



HAL
open science

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

Steffan Daniel Bos, Nico Lakenberg, Ruud van Der Breggen, Jeanine Houwing-Duistermaat, Margreet Kloppenburg, Anton J.M. de Craen, Marian Beekman, Ingrid Meulenbelt, P E.Slagboom

► **To cite this version:**

Steffan Daniel Bos, Nico Lakenberg, Ruud van Der Breggen, Jeanine Houwing-Duistermaat, Margreet Kloppenburg, et al.. A genome wide linkage scan reveals CD53 as an important regulator of innate TNF-alpha levels.. European Journal of Human Genetics, 2010, 10.1038/ejhg.2010.52 . hal-00528085

HAL Id: hal-00528085

<https://hal.science/hal-00528085>

Submitted on 21 Oct 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Steffan D. Bos¹, Nico Lakenberg¹, Ruud van der Breggen¹, Jeanine J. Houwing-
Duistermaat², Margreet Kloppenburg^{3,4}, Anton J.M. de Craen⁵, Marian Beekman¹,
Ingrid Meulenbelt¹, P. Eline Slagboom^{1,6}

Department of ¹Molecular Epidemiology, ²Medical Statistics, ³Rheumatology,
⁴Epidemiology, ⁵Gerontology and Geriatrics, LUMC, Leiden, The Netherlands.

⁶Netherlands Consortium for Healthy Ageing, The Netherlands

Corresponding author:

Steffan D. Bos, MSc

Leiden University Medical Centre

Section Molecular Epidemiology (S-05-P)

P.O. Box 9600

2300 RC Leiden, The Netherlands

Tel +31 71 5269736 Fax +31 71 5268280

E-mail: s.bos@lumc.nl

Abstract

Cytokines are major immune system regulators. Previously, innate cytokine profiles determined by LPS 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 patients (The GARP study) and their innate cytokine data on IL-1 β , IL-1Ra, IL-10 and 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. A SNP gene tagging method was applied to select SNPs on the basis of highest level of gene tagging and possible functional effects. QTDT was used to identify the SNPs associated to innate cytokine production. Initial association signals were modelled 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 to TNF α levels ($P = 0.001$). No association of this SNP was observed to osteoarthritis. A novel gene involved in the innate immune response of TNF α is identified. Genetic variation in this gene may play a role in diseases and disorders in which TNF α is intimately involved.

Keywords: linkage, osteoarthritis, immunity, TNF, GARP, CD53

Introduction

The immune system is a complex network of interacting pathways and signalling proteins which enables organisms to respond to pathogens as well as 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 pro-inflammatory cytokine response, subsequently followed by an anti-inflammatory response¹. 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 control³.

Based on innate *ex vivo* cytokine profiles, individuals can be characterized as pro- or anti-inflammatory and it has been shown that these profiles can be predisposing to diseases with an inflammatory component such as multiple sclerosis and cardiovascular events^{4, 5}. In osteoarthritis (OA), a pro-inflammatory profile may affect the articular cartilage homeostasis, which depends on a delicate balance of catabolic respectively anabolic activity induced 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. showed that patients of the Genetics osteoARthritis and Progression (GARP) study with OA at multiple joints sites simultaneously, have high innate IL-1 β and IL-1Ra and low innate IL-10 production as compared to controls⁸. Although a later study indicated that the mechanism underlying this association may be more complex, it confirmed the association of genetic variation associated to the innate cytokine levels to OA

features⁹. We and 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, 11, 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 upon LPS stimulation^{13, 14, 15, 16}. A large part of the heritability however, cannot be explained by the currently known genes.

Characterisation 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 proper balance of these cytokines. Through a better understanding of these elements more insight in underlying disease processes in diseases with an inflammatory component such as OA can be obtained, thereby enabling identification of putative therapeutic targets. In the present study we set off to discover such putative quantitative trait loci for innate cytokine levels by use of the available genome wide linkage data of subjects of the GARP study¹⁷, as well as data on their *ex vivo* LPS stimulated production of IL-1 β , IL-1Ra, IL-10 and TNF α ⁸. Confirmation by association analysis of innate cytokine levels was performed using 563 unrelated individuals of the Leiden 85-Plus study¹⁸. Identified genetic variation influencing the innate immunity profile was tested for association to OA and radiographic OA subtypes as assessed in the GARP study.

Materials & 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 on description 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 upon reaching the age of 85 years between September 1, 1997 and September 1, 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 study²⁰.

Phenotyping

In the GARP study and Leiden 85-Plus study, for most participants (N=370 and N=563 respectively) an *ex vivo* whole blood sample was stimulated with 10 ngml⁻¹ LPS and after a 4 hours incubation the sample was centrifuged and the TNF α levels were determined in the supernatant by use of an enzyme linked immunosorbent assay. In a second sample a similar protocol was performed with a 24 hour 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, 4, 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 3 additional microsatellite markers for each in the region of linkage (Supplementary Table 1). 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). PCR's 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, San Diego, CA). 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, 3 SNPs failed quality check due to 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 loci²¹. 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 by use of random gene dropping simulations in Merlin using 5000 reruns. The regions showing LOD scores over 2.5 were explored for candidate genes by use of the UCSC genome browser²², 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 genes which 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 browser²³. SNP selection was based on genetic position and function as well as potential to tag genetic variation present within these genes. Tagger software implemented in the Haploview program with settings “ $r^2 > 0.8$ ” and “pair wise tagging” was used to optimize tagging SNP selection²⁴. In the selection process we included only SNPs with minor allele frequencies over 0.05 in CEPH data. A prioritization was applied to SNPs in coding regions by forced inclusion of non-synonymous SNPs and lowest priority 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 QTDT²⁵. Initially, the -WEGA and -WEGD commands were applied to test for association given linkage on 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, BMI as covariates. Here, the random family effect models correlation between siblings of the GARP study due to shared genetic and environmental effects²⁶. 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 respectively for testing 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 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 effect variable to model familial dependencies. Dominant effects for the rare allele were tested by pooling heterozygotes with homozygotes for the rare allele.

Results

Characteristics

The characteristics of GARP and the study sample of the Leiden 85-Plus study where 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 as compared to 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 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 a LOD score of 2.5 (Figure 1A, 1B and 1C respectively). We did

not select candidate genes and single nucleotide polymorphism(SNP)s 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 1 A-D).

TNF α linkage and association analysis

Genome wide linkage analysis of innate TNF α levels revealed three regions with positive evidence for linkage with LOD scores over a LOD score of over 2.5 (Figure 1D), of which 1 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 $P = 3.0 \cdot 10^{-4}$, genome wide p-value 0.497) between markers D11S935 and D11S901 (width 53 megabases (Mb), Figure 2A). Furthermore on chromosome 17p13.2 (Figure 1D, peak 3) we observed a narrow linkage peak with maximum a LOD score of 3.38 (marker D17S938 $P = 4.0 \cdot 10^{-5}$, genome wide p-value 0.064) between markers D17S831 and D17S799 (width 5Mb, 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 $P = 3.0 \cdot 10^{-4}$, genome wide p-value 0.018) between markers D1S2868 and D1S484 (width 52Mb, 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 30-100% of the genotypic variation recorded in the HapMap database (Table 2). QTDT analysis indicated significant associations to 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 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 upon 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 to subjects of the Leiden 85-Plus study as controls using the dominant model (adjusted for age, sex and BMI, $P=0.142$), indicating that TNF α QTL locus did not confer susceptibility to OA.

Discussion & conclusion

Through a genome wide linkage scan we were able to identify SNP rs6679497 in *CD53* of which the minor allele associates to lower innate TNF α levels. It can be hypothesized that the specific genotype of rs6679497 predisposes or protect its carriers from diseases and disorders in which TNF α plays a substantial role.

Previously, it was shown that TNF α does not play a major role in the onset of OA⁸ and in line with this hypothesis we were unable to show associations of rs6679497 to OA as defined in the GARP study.

CD53 codes for cluster of differentiation 53, a leukocyte surface antigen. The protein family 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 viruses²⁷, 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 conditions²⁸. Furthermore, treatment of neutrophils with TNF α down-regulates the presence of the *CD53* antigens on the cell surface through a proteolytic mechanism²⁹. This indicates that the protein may play a substantial role in cellular stability and 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 death³⁰. 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 control³¹ 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 which characterizes leukocytes from normal and rheumatoid arthritis (RA) patients, *CD53* was found to be elevated on the RA lymphocytes surfaces³². 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 & 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 the SNP is only a proxy marker in LD with a causal (functional) polymorphism which could have a more obvious implication to the gene regulation or protein stability and functioning. This is substantiated by the fact that the *CD53* SNP explains only 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 the *ILI* cluster haplotypes were associated to IL-1 β bio-availability^{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 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 a LOD score of 2.5. More likely as was shown in a previous study³³, particularly the estimate of innate IL-1 β production upon 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 1 extreme value, which did not alter the linkage analysis results (data not shown). The use of Merlin-Regress²¹ 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 middle aged and elderly, therefore, further searches for genetic loci that influence the *ex vivo* innate cytokine profiles may benefit from the use of healthy young subjects in these searches.

Following a genome wide linkage analysis, 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* to 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 to a highly significantly lower innate TNF α level independent of age and sex effects. 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.

Acknowledgements

We acknowledge the support of the cooperating hospitals and referring rheumatologists, orthopaedic surgeons and general practitioners in our region for identifying eligible GARP patients, in random order: Dr. L.N.J.E.M. Coene, department of orthopedic surgery and Dr. H.K. Runday, department of rheumatology,

Leyenburg Hospital, the Hague; I. Speyer and Dr. M.L. Westedt, department of rheumatology, Bronovo Hospital, the Hague; Dr. D. van Schaardenburg, department of Rheumatology, Jan van Breemen Institute in Amsterdam; Dr. A.J. Peeters and Dr. D. van Zeben, department of rheumatology, Reinier de Graaf Hospital, Delft; Dr. E.J. Langelaan, department of orthopedic surgery, Rijnland Hospital in Leiderdorp and Dr. Y. Groeneveld, general practitioner, associated with the Leiden University Medical Center. In addition to the grant support of The Dutch League against Rheumatism (NR 04-1-403), Pfizer Inc., Groton, CT, USA and the Center of Medical Systems Biology (CMSB) provided generous financial support for this work.

The Leiden 85-plus Study is a collaborative project of the Department of Gerontology and Geriatrics (Rudi Westendorp, principal investigator) and the Department of Public Health and Primary Care (Jacobijn Gussekloo, principal investigator) of the Leiden University Medical Center, Leiden, the Netherlands.

Conflict of interest

The authors declare no conflicts of interest.

Reference List

1. Ohlsson, K., Linder, C., Lundberg, E., Axelsson, L.: Release of cytokines and proteases from human peripheral blood mononuclear and polymorphonuclear cells following phagocytosis and LPS stimulation. *Scand.J.Clin.Lab Invest* 1996; **56**: 461-470.
2. van der Linden, M. W., Huizinga, T. W., Stoeken, D. J., Sturk, A., Westendorp, R. G.: Determination of tumour necrosis factor-alpha and interleukin-10 production in a whole blood stimulation system: assessment of

laboratory error and individual variation. *J.Immunol.Methods* 1998; **218**: 63-71.

3. de Craen, A. J., Posthuma, D., Remarque, E. J., van den Biggelaar, A. H., Westendorp, R. G., Boomsma, D. I.: Heritability estimates of innate immunity: an extended twin study. *Genes Immun.* 2005; **6**: 167-170.
4. de Jong, B. A., Schrijver, H. M., Huizinga, T. W.*et al*: Innate production of interleukin-10 and tumor necrosis factor affects the risk of multiple sclerosis. *Ann.Neurol.* 2000; **48**: 641-646.
5. McGeer, P. L., McGeer, E. G.: Innate immunity, local inflammation, and degenerative disease. *Sci.Aging Knowledge Environ.* 2002; **2002**: re3-
6. Goldring, M. B.: Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. *Best.Pract.Res.Clin.Rheumatol.* 2006; **20**: 1003-1025.
7. Goldring, M. B., Otero, M., Tsuchimochi, K., Ijiri, K., Li, Y.: Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. *Ann.Rheum.Dis.* 2008; **67 Suppl 3**: iii75-iii82.
8. Riyazi, N., Slagboom, E., de Craen, A. J.*et al*: Association of the risk of osteoarthritis with high innate production of interleukin-1beta and low innate production of interleukin-10 ex vivo, upon lipopolysaccharide stimulation. *Arthritis Rheum.* 2005; **52**: 1443-1450.

9. Chapman, K., Loughlin, J.: Association of the interleukin-1 gene cluster with osteoarthritis of the hip: comment on the article by Meulenbelt et al and the letter by Smith et al. *Arthritis Rheum* 2006; **54**: 3722-3723.
10. Bos, S. D., Suchiman, H. E., Kloppenburg, M.*et al*: Allelic variation at the C-reactive protein gene associates to both hand osteoarthritis severity and serum high sensitive CRP levels in the GARP study. *Ann.Rheum.Dis.* 2007; **67**: 877-879.
11. Curran, J. E., Jowett, J. B., Elliott, K. S.*et al*: Genetic variation in selenoprotein S influences inflammatory response. *Nat.Genet.* 2005; **37**: 1234-1241.
12. Rafiq, S., Frayling, T. M., Murray, A.*et al*: A common variant of the interleukin 6 receptor (IL-6r) gene increases IL-6r and IL-6 levels, without other inflammatory effects. *Genes Immun.* 2007; **8**: 552-559.
13. Haukim, N., Bidwell, J. L., Smith, A. J.*et al*: Cytokine gene polymorphism in human disease: on-line databases, supplement 2. *Genes Immun.* 2002; **3**: 313-330.
14. Danis, V. A., Millington, M., Hyland, V. J., Grennan, D.: Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. *Clin.Exp.Immunol.* 1995; **99**: 303-310.
15. Wen, A. Q., Wang, J., Feng, K., Zhu, P. F., Wang, Z. G., Jiang, J. X.: Effects of haplotypes in the interleukin 1beta promoter on lipopolysaccharide-induced interleukin 1beta expression. *Shock* 2006; **26**: 25-30.

16. Temple, S. E., Lim, E., Cheong, K. Y.*et al*: Alleles carried at positions -819 and -592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with *Streptococcus pneumoniae*. *Immunogenetics* 2003; **55**: 629-632.
17. Meulenbelt, I., Min, J. L., Bos, S.*et al*: Identification of DIO2 as new susceptibility locus for symptomatic Osteoarthritis. *Hum.Mol.Genet.* 2008; **17**: 1867-1875.
18. van Exel, E., Gussekloo, J., Houx, P.*et al*: Atherosclerosis and cognitive impairment are linked in the elderly. The Leiden 85-plus Study. *Atherosclerosis* 2002; **165**: 353-359.
19. Riyazi, N., Meulenbelt, I., Kroon, H. M.*et al*: Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. *Ann.Rheum.Dis.* 2005; **64**: 438-443.
20. van Exel, E., Gussekloo, J., de Craen, A. J., Bootsma-van der, W. A., Frolich, M., Westendorp, R. G.: Inflammation and stroke: the Leiden 85-Plus Study. *Stroke* 2002; **33**: 1135-1138.
21. Abecasis, G. R., Cherny, S. S., Cookson, W. O., Cardon, L. R.: Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat.Genet.* 2002; **30**: 97-101.
22. Kuhn, R. M., Karolchik, D., Zweig, A. S.*et al*: The UCSC Genome Browser Database: update 2009. *Nucleic Acids Res.* 2009; **37**: D755-D761.

23. Frazer, K. A., Ballinger, D. G., Cox, D. R.*et al*: A second generation human haplotype map of over 3.1 million SNPs. *Nature* 2007; **449**: 851-861.
24. Goode, E. L., Fridley, B. L., Sun, Z.*et al*: Comparison of tagging single-nucleotide polymorphism methods in association analyses. *BMC.Proc.* 2007; **1 Suppl 1**: S6-
25. Abecasis, G. R., Cardon, L. R., Cookson, W. O.: A general test of association for quantitative traits in nuclear families. *Am.J.Hum.Genet.* 2000; **66**: 279-292.
26. Diggle P.J., Liang K.Y., Zeger S.L.: Analysis of longitudinal data. 1994;
27. Mollinedo, F., Fontan, G., Barasoain, I., Lazo, P. A.: Recurrent infectious diseases in human CD53 deficiency. *Clin.Diagn.Lab Immunol.* 1997; **4**: 229-231.
28. Kim, T. R., Yoon, J. H., Kim, Y. C.*et al*: LPS-induced CD53 expression: a protection mechanism against oxidative and radiation stress. *Mol.Cells* 2004; **17**: 125-131.
29. Mollinedo, F., Martin-Martin, B., Gajate, C., Lazo, P. A.: Physiological activation of human neutrophils down-regulates CD53 cell surface antigen. *J.Leukoc.Biol.* 1998; **63**: 699-706.
30. Yunta, M., Lazo, P. A.: Apoptosis protection and survival signal by the CD53 tetraspanin antigen. *Oncogene* 2003; **22**: 1219-1224.

31. Hernandez-Torres, J., Yunta, M., Lazo, P. A.: Differential cooperation between regulatory sequences required for human CD53 gene expression. *J Biol.Chem.* 2001; **276**: 35405-35413.
32. Pedersen-Lane, J. H., Zurier, R. B., Lawrence, D. A.: Analysis of the thiol status of peripheral blood leukocytes in rheumatoid arthritis patients. *J Leukoc.Biol.* 2007; **81**: 934-941.
33. Smith, A. J., Keen, L. J., Billingham, M. J.*et al*: Extended haplotypes and linkage disequilibrium in the IL1R1-IL1A-IL1B-IL1RN gene cluster: association with knee osteoarthritis. *Genes Immun.* 2004;
34. Moxley, G., Han, J., Stern, A. G., Riley, B. P.: Potential influence of IL1B haplotype and IL1A-IL1B-IL1RN extended haplotype on hand osteoarthritis risk. *Osteoarthritis.Cartilage.* 2007; **15**: 1106-1112.
35. Scanzello, C. R., Plaas, A., Crow, M. K.: Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Curr.Opin.Rheumatol.* 2008; **20**: 565-572.

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 ¹
No. 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) ¹All subjects of the Leiden 85-Plus study were 85 years old at sampling.

Table 2. Genes and selected SNPs in Linkage Peak, TNF α

Chromosome	Gene (coverage ¹)	Short description ²	SNP	Position	QTD ³	QTD dom ⁴
1	CSF1 (58%)	Granulocyte/macrophage colony-stimulating factors are cytokines that act in hematopoiesis by controlling the production, differentiation, and function of 2 related white cell populations of the blood, the granulocytes and the monocytes-macrophages	rs915357	Intron	0.055 ⁺	0.060 ⁺
			rs333968	Intron		
			rs333970	Intron		
			rs3738760	Coding Exon		
	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	rs10494122	Intron	0.012*	0.009**
			rs10857833	Intron		
			rs6679497	Intron		
			rs4839581	Intron		
	FAM19A3 (100%)	Contains conserved cysteine residues at fixed positions, and are distantly related to MIP-1alpha, a member of the CC-chemokine family.	rs4450019	Intron	0.019*	0.033*
			rs11102524	Intron		
11	MADD (68%)	Tumor necrosis factor alpha (TNF-alpha) is a signalling 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-alpha 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		

17	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*
			rs2074225	Coding exon*
	rs1050922	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.	rs3862667	Intron
			rs572350	Intron
			rs671444	Intron
			rs12364244	Intron
			rs637186	Coding exon*
	GPS2 (100%)	This gene encodes a protein involved in G protein-mitogen-activated protein kinase (MAPK) signalling 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*	
rs6608	3'UTR			
CD68 (50%)	This gene encodes a 110-kD transmembrane glycoprotein that is highly expressed by human	rs9896688	Intron boundary	

	monocytes and tissue macrophagesThe protein is also a member of the scavenger receptor family.	rs9901673	Coding Exon*
	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.	rs9901675	Coding Exon*

[†] P < 0.1; * P < 0.05; ** P < 0.01

¹ Gene coverage was based on genetic variation present in the HapMap database build 18.

² Short description adapted from gene ontology description provided in the UCSC genome graph tool.

³ P values reported by QTDT using command WEGA (additive model), tests for association given linkage on a specific locus.

⁴ 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) ¹	Log(TNF α)	N (12) ¹	Log(TNF α)	N(22) ¹	Log(TNF α)	P LMM ²	P LMM ³
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*	

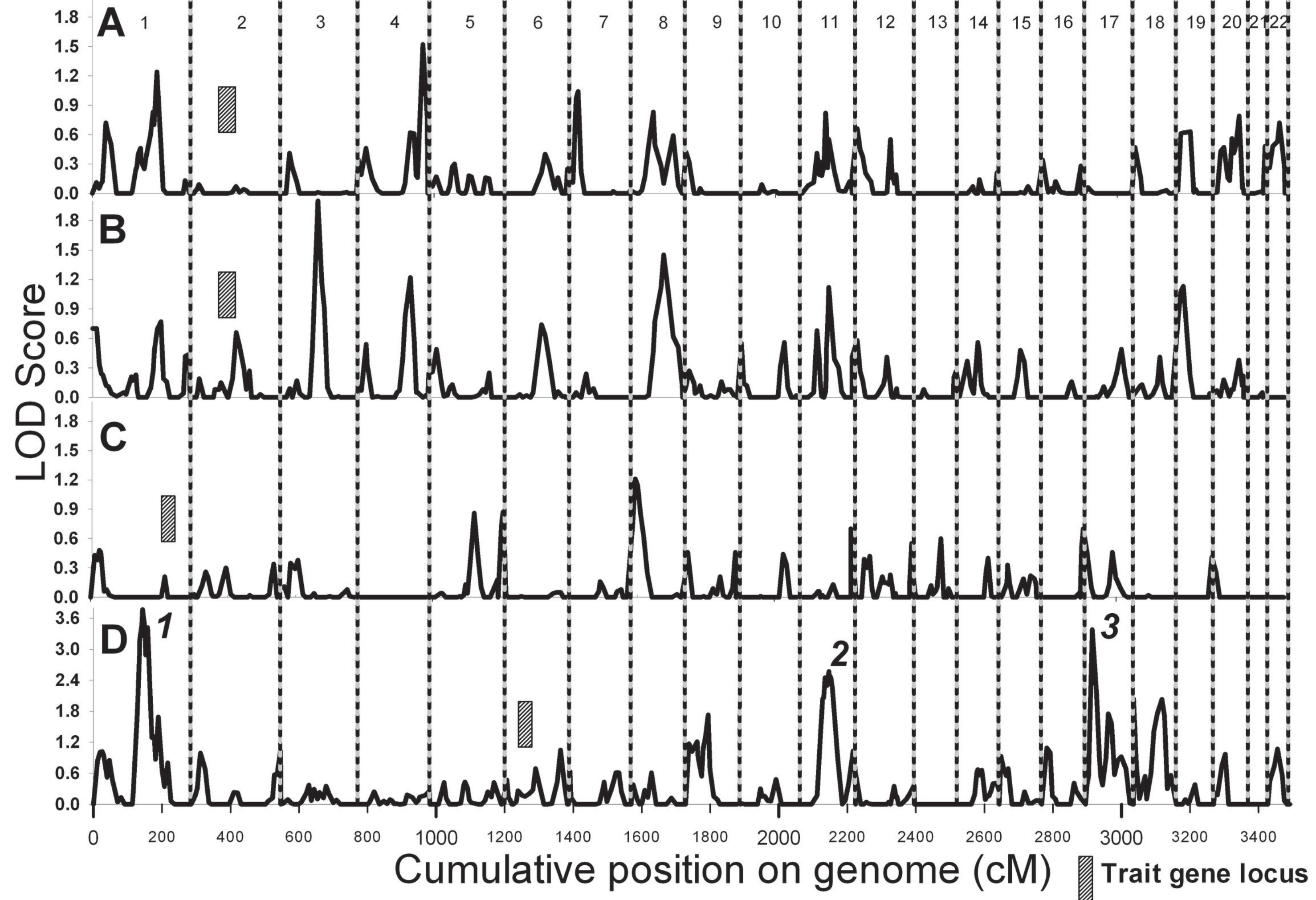
⁺ P < 0.1; * P < 0.05; ** P < 0.01

¹ 11=homozygote common allele; 12=heterozygote; 22=homozygote rare allele

² Modelling dominant effect, corrected for familial relationship, age, sex and BMI

³ Modelling dominant effect combining both studies, corrected for study differences in level, familial relationship, age, sex and BMI

Chromosome number



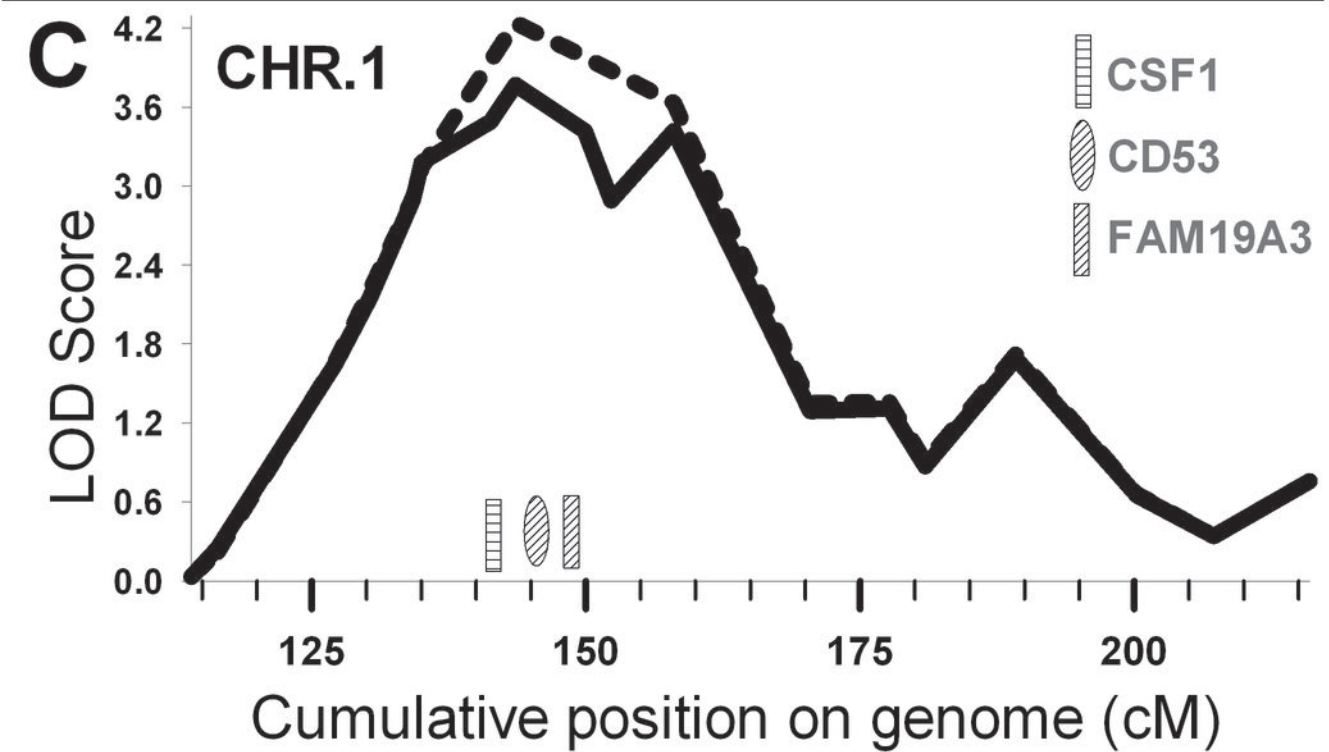
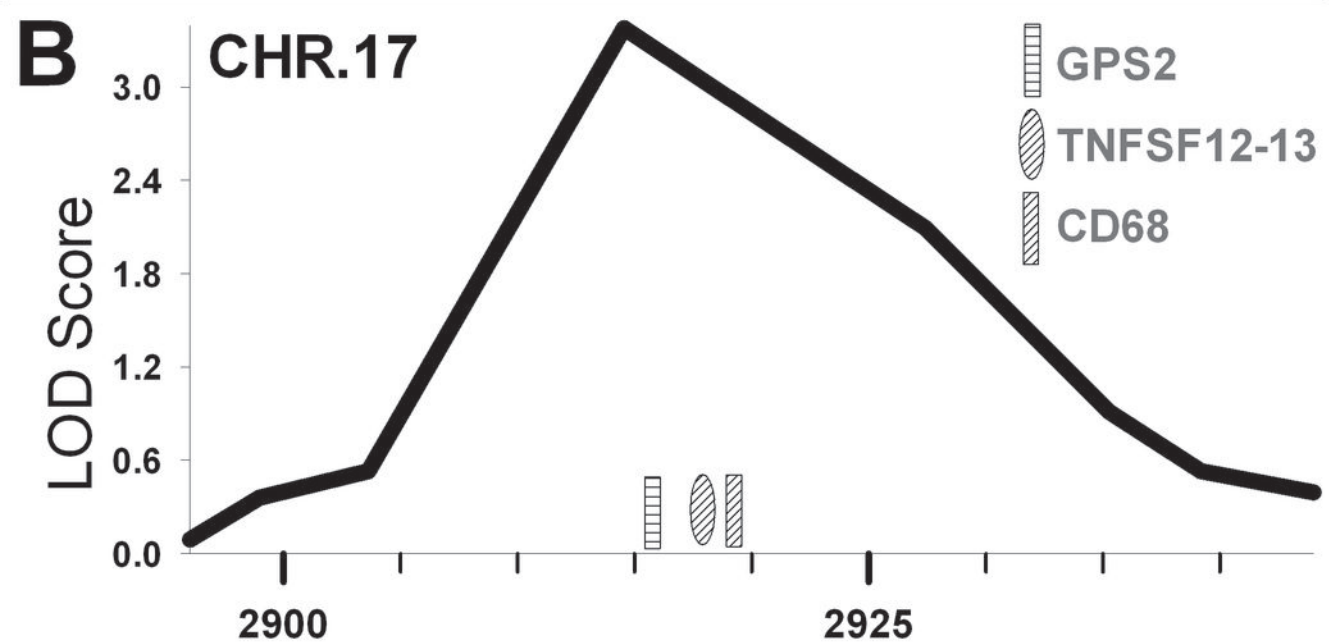
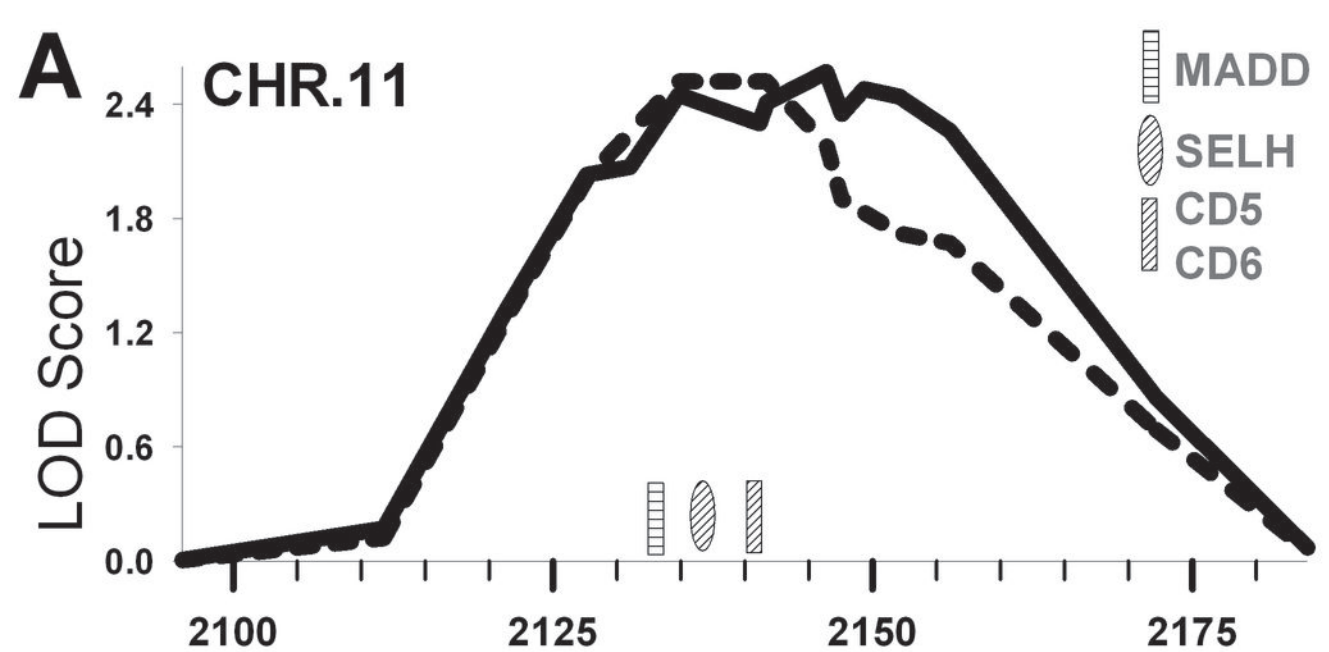


Figure Legends

Figure 1. LOD scores for genome wide linkage analyses for QTL's of (A) IL-1 β , (B) IL-1Ra, (C) IL-10 and (D) TNF α .

Figure 2. Detailed view of initial and fine mapped linkage peaks identified on chromosome 11 (Pane A, peak 2), chromosome 17 (Pane B, peak 3) and chromosome 1 (Pane C, peak 1). Schematically represented are the tested genes positions in the linkage area. The dotted lines represent the initial linkage signal, whereas the solid lines represent the finemapped linkage signal.