

ARTICLE

A genome-wide linkage scan reveals CD53 as an important regulator of innate TNF- α levels

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Cytokines are major immune system regulators. Previously, innate cytokine profiles determined by lipopolysaccharide stimulation were shown to be highly heritable. To identify regulating genes in innate immunity, we analyzed data from a genome-wide linkage scan using microsatellites in osteoarthritis (OA) patients (The GARP study) and their innate cytokine data on interleukin (IL)-1 β , IL-1Ra, IL-10 and tumor necrosis factor (TNF) α . A confirmation cohort consisted of the Leiden 85-Plus study. In this study, a linkage analysis was followed by manual selection of candidate genes in linkage regions showing LOD scores over 2.5. An single-nucleotide polymorphism (SNP) gene tagging method was applied to select SNPs on the basis of the highest level of gene tagging and possible functional effects. QTD was used to identify the SNPs associated with innate cytokine production. Initial association signals were modeled by a linear mixed model. Through these analyses, we identified 10 putative genes involved in the regulation of TNF α . SNP rs6679497 in gene *CD53* showed significant association with TNF α levels ($P=0.001$). No association of this SNP was observed with OA. A novel gene involved in the innate immune response of TNF α is identified. Genetic variation in this gene may have a role in diseases and disorders in which TNF α is closely involved.

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INTRODUCTION

The immune system is a complex network of interacting pathways and signaling proteins that enables organisms to respond to pathogens and to many other events that challenge homeostasis. The immune system is regulated through cytokines, which are mainly secreted by lymphocytes. The ability of lymphocytes to produce cytokines can be characterized by *ex vivo* stimulation with, for example, the bacterial surface molecule lipopolysaccharide (LPS). This ligand triggers *ex vivo* lymphocytes to produce a maximal proinflammatory cytokine response, subsequently followed by an anti-inflammatory response.¹ The as-such-determined maximal cytokine production profile has been shown to be stable² and to contain a significant heritable component estimated between 53 and 86%, indicating a strong genetic control.³

On the basis of innate *ex vivo* cytokine profiles, individuals can be characterized as pro or anti-inflammatory and it has been shown that these profiles can predispose to diseases with an inflammatory component such as multiple sclerosis and cardiovascular events.^{4,5} In osteoarthritis (OA), a proinflammatory profile may affect the articular cartilage homeostasis, which depends on a delicate balance between catabolic and anabolic activity induced, respectively, by pro- (tumor necrosis factor (TNF) α , interleukin (IL)-1 β) and anti-inflammatory (IL-10 and IL-1 receptor antagonist (Ra)) cytokines.^{6,7} In line with this hypothesis, Riyazi *et al*⁸ showed that patients of the Genetics osteoArthritis and Progression (GARP) study, with OA at

multiple joint sites simultaneously, have high innate IL-1 β and IL-1Ra and low innate IL-10 production compared with controls. Although a later study indicated that the mechanism underlying this association may be more complex, it confirmed the association of genetic variation of the innate cytokine levels with OA features.⁹ We, along with others, have shown that genetic variation of genes involved in the regulation of the immune system may be reflected by a specific profile of circulating plasma inflammatory markers.^{10–12} Furthermore, it was shown that DNA variants within the *IL10* gene and genes of the *IL-1* cluster may be responsible for a part of the variation in the heritable innate *ex vivo* cytokine production on LPS stimulation.^{13–16} However, a large part of the heritability cannot be explained by the currently known genes.

Characterization of the genes that explain a considerable part of the individual variation in the innate cytokine profiles may shed more light on the regulatory elements designed to obtain or maintain a proper balance of these cytokines. Through a better understanding of these elements, more insight into the underlying disease processes in diseases with an inflammatory component such as OA can be obtained, thereby enabling the identification of putative therapeutic targets. In this study, we set off to discover such putative quantitative trait loci for innate cytokine levels using the available genome-wide linkage data of subjects of the GARP study,¹⁷ as well as data on their *ex vivo* LPS-stimulated production of IL-1 β , IL-1Ra, IL-10 and TNF α .⁸ Innate cytokine levels were confirmed by association analysis using

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563 unrelated individuals of the Leiden 85-Plus study.¹⁸ Identified genetic variation influencing the innate immunity profile was tested for association with OA and radiographic OA subtypes, as assessed in the GARP study.

MATERIALS AND METHODS

Study subjects

The GARP study consists of 191 Caucasian sibling pairs affected predominantly by symptomatic OA at multiple joint sites. Characteristics of the GARP study are listed in Table 1. Details of the phenotype and data collection are described by Riyazi *et al.*¹⁹ As a confirmation cohort, we used the Leiden 85-Plus study, which consists of inhabitants of Leiden (Netherlands), who were asked to participate in this study on reaching the age of 85 years between 1 September 1997 and 1 September 1999. The response rate was 87% and in total 599 individuals were included in this study. OA data are not available for the Leiden 85-Plus study.²⁰

Phenotyping

For most participants ($N=370$ and 563 , respectively) in the GARP study and Leiden 85-Plus study, an *ex vivo* whole-blood sample was stimulated with 10-ng/ml LPS, and, after a 4 h incubation, the sample was centrifuged and the TNF α levels were determined in the supernatant using an enzyme-linked immunosorbent assay. In a second sample, a similar protocol was performed with a 24 h incubation, after which the plasma levels of IL-1 β , IL-1Ra and IL-10 were determined.^{1,19} In concordance with previous studies,^{3–5} the *ex vivo*-LPS-stimulated cytokine levels were not normally distributed and influenced by gender. In our analyses, log-transformed cytokine levels were used and analyses were adjusted for sex.

Genotyping

Previously, a genome-wide microsatellite scan was performed in the GARP study to identify new OA susceptibility loci; a detailed description of the genotyping methods and control policy has been described by Meulenbelt *et al.*¹⁷ In short, 417 microsatellite markers on an average spacing of 10 cM across the genome were measured. Initial linkage peaks were identified and two peaks were fine mapped by typing three additional microsatellite markers for each in the region of linkage (Supplementary Table 1). Single-nucleotide polymorphism (SNP) multiplex genotyping assays were designed using Assay Designer software 3.1. iPlex assays were measured on the Sequenom MassARRAY system (Sequenom, San Diego, CA, USA). PCRs were carried out in a final volume of 5 μ l and contained standard reagents and 5 ng of dried genomic DNA. Genotypes were called using the Genotyper v3.1 software (Sequenom). All SNPs were checked for deviations from Hardy–Weinberg equilibrium and approximately 8% of the subjects were genotyped twice as a check for genotyping and calling consistency. Of the 47 genotyped SNPs in this study, three SNPs failed quality check because of low amplification, bad cluster separation or low confidence in called genotypes, and were excluded from further analysis.

Linkage analysis and candidate gene selection

The GARP microsatellite genotype data and log-transformed LPS-stimulated levels were analyzed using the variance components option implemented

in Merlin to assess linkage of the levels to genetic loci.²¹ Merlin output files were modified to tab delimited files with LOD score per marker to facilitate uploading to a custom track in the UCSC genome browser genome graph function. The significance level of linkage peaks was assessed using random gene dropping simulations in Merlin using 5000 reruns. The regions showing LOD scores over 2.5 were explored for candidate genes using the UCSC genome browser,²² where the individual markers' LOD scores were uploaded on a custom track. All UCSC-annotated genes in the 1-LOD-drop region within the flanking areas of a linkage peak over 2.5 were considered for possible involvement in the cytokine response. This manual selection of genes was based on location within the linkage region, GO terms and Swissprot description provided in the UCSC genome graph function. Genes selected were those that are described as being involved in immune system communication, antigen recognition and immune response. We selected 10 positional candidate genes (three linkage areas). Candidate genes were subsequently tagged using SNPs selected from the International HapMap Project genome browser.²³ SNP selection was based on genetic position and function as well as on the potential to tag genetic variation present within these genes. Tagger software implemented in the Haploview program with settings ' $r^2 > 0.8$ ' and 'pairwise tagging' was used to optimize tagging SNP selection.²⁴ In the selection process, we included only SNPs with minor allele frequencies over 0.05 in CEPH data. SNPs in coding regions were prioritized by forced inclusion of nonsynonymous SNPs and the lowest priority was given to downstream SNPs. In total, 47 SNPs were selected for genotyping.

Association analysis of quantitative innate cytokine levels

The GARP data were analyzed for association of LPS levels to SNPs in the candidate genes using QTDT.²⁵ Initially, –WEGA and –WEGD commands were applied to test for association on the basis of linkage to a specific locus under an additive and dominant model, respectively. To test for possible population stratification, we used the –AP –WEGA or –AP –WEGD command. Furthermore, a linear mixed model was used to model the association with the SNP in the GARP and Leiden 85-Plus data including sex, age and BMI as covariates. Here, the random family effect shows correlation between siblings of the GARP study due to shared genetic and environmental effects.²⁶ In the combined GARP and Leiden 85-Plus analysis, in addition to family numbers and covariates sex, age and BMI, we included study identifiers in the model to correct for putative batch differences. Genotypes were coded as 0 (homozygote common allele), 1 (heterozygote) and 2 (homozygote rare allele) to test an additive model and 0, 1 and 1 to test a dominant model with one degree of freedom. All reported P -values are nominal P -values uncorrected for multiple testing for the reader's interpretation of the results, unless mentioned otherwise.

Qualitative association analysis of OA status and SNPs

To assess the association of SNPs to OA at multiple joint sites as defined in the GARP study, a logistic regression was performed in STATA/SE 8.0, using the Leiden 85-Plus sample as a reference sample, whereby we used family numbers in the GARP study as a random effects variable to model familial dependencies. Dominant effects for the rare allele were tested by pooling heterozygotes with homozygotes for the rare allele.

Table 1 Characteristics GARP and Leiden 85-Plus Study

	N	The GARP Study	N	Leiden 85-Plus Study
No.	—	382	—	563
Age, mean \pm SD*	—	60.4 \pm 7.6	—	85 ^a
No. of women (frequency)*	—	301 (0.81)	—	375 (0.67)
Mean Log (IL1 β) \pm SD* (variance)	370	3.49 \pm 0.30 (0.088)	559	3.54 \pm 0.38 (0.142)
Mean Log (IL1Ra) \pm SD* (variance)	369	4.37 \pm 0.15 (0.023)	560	4.55 \pm 0.20 (0.041)
Mean Log (IL10) \pm SD (variance)	369	2.87 \pm 0.19 (0.035)	560	2.85 \pm 0.30 (0.090)
Mean Log (TNF α) \pm SD* (variance)	368	3.87 \pm 0.18 (0.032)	561	3.98 \pm 0.21 (0.045)

*Significant difference between studies (t -test).

^aAll subjects of the Leiden 85-Plus Study were 85 years old at sampling.

RESULTS

Characteristics

The characteristics of GARP and the study sample of the Leiden 85-Plus study, in which both innate cytokine production levels and genotypes were available, are shown in Table 1. The participants of the Leiden 85-Plus study were significantly older compared with the GARP participants ($P < 0.01$), and we observed significant differences in the transformed LPS-stimulated levels between the GARP and Leiden 85-Plus participants for IL-1 β ($P = 0.034$), IL-1Ra ($P < 0.01$) and TNF α ($P < 0.01$). To check for age dependencies of the LPS-stimulated cytokine profiles, the correlation with age was analyzed. We observed no significant correlations of age and IL-1 β , IL-10 or IL-1Ra. However, TNF α showed a significant ($P = 0.037$) correlation with age with a Pearson correlation coefficient of 0.11 (data not shown). The older subjects of the GARP study had on an average a higher LPS-stimulated TNF α level.

IL-1 β , IL-1Ra and IL-10 linkage and association analysis

The genome-wide linkage analysis to find quantitative trait loci involved in innate IL-1 β , IL-1Ra and IL-10 analyses using variance components did not reveal any evidence for linkage above an LOD score of 2.5 (Figure 1a–c, respectively). We did not select candidate genes and SNPs for follow-up analysis of these traits. No substantial evidence for linkage was observed at the loci encoding cytokines IL-1 β and IL-1Ra (2q13), IL-10 (1q32.1) or TNF α (6p21.33) (Figure 1a–d).

TNF α linkage and association analysis

Genome-wide linkage analysis of innate TNF α levels revealed three regions with a positive evidence for linkage with LOD scores over 2.5 (Figure 1d), of which one peak reached a genome-wide linkage significance level. The linkage peak on chromosome 11q12.1 (Figure 1d, peak 2) was fine mapped using three microsatellite markers, and after fine mapping showed a maximum LOD score of 2.57 (marker D11S1314, $P = 3.0 \times 10^{-4}$, genome-wide P -value 0.497) between markers D11S935 and D11S901 (width 53 Mb, Figure 2a). Furthermore, on chromosome 17p13.2 (Figure 1d, peak 3), we observed a narrow linkage peak with a maximum LOD score of 3.38 (marker D17S938, $P = 4.0 \times 10^{-5}$, genome-wide P -value 0.064) between markers D17S831 and D17S799 (width 5 Mb, Figure 2b), which was not fine mapped. The largest peak on chromosome 1p13.3 (Figure 1d, peak 1) was fine mapped using three additional microsatellite markers and after fine mapping showed a maximum LOD score of 3.77 (marker D1S2726, $P = 3.0 \times 10^{-4}$, genome-wide P -value 0.018) between markers D1S2868 and D1S484 (width 52 Mb, Figure 2c). Within these linkage peaks, we selected 10 candidate genes (Table 2). On chromosome 11, we selected candidate genes *MADD*, *SELH*, *CD6* and *CD5* on chromosome 17 *GPS2*, *TNFA-SF12/13* and *CD68* and on chromosome 1 *CSF1*, *CD53* and *FAM19A3*. The genes were tagged using 44 haplotype tagging SNPs, thereby tagging from 30 to 100% of the genotypic variation recorded in the HapMap database (Table 2). QTD analysis indicated significant associations

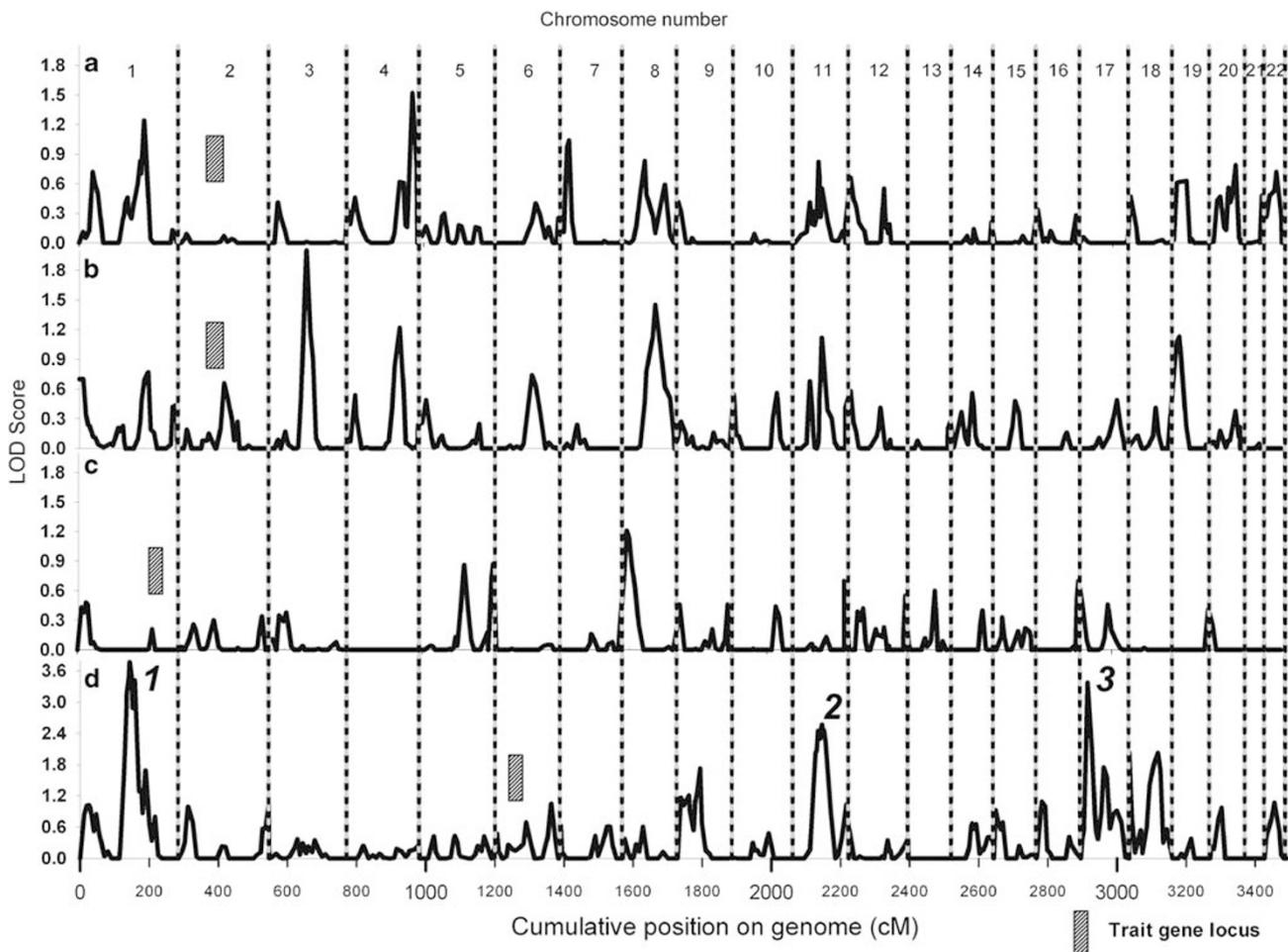


Figure 1 LOD scores for genome-wide linkage analyses for QTLs of (a) IL-1 β , (b) IL-1Ra, (c) IL-10 and (d) TNF α .

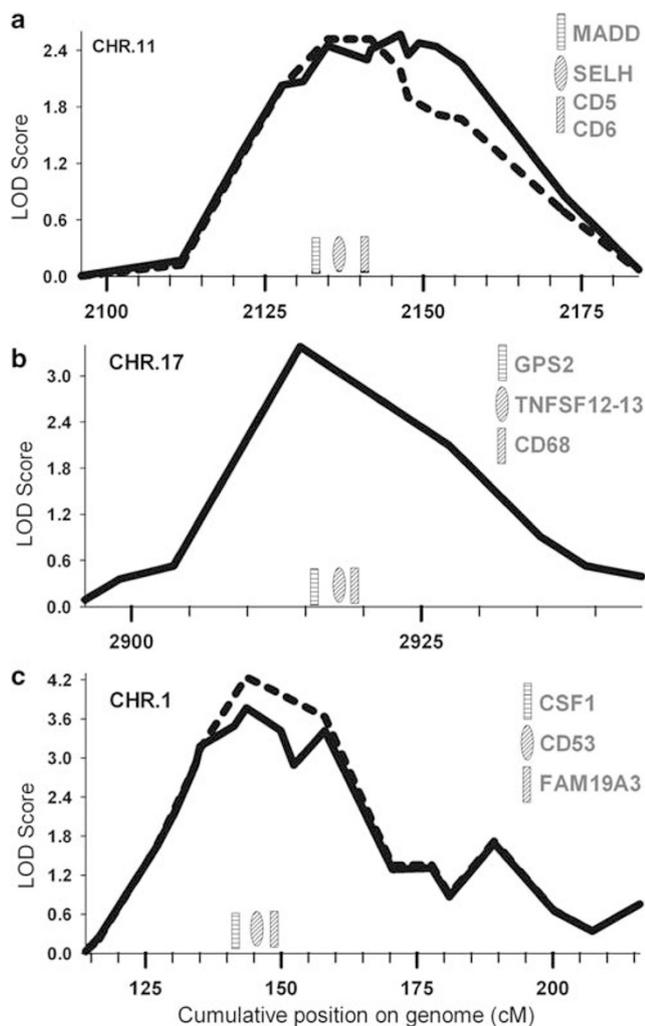


Figure 2 A detailed view of the initial and fine-mapped linkage peaks identified on chromosome 11 (panel a, peak 2), chromosome 17 (panel b, peak 3) and chromosome 1 (panel c, peak 1). Schematically represented are the tested gene positions in the linkage area. Dotted lines represent the initial linkage signal, whereas solid lines represent the fine-mapped linkage signal.

with TNF α levels for SNPs in *CD53* and *FAM19A3* (Table 2). We were unable to model the observed associations of *FAM19A3* in a linear mixed model; however, when a dominant linear mixed model was fitted for *CD53* rs6679497, we again observed a significant association in both the GARP and Leiden 85-Plus studies separately ($P=0.013$ and 0.032 , respectively, Table 3). When we combined the data of both studies in a linear mixed model using a dominant model, we observed a highly significant association of rs6679497 ($P=0.001$, Table 3). The association remains significant after a Bonferroni correction to account for the 11 SNPs tested on the locus (corrected P -value= 0.012), or accounting for all 44 SNPs selected on the linkage analysis (corrected P -value= 0.047). The minor allele of this intron SNP (frequency 0.48) associated with significantly lower innate TNF α levels.

Association analysis of rs6679497 to OA

No significant association was observed for *CD53* rs6679497 when GARP subjects (cases) were compared with subjects of the Leiden

85-Plus study as controls using the dominant model (adjusted for age, sex and BMI, $P=0.142$), indicating that the TNF α QTL locus did not confer susceptibility to OA.

Discussion and Conclusion

Through a genome-wide linkage scan, we identified SNP rs6679497 in *CD53*, of which the minor allele associates with lower innate TNF α levels. It can be hypothesized that the specific genotype of rs6679497 predisposes or protects its carriers from diseases and disorders in which TNF α has a substantial role. Previously, it was shown that TNF α does not have a major role in the onset of OA⁸ and in line with this hypothesis, we were unable to show associations of rs6679497 with OA as defined in the GARP study.

CD53 codes for cluster of differentiation 53, a leukocyte surface antigen. The protein family to which this cell surface glycoprotein belongs to is known to complex with integrins, cellular components involved in cell–cell and cell–matrix interactions. *CD53* deficiency has been linked to recurring infectious diseases caused by bacteria, fungi and viruses;²⁷ susceptibility to these might be increased for carriers of the minor allele of rs6679497. The protein is implicated in elevated cellular glutathione in response to LPS activation and may increase cell survival under UV-B and oxidative conditions.²⁸ Furthermore, treatment of neutrophils with TNF α downregulates the presence of *CD53* antigens on the cell surface through a proteolytic mechanism.²⁹ This indicates that the protein may have a substantial role in cellular stability and in the inflammatory response to adverse conditions. Furthermore, the protective effect of ligated *CD53* on the cellular surface may help specific tumors to escape from programmed cell death.³⁰ Although rs6679497 or any of the SNPs in its LD block are investigated for effects on the expression levels or protein function, the gene is under strong genetic control,³¹ and genetic variation might have a role in tumor biology or other diseases. Such a relation can readily be elucidated through investigation of this marker SNP in cancer cohorts. Furthermore, in a study that characterizes leukocytes from normal and rheumatoid arthritis (RA) patients, *CD53* was found to be elevated on the RA lymphocytes surfaces.³² Investigating the role of this gene in relation to this and other TNF α -driven diseases later in life may show protective effects of the rs6679497 minor allele.

The SNP *CD53* rs6679497 resides within the intron of the gene, which shows low levels of conservation across species, and it is in an LD block across several introns and exons encompassing at least 23 other intron SNPs (recorded in the HapMap phase 1 and 2 data). Several transcripts are known as recorded in the UCSC database (accession number ENSG00000143119); however, only a proportion of these will actually be translated into a protein. Given the current level of total variation tagging (52%), it is likely that the SNP is only a proxy marker in LD with a causal (functional) polymorphism, which could have a more obvious implication on gene regulation or protein stability and functioning. This is substantiated by the fact that the *CD53* SNP explains only a part of the linkage as determined in QTDT analyses (results not shown). To find the true functional variant, a more detailed analysis of this gene by sequencing or SNP saturation is necessary. Alternatively, in our candidate gene approach, we may have missed additional genetic variation at the loci of interest because of a knowledge bias on both the presence and role of genes at these loci.

Although it was shown previously that *IL1* cluster haplotypes were associated with IL-1 β bioavailability,^{33,34} we observed no evidence of linkage on the genetic loci for the respective cytokines' genes, the *IL-1* gene cluster on chromosome 2, *IL-10* on chromosome 1 or *TNF* on chromosome 6. Possibly, the genetic variation in LPS-stimulated cytokine levels explained by these loci is not readily detected by

Table 2 Genes and selected SNPs in linkage peak, TNF α

Chromosome	Gene (coverage) ^a	Short description ^b	SNP	Position	QTDT add ^c	QTDT dom ^d
1	CSF1 (58%)	Granulocyte/macrophage colony-stimulating factors are cytokines that act in hematopoiesis by controlling the production, differentiation, and function of two related white cell populations of the blood, the granulocytes and the monocytes-macrophages.	rs915357	Intron	0.055 ⁺	0.060 ⁺
			rs333968	Intron		
			rs333970	Intron		
	CD53 (52%)	Cell surface glycoprotein that is known to complex with integrins. Familial deficiency of this gene has been linked to an immunodeficiency associated with recurrent infectious diseases caused by bacteria, fungi and viruses. Alternative splicing results in multiple transcript variants encoding the same protein.	rs3738760	Coding exon		
			rs10494122	Intron		
			rs10857833	Intron		
			rs6679497	Intron	0.012 [*]	0.009 ^{**}
	FAM19A3 (100%)	Contains conserved cysteine residues at fixed positions, and are distantly related to MIP-1 α , a member of the CC-chemokine family.	rs4839581	Intron		
			rs3790722	Intron		
			rs4450019	Intron	0.019 [*]	0.033 [*]
rs11102524			Intron			
11	MADD (68%)	Tumor necrosis factor alpha (TNF α) is a signaling molecule that interacts with one of two receptors on cells targeted for apoptosis. The protein encoded by this gene is a death domain-containing adaptor protein that interacts with the death domain of TNF α receptor 1 to activate mitogen-activated protein kinase (MAPK) and propagate the apoptotic signal.	rs7114704	Intron		
			rs10501320	Intron		
			rs10501321	Intron		
			rs10838689	Intron		
			rs2290149	Intron		
			rs11039183	Intron		
			rs753993	Intron		
	SELH (100%)	This gene encodes a selenoprotein, which contains a selenocysteine (Sec) residue at its active site.	rs9420	Intron boundary		
			rs3017889	Downstream		
	CD6 (30%)	CD6 is a monomeric 105- or 130-kD membrane glycoprotein that is involved in T-cell activation.	rs2905504	Intron		
			rs11230550	Intron		
			rs11230553	Intron		
			rs2283263	Intron		
			rs11230559	Intron		
			rs11230563	Coding exon [*]		
	CD5 (68%)	Human T-cell surface glycoprotein of relative molecular mass (Mr) 67 000, has been implicated in the proliferative response of activated T cells and in T-cell helper function.	rs2074225	Coding exon [*]		
			rs1050922	Coding exon		
			rs3862667	Intron		
			rs572350	Intron		
			rs671444	Intron		
			rs12364244	Intron		
			rs637186	Coding exon [*]		
17	GPS2 (100%)	This gene encodes a protein involved in G protein-mitogen-activated protein kinase (MAPK) signaling cascades.	rs2270981	Coding exon		
			rs2292064	Coding exon		
	TNFA-SF (80%)	This gene encodes a member of the tumor necrosis factor superfamily. It encodes a hybrid protein composed of the cytoplasmic and transmembrane domains of family member 12 fused to the C-terminal domain of family member 13. The hybrid protein is membrane anchored and presents the receptor-binding domain of family member 13 at the cell surface. It stimulates cycling in T- and B-lymphoma cell lines.	rs9899183	Intron		
			rs12937543	Promoter		
			rs4968211	Promoter		
			rs11552708	Coding Exon [*]		
			rs3803800	Coding Exon [*]		
	CD68 (50%)	This gene encodes a 110-kD transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. The protein is also a member of the scavenger receptor family. Scavenger receptors typically function to clear cellular debris, promote phagocytosis, and mediate the recruitment and activation of macrophages. Alternative splicing results in multiple transcripts encoding different isoforms.	rs6608	3'UTR		
			rs9896688	Intron boundary		
			rs9901673	Coding exon [*]		
			rs9901675	Coding exon [*]		

⁺ $P < 0.1$; ^{*} $P < 0.05$; ^{**} $P < 0.01$.

^aGene coverage was based on genetic variation present in the HapMap database build 18.

^bShort description adapted from gene ontology description provided in the UCSC genome graph tool.

^c P -values reported by QTDT using command WEGA (additive model), tests for association given linkage on a specific locus.

^d P -values reported by QTDT using command WEGD (dominant model), tests for association given linkage on a specific locus.

Table 3 Genotype analysis and linear mixed model for CD53 SNP rs6679497, assuming a dominant model of association

Study	N (11) ^a	Log(TNF α)	N (12) ^a	Log(TNF α)	N (22) ^a	Log(TNF α)	P LMM ^b	P LMM ^c
GARP	86	3.90	178	3.86	99	3.85	0.013*	0.001**
Leiden 85-Plus	312	4.01	564	3.97	238	3.97	0.032*	

* $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.^a11=homozygote common allele; 12=heterozygote; 22=homozygote rare allele.^bModeling dominant effect, corrected for familial relationship, age, sex and BMI.^cModeling dominant effect combining both studies, corrected for study differences in level, familial relationship, age, sex and BMI.

linkage analysis, which is known to be most suitable to detect loci that explain a major part of genetic variation. Furthermore, our linkage analyses of innate IL-1 β , IL-1Ra and IL-10 levels in general revealed only moderate linkage peaks up to an LOD score of 2.5. More likely, as was shown in a previous study,³³ the estimate of innate IL-1 β production on LPS stimulation may not be entirely independent of the OA disease status, possibly by sensitization of the Toll-like receptor pathways as a result of disease activity³⁵ or otherwise sensitization of the response by lymphocytes, which may have interfered with the current linkage analysis by introducing cohort heterogeneity or bias. In the linkage analyses, we checked whether the levels in the GARP study sample were normally distributed to facilitate powerful linkage analysis using the variance component option. The innate levels of IL-1 β , IL-10 and TNF α were normally distributed, whereas innate IL-1Ra levels were normally distributed after removal of one extreme value, which did not alter the linkage analysis results (data not shown). The use of Merlin-Regress,²¹ which may be more appropriate for use in highly selected samples, showed a similar pattern of LOD scores for all traits, with slightly lower maximum LOD scores. It remains possible that the observed association only occurs in the middle aged and elderly; therefore, further searches for genetic loci that influence *ex vivo* innate cytokine profiles may benefit from the use of healthy young subjects in these searches.

Following a genome-wide linkage analysis, namely, an association analysis of positional candidate gene SNPs within the 1-LOD-drop interval of a linkage peak, we show a consistent association of SNP rs6679497 in CD53 with innate TNF α levels in both the GARP study ($P=0.013$) and the confirmation cohort consisting of Leiden 85-Plus participants ($P=0.032$). A dominant linear mixed model analysis on combined data from the GARP and Leiden 85-Plus study showed that the minor allele of this SNP associated with a highly significantly lower innate TNF α level independent of age and sex. In diseases with a large TNF α component, such as inflammatory bowel disease or rheumatoid disorders, the minor allele of rs6679497 might exert a protective effect in susceptibility or severity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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