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ORIGINAL ARTICLE

A genome-wide association study identifies a new locus associated with the response to anti-TNF therapy in rheumatoid arthritis

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Anti-Tumor Necrosis Factor (anti-TNF) drugs are biologic agents commonly used to treat rheumatoid arthritis (RA). However, anti-TNFs are not effective in approximately one out of four treated patients. We conducted a Genome-Wide Association Study (GWAS) to identify the genetic variation associated with the response to anti-TNF therapy in RA. In the discovery stage, 372 RA patients treated with an anti-TNF agent (infliximab, adalimumab or etanercept) were analyzed and treatment response was defined at 12 weeks of therapy. We found a genome-wide significant association in the *MED15* gene with the response to etanercept ($P < 1.5e\text{-}8$). Using an independent cohort of 245 RA patients, we performed a replication study of the most significant GWAS associations. We replicated the association at the *MED15* locus and found suggestive evidence of association in the previously associated *MAFB* locus. The results of this study suggest novel mechanisms associated with the response to anti-TNF therapies.

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INTRODUCTION

Anti-Tumor Necrosis Factor (anti-TNF) agents are the most used biologic drugs in rheumatoid arthritis (RA) management. After the failure of conventional disease-modifying anti-rheumatic drugs, anti-TNF agents are the predominant treatment option.[1] However, there is considerable heterogeneity in the response to this therapy, and approximately 25% of anti-TNF-treated patients do not show significant clinical improvement. The high cost of this therapy and the possibility of alternative biologic treatments of similar efficacy[2,3] are raising the need for biomarkers of response to anti-TNF therapy.

Clinical and epidemiological factors like sex, age at onset, rheumatoid factor and anti-cyclic citrullinated antibodies have been shown to be weak predictors of the response to anti-TNF therapy.[4] Consequently, molecular features like DNA variation,[5] gene expression profiles[6] or metabolite levels[7] are increasingly being studied to characterize new biomarkers for anti-TNF response. Genome-Wide Association Study (GWAS) has been very successful at identifying the common genetic variation associated with the risk of developing disease. To date, more than 100 loci have been associated with RA susceptibility.[8] Although these loci suggest new pathways and potential new drug targets, there is yet no consistent association between these loci and treatment response.[9]

The power of the GWAS approach has been recently used to identify new genomic regions associated with the differential response to anti-TNF agents.[10–14] So far, only two loci have shown sufficient statistical significance to be considered true treatment response biomarkers.[13,15,16] Single nucleotide polymorphism (SNP) rs6427528, located in the 3′UTR of the *CD84* gene, was found to be associated with the response to anti-TNF therapy as well with *CD84* gene expression regulation. Importantly, the association was found to be specific for etanercept-treated patients and was not observed in adalimumab- or infliximab-treated patients.[13] More recently, the *PDE3A-SLCO1C1* locus on chromosome *12p12* showed a genome-wide significant ($P < 5e\text{-}8$) association with anti-TNF treatment response in RA.[15] Although the statistical significance was highest when combining all anti-TNF therapies, the genetic association was only statistically significant in the infliximab and etanercept-treated subsets of patients. Together, these results suggest that the genetic variation associated with the response to anti-TNF agents in RA is heterogeneous and at least partially depends on the type of anti-TNF agent.

Here we report a GWAS and validation study for anti-TNF response with a total of 606 Caucasian European RA patients. There is previous strong evidence of treatment-specific genetic variation associated with the clinical response. To this end, we have also analyzed the association with each anti-TNF therapy.

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PATIENTS AND METHODS

GWAS and replication study cohorts

A total of 372 RA patients treated with an anti-TNF agent (adalimumab, etanercept or infliximab) as their first biologic therapy were recruited in the discovery phase. The patients were selected by the Immune-Mediated Inflammatory Disease Consortium (IMIDC),[15] which includes a network of rheumatology departments from 12 different Spanish University Hospitals. All patients were diagnosed as having RA according to the American College of Rheumatology classification criteria,[17] had undergone >2 years of follow-up since diagnosis and were naïve to anti-TNF therapy (or any other) biologic therapy. All cases were Caucasian European and with all four grandparents born in Spain.

An independent cohort of 245 RA patients treated with an anti-TNF therapy and also selected by the IMIDC was used to validate the most significant genetic associations from the GWAS. The patient recruitment criteria were the same as for the discovery phase.

Informed consent was obtained from all participants, and protocols were reviewed and approved by local institutional review boards. The present study was conducted according to the Declaration of Helsinki principles.

GWAS and replication genotyping

The genotyping of $>550{,}000$ SNPs for each patient in the discovery phase was performed using the Illumina Quad610 Beadchip platform (Illumina, San Diego, CA, US). GWAS genotyping was performed at the Centro Nacional de Genotipado (CeGen, Spain) using the recommended protocol. After excluding mitochondrial, X and Y chromosome SNPs, a total of 600,470 markers were considered for GWAS analysis. The SNP genotype calling was performed using Illumina GenomeStudio software v2010.1 (Illumina). Only samples that had $>95\%$ completion rate (99% samples) were used in the association analysis. SNPs with $<95\%$ call rate (0.6%) or a minor allele frequency (MAF) <0.05 (6.2%) were excluded from the analysis. Using a previously published cohort of healthy control individuals from the same European ancestry[18] and genotyped with the same platform, we calculated the principal components of variation using EIGENSTRAT software v3 (Alkes Price Lab, Harvard University, MA, USA; http://www.hsph.harvard.edu/alkes-price/software/).[19] Using the top 10 axes of variation we excluded the genetic outliers (that is, defined as those individuals at >6 s.d. from the center of any of the estimated principal components, $n=11$ patients). Supplementary Figure S1 shows the distribution of the control and RA cohorts according to the two first principal components of variation. SNPs that failed the Hardy–Weinberg equilibrium test in the control cohort (99.5% of SNPs, $P>0.0001$ in controls) were also excluded from the downstream analysis.

Replication genotyping was performed at the HudsonAlpha Institute for Biotechnology (Huntsville, Alabama, US) using the Illumina GoldenGate assay (Illumina) according to the recommended protocol. Genotype calling was performed using GStream genotyping software v1.0 (Rheumatology Research Group, Vall d'Hebron Hospital, Barcelona, Spain; http://www.urr.cat/software/).[20] Similar quality control measures were applied, including genotyping call rate $>90\%$ and sample completion rate $>80\%$. Five percent of the samples were genotyped in duplicate to estimate the genotyping error rate ($<1\%$ error rate).

Statistical analysis

After the quality control analysis, we performed the GWAS for anti-TNF response in RA. Genome-wide association analyses were performed using PLINK software (Purcell Lab, MA, USA; http://pngu.mgh.harvard.edu/~purcell/plink/).[21] The response to anti-TNF treatment was measured at week 12 and was based on the European League Against Rheumatism (EULAR) response criteria.[22] The European League Against Rheumatism criteria define the response to treatment using the change in the disease activity induced by the treatment as well as the end-point disease activity. Using these two parameters, RA patients are categorized into good, moderate or nonresponders.

According to the findings of the most recent pharmacogenetic studies in RA,[13,15,23] there is strong evidence supporting the existence of anti-TNF drug-specific genetic association. Consequently, we conducted genome-wide analysis following two different approaches for the identification of loci associated with anti-TNF response in RA. First, we performed a global GWAS including all three treatments (that is, infliximab, etanercept and adalimumab). In order to increase the power of this study, we compared those patients having a more extreme response to therapy (that is, European League Against Rheumatism good responders vs. non-responders). This approach has been previously used for the identification of genetic variants associated with anti-TNF response.[12,15] Second, we performed a separate GWAS for each anti-TNF drug included in this study. Given the limited sample size, and similar to previous studies,[10,12] good and moderate European League Against Rheumatism responders were merged into a single anti-TNF responder group. Finally, based on the previous evidence of a shared genetic association between the response to etanercept and infliximab,[15] we performed the combined GWAS of these anti-TNF agents. In all 5 GWAS, the association to treatment response was tested using the allelic $\chi 2$ test.

Power calculations were performed using the R package 'Genetics Design' v1.34 (Bioconductor; http://www.bioconductor.org/).[24] Using an $\alpha=5e\text{-}8$, a MAF = 0.2 and a multiplicative genetic model, we estimated an $\geqslant 80\%$ power to detect effect sizes of OR $\geqslant 2.8$ for the global extreme anti-TNF association, OR $\geqslant 7$ for the adalimumab group of patients, OR $\geqslant 4$ for infliximab, OR $\geqslant 4.9$ for etanercept and OR $\geqslant 2.8$ for the combined etanercept–infliximab group.

The selection of loci for replication was based on the level of statistical significance reached in the GWAS ($P<1e\text{-}7$). In order to include refine and identify further significant associated loci, all loci harboring an SNP with a *P*-value $<1e\text{-}5$ were selected to impute the genomic region. For this objective, SHAPEIT v1 (Oxford University, UK; https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html)[25] and IMPUTE2 (Oxford University, UK; https://mathgen.stats.ox.ac.uk/impute/impute_v2.html)[26] programs were used to increase the density of SNPs in each region based on the 1000 Genomes Project reference panel.[27] With this approach, an average >5 increase in the density of SNPs was obtained for each associated region. At each region, if an imputed SNP reached the significance threshold (that is, $P<1e\text{-}7$) and its imputation quality metric was good (information statistic >0.9), it was selected for replication. The association of each locus to treatment response was determined using the same statistical association test as in the discovery cohort. Statistical analysis in this phase was performed using R v2.15 (The R Foundation; http://www.r-project.org/) and plots were generated using LocusZoom (University of Michigan, MI, USA; http://csg.sph.umich.edu/locuszoom/).[28]

RESULTS

The epidemiological and clinical characteristics of the GWAS and replication patient cohorts are shown in Table 1. After applying the quality control criteria, a total of 511,754 SNPs genotyped in 361 RA patients were available for GWAS. Seventy-four percent of patients were responders at week 12 of anti-TNF therapy. In total, 132 patients were treated with etanercept, 132 with infliximab and 96 with adalimumab. With this data set we used the different GWAS approaches for the identification of genetic markers for anti-TNF response. Quantile–quantile plots showed no systematic inflation of *P*-values (Supplementary Figure S2).

Table 1. Clinical characteristics of the GWAS and replication patient cohorts

Clinical variable	*GWAS cohort*	*Replication cohort*
Age at treatment, mean ± s.d.	53.3 ± 11.8	51.7 ± 12.4
Age at diagnosis, mean ± s.d.	43.2 ± 12.5	43.3 ± 13.2
Female, *n* (%)	298(82.6%)	202(82.5%)
Disease duration (years), mean ± s.d.	10.1 ± 8.3	8.4 ± 7.3
Anti-CCP positive[a], *n* (%)	76(78.1%)	135(64.3%)
RF positive[b], *n* (%)	282(78.3%)	178(78.1%)
Baseline DAS28, mean ± SD	5.6 ± 1.1	5.4 ± 1.3
Treated with infliximab, *n* (%)	132(36.6%)	76(31.1%)
Treated with etanercept, *n* (%)	133(36.8%)	114(46.7%)
Treated with adalimumab, *n* (%)	96(26.6%)	54(22.1%)
EULAR nonresponders, *n* (%)	92(25.5%)	64(26.2%)
EULAR moderate responders, *n* (%)	152(42.1%)	99(40.6%)
EULAR good responders, *n* (%)	117(32.4%)	81(33.2%)

Abbreviations: Anti-CCP: anti-cyclic citrullinated peptide antibodies; DAS28: Disease Activity Score for 28 joints; EULAR: European League Against Rheumatoid Arthritis response criteria; GWAS, Genome-Wide Association Study; RF: rheumatoid factor antibodies. [a]ACPA status available in 96.1% patients. [b]RF status available in 99.7% patients.

Table 2. Summary of GWAS and replication study results for anti-TNF response in RA

SNP	*Locus*	*Analysis*	*Chr*	*Bp*	*MAF*	*GWAS OR(95%CI)*	*GWAS P*	*REPL OR(95%CI)*	*REPL P*
rs6941263	*ARMC2*	Global extreme	6	109 369 880	0.16	0.2 (0.11–0.37)	6.22E-08	0.83 (0.28–2.47)	0.77
rs113878252	*MED15*	Etanercept	22	20 895 927	0.1	0.09 (0.04–0.24)	1.24E-08	0.47 (0.23–0.99)	0.039[a]
rs6065221	*MAFB*	Etanercept/infliximab	20	38 326 726	0.14	0.24 (0.13–0.41)	6.26E-08	0.58 (0.19–1.76)	0.056

Abbreviations: Bp, basepair; Chr, chromosome; CI, confidence interval for odds ratio; GWAS OR, Odds ratio in GWAS; GWAS P, *P*-value in GWAS; MAF, minor allele frequency in the patient cohort; REPL OR, odds ratio in replication study; REPL P, *P*-value in the replication study; SNP, single nucleotide polymorphism.
[a]validation for *MED15* locus association was performed using SNP rs4821915 (Bp 20,891,545).

A total of 3 SNPs were found to meet the significance threshold in the GWASs (Table 2). From these, the *MED15* SNP rs113878252 association with etanercept reached a genome-wide level of statistical significance ($P = 1.24e\text{-}8$, OR(95%CI) = 0.09(0.04–0.24)), Figure 1). The other two highly significant SNPs were rs6941263 (*ARMC2* locus, $P = 6.22e\text{-}8$, OR(95%CI) = 0.2(0.11–0.37)) and rs6065221 (*MAFB* locus, $P = 6.26e\text{-}8$, OR(95%CI) = 0.24(0.13–0.41)), and were found in the global and etanercept–infliximab GWASs, respectively.

The validation cohort consisted of 181 responders (73.9%) and 64 nonresponders to anti-TNF therapy (26.1%). From these, 115 RA patients had been treated with etanercept, 76 with infliximab and 54 with adalimumab. *MED15* SNP rs113878252 association with etanercept could not be analyzed in the selected platform and, consequently, the closest most significant non-imputed SNP was selected for replication (that is, SNP rs4821915 at 4.3 kb, $P = 2.26e\text{-}6$).

Using the independent patient cohort we validated the association at *MED15* with the response to etanercept ($P < 0.05$, same direction of effect) and found suggestive evidence for *MAFB* ($P = 0.056$, same direction of effect).

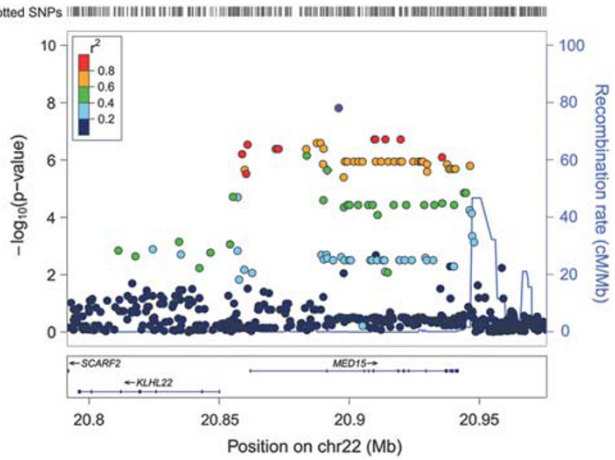

Figure 1. Association results for MED15 locus with the response to etanercept. Regional association plot with the significance of the SNPs (i.e., $-\log_{10}$(*P*-value), y-axis) as a function of the basepair location on chromosome 22q11.2 (x-axis). The variant most associated with the response to etanercept, SNP rs113878252, is colored in purple and the remaining SNPs are colored according to the linkage disequilibrium (r^2 value) with this SNP. The estimated recombination rates (centiMorgans/Megabase, right y-axis) are plotted as a continuous background line (blue). The genes at MED15 locus are also shown.

DISCUSSION

The introduction of anti-TNF agents has been a revolution in the management of RA. However, this therapeutic approach is not effective in all patients, and consequently the disease activity might still progress, destroying joint tissues and causing an important reduction in the quality of life of patients. Therefore, there is a need to find markers that can help identify those patients with a higher probability of being nonresponsive to anti-TNF therapy and who can benefit from alternative therapeutic strategies. In the present study we have used the GWAS approach to identify new loci associated with anti-TNF response in RA. We have identified a genome-wide significant association between the *MED15* locus and the response to etanercept ($P = 1.24e\text{-}8$). Using an independent cohort of patients we have performed a validation study of the most significant results in the GWAS. In this validation stage we have replicated the association of the *MED15* locus with the response to etanercept, and we have found suggestive evidence for the *MAFB* locus with the response to etanercept/infliximab. The results of this study therefore contribute to the characterization to the complex genetic basis of anti-TNF response in RA.

The mediator complex subunit 15 gene (*MED15*) encodes for a subunit of the multiprotein complexes PC2 and ARC/DRIP and might participate in RNA polymerase II transcription.[29] Also called *ARC105*, it has been shown to be an essential mediator of TGF-Beta signal transduction, enhancing the response to this cytokine.[30] DiGeorge Syndrome, a disease caused by the deletion of the chromosome 22 region harboring *MED15*, is characterized by an increased prevalence of autoimmune diseases, including juvenile rheumatoid arthritis.[31] Of relevance, gene expression data from different human tissues show a marked expression of this gene in CD8+ T cells compared with other human cell types or tissues[32] (Supplementary Figure S3). Although traditionally considered a CD4+ T-cell-mediated disease,[33] RA is now known to involve many other different cell types and immune subsets. Recent evidence shows that CD8+ T cells have a marked role in the disease and amount to as much as 40% of the RA synovial fluid T-cell infiltrate.[34,35] The formation of ectopic germinal centers in the synovial membrane, which are associated with a worse prognosis in RA, have been shown to require CD8+ T cell activity. Of relevance, treating RA mouse models with anti-CD8+ antibodies leads to synovial follicle disintegration and the reduction of multiple cytokines including TNFalpha.[36] It is therefore likely that genetic variation influencing CD8+ T-cell activity could influence the resistance or efficacy to cytokine blocking treatments such as anti-TNF agents. Future studies confirming the functional implication of *MED15* in RA and the response to anti-TNF agents are warranted.

In the present GWAS we have found highly suggestive evidence for the association between variation in the chromosome region *20q11–13* and the response to etanercept and infliximab. This region harbors the v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B gene (*MAFB*), a transcription factor that is crucial in the hematopoietic cell differentiation of monocytes–macrophages.[37] Importantly, the first GWAS to study the genetic variation associated with the response to anti-TNF found also a strong association signal in the *MAFB* locus.[10] In particular, they found an association with SNP rs6028945, which is at ~ 56 kb from our associated SNP rs6065221. In our data set we did not find

evidence of association between rs6028945 SNP and response to treatment (data not shown); however, the low LD between both markers ($r^2 = 0.03$, CEU Hapmap population) could explain the lack of concordance. In the previous GWAS, however, all three anti-TNF treatments were considered together. This result suggests that *MAFB* locus association with the response to anti-TNF therapy is heterogeneous and might include different regions on the same locus according to the type of anti-TNF agents considered. Further support from independent patient cohorts will be necessary to confirm the nature of this pharmacogenetic association.

In the present GWAS we have identified a new genetic region associated with the response to anti-TNF therapy and we have found additional evidence for a previously associated locus. These two genetic associations are treatment-specific, highlighting the importance of separately analyzing the genetic contribution of each therapy in RA. This study provides additional support for the existence of different biological mechanisms through which anti-TNF agents exert their immunomodulatory role. The results of this study are an important contribution to the pharmacogenetics of this prevalent disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Taylor PC, Feldmann M. Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis. *Nat Rev Rheumatol* 2009; **5**: 578–582.
2 Mease P, Genovese MC, Gladstein G, Kivitz AJ, Ritchlin C, Tak PP *et al.* Abatacept in the treatment of patients with psoriatic arthritis: results of a six-month, multicenter, randomized, double-blind, placebo-controlled, phase II trial. *Arthritis Rheum* 2011; **63**: 939–948.
3 Emery P, Keystone E, Tony HP, Cantagrel A, van Vollenhoven R, Sanchez A *et al.* IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* 2008; **67**: 1516–1523.
4 Potter C, Hyrich KL, Tracey A, Lunt M, Plant D, Symmons DP *et al.* Association of rheumatoid factor and anti-cyclic citrullinated peptide positivity, but not carriage of shared epitope or PTPN22 susceptibility variants, with anti-tumour necrosis factor response in rheumatoid arthritis. *Ann Rheum Dis* 2009; **68**: 69–74.
5 Plant D, Wilson AG, Barton A. Genetic and epigenetic predictors of responsiveness to treatment in RA. *Nat Rev Rheumatol* 2014; **10**: 329–337.
6 Julia A, Erra A, Palacio C, Tomas C, Sans X, Barcelo P *et al.* An eight-gene blood expression profile predicts the response to infliximab in rheumatoid arthritis. *PLoS One* 2009; **4**: e7556.
7 Kapoor SR, Filer A, Fitzpatrick MA, Fisher BA, Taylor PC, Buckley CD *et al.* Metabolic profiling predicts response to anti-tumor necrosis factor alpha therapy in patients with rheumatoid arthritis. *Arthritis Rheum* 2013; **65**: 1448–1456.
8 Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014; **506**: 376–381.
9 Julia A, Marsal S. The genetic architecture of rheumatoid arthritis: from susceptibility to clinical subphenotype associations. *Curr Top Med Chem* 2013; **13**: 720–731.
10 Liu C, Batliwalla F, Li W, Lee A, Roubenoff R, Beckman E *et al.* Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. *Mol Med* 2008; **14**: 575–581.
11 Plant D, Bowes J, Potter C, Hyrich KL, Morgan AW, Wilson AG *et al.* Genome-wide association study of genetic predictors of anti-tumor necrosis factor treatment efficacy in rheumatoid arthritis identifies associations with polymorphisms at seven loci. *Arthritis Rheum* 2011; **63**: 645–653.
12 Krintel SB, Palermo G, Johansen JS, Germer S, Essioux L, Benayed R *et al.* Investigation of single nucleotide polymorphisms and biological pathways associated with response to TNFalpha inhibitors in patients with rheumatoid arthritis. *Pharmacogenet Genomics* 2012; **22**: 577–589.
13 Cui J, Stahl EA, Saevarsdottir S, Miceli C, Diogo D, Trynka G *et al.* Genome-wide association study and gene expression analysis identifies CD84 as a predictor of response to etanercept therapy in rheumatoid arthritis. *PLoS Genet* 2013; **9**: e1003394.
14 Umicevic Mirkov M, Cui J, Vermeulen SH, Stahl EA, Toonen EJ, Makkinje RR *et al.* Genome-wide association analysis of anti-TNF drug response in patients with rheumatoid arthritis. *Ann Rheum Dis* 2012; **72**: 1375–1381.
15 Acosta-Colman I, Palau N, Tornero J, Fernandez-Nebro A, Blanco F, Gonzalez-Alvaro I *et al.* GWAS replication study confirms the association of PDE3A-SLCO1C1 with anti-TNF therapy response in rheumatoid arthritis. *Pharmacogenomics* 2013; **14**: 727–734.
16 Marquez A, Ferreiro-Iglesias A, Davila-Fajardo CL, Montes A, Pascual-Salcedo D, Perez-Pampin E *et al.* Lack of validation of genetic variants associated with anti-tumor necrosis factor therapy response in rheumatoid arthritis: a genome-wide association study replication and meta-analysis. *Arthritis Res Ther* 2014; **16**: R66.
17 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315–324.
18 Julia A, Domenech E, Ricart E, Tortosa R, Garcia-Sanchez V, Gisbert JP *et al.* A genome-wide association study on a southern European population identifies a new Crohn's disease susceptibility locus at RBX1-EP300. *Gut* 2012; **62**: 1440–1445.
19 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; **38**: 904–909.
20 Alonso A, Marsal S, Tortosa R, Canela-Xandri O, Julia A. GStream: improving SNP and CNV coverage on genome-wide association studies. *PLoS One* 2013; **8**: e68822.
21 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
22 Fransen J, van Riel PL. The Disease Activity Score and the EULAR response criteria. *Clin Exp Rheumatol* 2005 Suppl 5 **23**: S93–S99.
23 Montes A, Perez-Pampin E, Narvaez J, Canete JD, Navarro-Sarabia F, Moreira V *et al.* Association of FCGR2A with the response to infliximab treatment of patients with rheumatoid arthritis. *Pharmacogenet Genomics* 2014; **24**: 238–245.
24 Qiu W, Lazarus R Power Calculation for Testing If Disease is Associated with Marker in a Case-Control Study Using the GeneticsDesign Package: Cambridge 2010.
25 Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods* 2012; **9**: 179–181.
26 Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; **5**: e1000529.
27 Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491**: 56–65.
28 Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP *et al.* LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010; **26**: 2336–2337.
29 Naar AM, Beaurang PA, Zhou S, Abraham S, Solomon W, Tjian R. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* 1999; **398**: 828–832.
30 Kato Y, Habas R, Katsuyama Y, Naar AM, He X. A component of the ARC/Mediator complex required for TGF beta/Nodal signalling. *Nature* 2002; **418**: 641–646.
31 Jawad AF, McDonald-Mcginn DM, Zackai E, Sullivan KE. Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *J Pediatr* 2001; **139**: 715–723.
32 Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM. A census of human transcription factors: function, expression and evolution. *Nat Rev Genet* 2009; **10**: 252–263.
33 Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; **423**: 356–361.
34 McInnes IB. Leukotrienes, mast cells, and T cells. *Arthritis Res Ther* 2003; **5**: 288–289.
35 Ortiz AM, Laffon A, Gonzalez-Alvaro I. CD69 expression on lymphocytes and interleukin-15 levels in synovial fluids from different inflammatory arthropathies. *Rheumatol Int* 2002; **21**: 182–188.
36 Kang YM, Zhang X, Wagner UG, Yang H, Beckenbaugh RD, Kurtin PJ *et al.* CD8 T cells are required for the formation of ectopic germinal centers in rheumatoid synovitis. *J Exp Med* 2002; **195**: 1325–1336.
37 Gemelli C, Montanari M, Tenedini E, Zanocco Marani T, Vignudelli T, Siena M *et al.* Virally mediated MafB transduction induces the monocyte commitment of human CD34+ hematopoietic stem/progenitor cells. *Cell Death Differ* 2006; **13**: 1686–1696.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)