         0.266         4.4E-03         1.06         0.045 <i>j</i> 22.1         1103,4021 <i>IFNBI</i> T         G         4.7E-04         1.12         0.310         0.31         0.27         1.01         0.31         4.4E-03         1.06         0.86 <i>j</i> 22.1         7.223,143 <i>PIBFI</i> A         G         4.3E-04         1.12         0.310         0.31         0.27         0.31         1.06         0.36         0.4E-03         1.06         0.86 <i>j</i> 22.1         7.223,143 <i>PIBFI</i> A         G         0.341         0.27         0.31         0.31         0.36					Major	Minor			Control	Case			Control	Case			d
q36.1         149,676,235         RARKES2         G         A.1E-04         1.12         0.259         0.281         1.02         0.266         4.4E-03         1.06         0.045           p21.3         21,034,021 <i>IFNBI</i> T         G         4.7E-04         1.12         0.330         0.331         0.24         1.02         0.306         4.4E-03         1.06         0.864           q22.1         21,034,021 <i>IFNBI</i> A         G         4.3E-04         1.12         0.310         0.311         0.24         1.02         0.317         0.317         4.4E-03         1.06         0.864           q22.1         72.223,143 <i>PIBF1</i> A         G         4.3E-04         1.12         0.319         0.341         0.24         1.06         0.317         0.317         0.316         1.06         0.164           2221         47,140,263 <i>TTC7A</i> C         A         6.319         0.241         0.24         0.31         0.241         0.31         0.316         0.164         0.16         0.144           2721         101,353,793 <i>ILIRLI</i> A         6         0.241         0.24         0.29         0.241         0.10	a	713.2	10,310,751	ICAMI, ICAM3	Т	Ð	1.0E-04	1.15	0.224	0.245	0.09	1.03	0.229	0.233	6.9E-04	1.07	0.60
<i>p</i> 21.3         21,034,021 <i>IFNBI</i> T         G         4.7E-04         1.12         0.300         0.331         0.24         1.02         0.301         4.4E-03         1.06         0.86 <i>q</i> 22.1         72,223,143 <i>PIBF1</i> A         G         4.3E-04         1.12         0.319         0.341         0.27         1.01         0.317         0.316         1.05         0.14 <i>2p21</i> 47,140,263 <i>TTC7A</i> C         A         6.9E-04         1.13         0.210         0.27         1.01         0.317         0.316         1.05         0.14 <i>q12.1</i> 102,553,793 <i>ILJRL1</i> A         G         8.2E-04         1.13         0.210         0.274         1.01         0.317         0.221         0.14         1.06         0.14         1.06         0.14 <i>q12.1</i> 102,553,793 <i>ILJRL1</i> A         G         8.2E-04         0.89         0.307         0.274         0.93         1.06         0.143         1.06         0.143 <i>q12.1</i> 102,556,433 <i>ILJRL1</i> A         9.8E-05         0.81         0.093         0.94         1.06	6	<sub>1</sub> 36.1	149,676,235	RARRES2	G	А	9.1E-04	1.12	0.259	0.282	0.23	1.02	0.260	0.266	4.4E-03	1.06	0.045
qq22.1         72,223,143         PIBF1         A         G         4.3E-04         1.12         0.319         0.341         0.317         0.317         0.315         0.008         1.05         0.14           2p21         47,140,263         TTC7A         C         A         6.9E-04         1.13         0.210         0.27         0.44         1.00         0.211         0.014         1.06         0.14           2q12.1         102,353,793         ILIRL1         A         G         8.2E-04         0.89         0.307         0.24         1.09         0.225         0.308         0.483         0.99         0.143           12q12         102,353,793         ILIRL1         A         G         8.2E-04         0.89         0.307         0.24         1.09         0.295         0.308         0.483         0.99         0.043           12q12         102,353,793         ILIRL1         A         9.8         0.307         0.274         0.93         1.04         1.06         0.233         0.94         0.94         0.034           12q12         102,353,793         ILIRL1         A         9.8         0.993         0.94         0.993         0.94         0.994         0.934	<u>a</u>	21.3	21,034,021	IFNBI	Т	G	4.7E-04	1.12	0.300	0.331	0.24	1.02	0.297	0.301	4.4E-03	1.06	0.86
ZpZ1         47,140,263         TTC7A         C         A         6.9E-04         1.13         0.210         0.227         0.44         1.00         0.221         0.014         1.06         0.145         1.06         0.121         1.06         0.143         1.06         0.121         1.06         0.143         1.06         0.214         1.06         0.143         1.06         0.143         1.06         0.143         1.06         0.143         0.093         0.014         1.06         0.133           12q12         102,353,793         1LIKL1         A         G         8.2E-04         0.89         0.307         0.274         0.93         1.04         0.238         0.93         0.433         0.043         0.93         0.033           12q12         42,568,433         ILIKL1         A         9.8E-05         0.81         0.093         0.94         1.06         0.143         0.99         0.023           12q13         74,758,649         C         A         2.77         0.031         0.94         0.041         0.041         0.041         0.042         0.042           12q13         74,758,649         C         A         2.76         0.081         0.94         0.94	2	<sub>1</sub> 22.1	72,223,143	PIBFI	А	G	4.3E-04	1.12	0.319	0.341	0.27	1.01	0.317	0.315	800.0	1.05	0.14
<i>q12.1</i> 102,353,793 <i>IL1RL1</i> A         G         8.2E-04         0.89         0.307         0.274         0.93         1.04         0.295         0.308         0.483         0.99         0.093         0.003 <i>12q12</i> 42,568,433 <i>IRAK4</i> G         A         9.8E-05         0.81         0.098         0.082         0.94         1.08         0.099         0.105         0.04         0.004         0.002 <i>6q13</i> 74,758,649         CD109         T         A         2.068         0.068         0.081         0.94         0.94         0.071         0.14         1.05         0.0155		2p21	47,140,263	<i>TTC7A</i>	С	Α	6.9E-04	1.13	0.210	0.227	0.44	1.00	0.221	0.221	0.014	1.06	0.14
12q12         42,568,433         IRAK4         G         A         9.8E-05         0.81         0.098         0.082         0.94         1.08         0.099         0.105         0.06         0.94         0.002           6q13         74,758,649         CD109         T         A         2.7E-05         1.27         0.068         0.081         0.94         0.94         0.079         0.071         0.14         1.05         0.015	39	<i><sub>112.1</sub></i>	102,353,793	ILIRLI	А	G	8.2E-04	0.89	0.307	0.274	0.93	1.04	0.295	0.308	0.483	66'0	0.023
6q13 74,758,649 CD109 T A 2.7E-05 1.27 0.068 0.081 0.94 0.94 0.079 0.071 0.14 1.05 0.0155	1	2q12	42,568,433	IRAK4	G	А	9.8E-05	0.81	0.098	0.082	0.94	1.08	0.099	0.105	0.06	0.94	0.002
	Ĩ	6q13	74,758,649	CD109	Т	Υ	2.7E-05	1.27	0.068	0.081	0.94	0.94	0.079	0.071	0.14	1.05	0.0155

These SNPs are close to other loci already associated to autoimmune disease.

# Table 3

# Tested SNPs near other alleles associated with autoimmune diseases

Seven of the 22 SNPs tested are near loci already associated with autoimmune diseases. In the first three columns we list the SNPs, cytogenetic location, and the likely candidate gene. In the next three columns we list the published SNP, the attributed gene, and the disease associations. In the final three columns we list the physical distance and measures of LD. For PTPRC the published SNP is rare, and LD cannot be accurately assessed.

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SNP				Published	I SNP	Pro	ximity	
D	Chr	Gene	IJ	Gene	Disease Associations	Distance (kb)	r2	D,
rs12746613	1q23.3	FCGR2A	rs1801274	FCGR2A	Systemic Lupus Erythematosus	12.7	0.19	1.00
rs394581	6q25.3	TAGAP	rs1738074	TAGAP	Celiac Disease, Type I Diabetes	16.5	0.32	0.73
rs10919563	1q31.3	PTPRC	rs17612648	PTPRC	Multiple Sclerosis	34.5		
rs7234029	18p11.21	PTPN2	rs478582	PTPN2	Type I Diabetes	41.1	0.14	1.00
rs1980422	2q33.2	CD28	rs3087243	CTLA4	Type I Diabetes, Rheumatoid Arthritis	128.5	0.04	0.40
rs548234	6q21	PRDMI	rs7746082	PRDMI	Crohn's Disease	132.8	0.01	0.08
rs11586238	Ip13.I	CD2	rs2300747	CD58	Multiple Sclerosis	158.9	0.01	0.29