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Genetics of Serum Concentrations of IL-6 and TNFα in Systemic Lupus Erythematosus and Rheumatoid Arthritis: A Candidate Gene Analysis

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Abstract

Elevated concentrations of inflammatory mediators are characteristic of autoimmune disease accompanied by chronic or recurrent inflammation. We examined the hypothesis that mediators of inflammation known to be elevated in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are associated with genetic polymorphism previously identified in studies of inflammatory disease.

Serum interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα concentrations in patients with SLE (n=117) or RA (n=164) and in inflammatory disease-free control subjects (n=172) were measured by multiplex ELISA. Candidate genes were chosen from studies of autoimmune and inflammatory disease. Genotypes were determined for 345 SNP markers in 75 genes. Association between serum analytes and single alleles was tested by linear regression. Polymorphisms in several genes were associated with IL-6 levels (including *IL10, TYK2* and *CD40L* in SLE and *DRB1*, *NOD2* and *CSF1* in RA) or with TNFα levels (including *TNFSF4* and *CSF2* in SLE and *PTPN2, DRB1* and *NOD2* in RA). Some associations were shared between disease and control groups or between IL-6 and TNFα within a group.

In conclusion, variation in genes implicated in disease pathology is associated with serum IL-6 or TNFα concentration. Some genetic associations are more apparent in healthy controls than in SLE or RA, suggesting dysregulation of the principal mediators of chronic inflammation in disease. Susceptibility genes may affect inflammatory response with variable effect on disease etiology.

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Keywords

Systemic lupus erythematosus; rheumatoid arthritis; genes; snp; TNFα; IL-6

Introduction

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases of complex, heterogeneous etiology and pathology associated with chronic inflammation;[1, 2] each disease has a substantial genetic component.[3] Many cytokines play an important role in RA and SLE and TNFα and IL-6 are major inflammatory mediators of pathology in both SLE and RA.[1, 2, 4, 5] The pleiotropic nature of both TNFα and IL-6 in mediating inflammation, as well as their participation in multiple regulatory pathways, complicates any straightforward interpretation of mechanism in chronic autoimmune disease.

It is clear, however, that serum concentrations of these mediators are substantially elevated in SLE and RA compared to controls, and to some extent, pathological features can be correlated to these and other biomarkers and quantifiable clinical variables. Increased concentrations of these pro-inflammatory cytokines are associated with several autoimmune disorders. Some disease susceptibility genes are also associated with multiple autoimmune disorders.[3] This leads to the hypothesis that variation in these genes may be associated with cytokine level. Correlation of inflammatory mediator levels with genetic variation could reveal novel aspects of disease pathology and lead to additional hypotheses of mechanism.[6–8] The elaboration of these relationships may further our understanding of differential pathogenesis within autoimmune disease.

This study focuses on IL-6 and TNFα because these two cytokines are increased in patients with RA and SLE and are therapeutic targets of many biologic agents. Furthermore, the genetic determinants of these two cytokines have been studied in other inflammatory conditions, cancer and cardiovascular disease.[9–11]

We examined the association of serum IL-6 and TNFα levels with genetic variation involved in susceptibility to autoimmune disorders. Our hypothesis is that genetic associations between pro-inflammatory cytokine levels and polymorphism in susceptibility genes will identify differences in the control of inflammation in autoimmune disease and suggest mechanisms of dysregulation in disease pathogenesis.

Methods

Study Population

Patients with SLE or RA and subjects without inflammatory disease were 18 years of age or older, and patients fulfilled the American College of Rheumatology classification criteria for SLE or RA.[12, 13] The subjects are participants in ongoing studies of cardiovascular risk in cohorts of patients with rheumatic disease; details regarding recruitment and methodological procedures have been described.[14, 15] By design, there were two separate groups of control subjects without inflammatory disease which were frequency-matched for age, race and sex

to patients with SLE or RA. In the present study we combined these to form a single control group to allow comparison of serum biomarker concentrations and genetic markers among the three groups with appropriate statistical adjustment for demographic variables. The study was approved by the Vanderbilt University Institutional Review Board and subjects gave written informed consent.

Clinical Measurements, Biomarker Assays, and Genotyping

Clinical information and laboratory data were obtained as described.[16–19] Serum concentrations of TNFα and IL-6 were measured by multiplex ELISA (Millipore) in study participants (117 SLE, 164 RA and 172 controls).

Disease activity was quantified using the modified disease activity score that includes 28 joint counts (DAS28)[20] in patients with RA and the systemic lupus erythematosus disease activity index (SLEDAI).[21]

Our primary hypothesis that indicators of inflammation known to be elevated in SLE and RA are associated with some of the genes implicated in autoimmune disease pathology required a candidate selection process. Seventy-five genes of relevance to autoimmune disease susceptibility and chronic inflammatory conditions were chosen from the published literature. Genotypes were determined for 345 SNP markers in these 75 genes. Supplementary Table 1 lists the genes, selected SNP markers of each designated by genome position, and observed minor allele frequencies (maf). Tagging SNP markers were selected based upon HapMap CEU subject data ($R^2 \geq 0.8$, maf >0.05) for 59 genes, encompassing 3–20 kb upstream to 1–10 kb downstream of each respective coding region. Separately, 16 genes were represented by a single SNP selected from published inflammatory and autoimmune disease association studies.

DNA was extracted from whole blood samples using Puregene DNA Purification reagents (Gentra) and standard protocols on an Autopure LS instrument (Qiagen). Quantitation was done with a Nanodrop 2000 instrument (Thermo Scientific). Samples were diluted to 50 ng/ul for genotype assay. Genotyping was done using GoldenGate assay on a Beadstation 500 instrument (Illumina).

Statistical Analysis

Descriptive statistics were calculated as percentage (%), mean with standard deviation (mean ± SD), or median with interquartile range (median [IQR]) according to the distribution of the variables. Genotype data was recoded to 0, 1, 2 for analysis with each minor allele assigned a value of one unit. Values for concentrations of inflammatory mediators were $\log_{10}$-transformed when used in genetic association analysis in order to normalize distribution. Linear regression was used to measure association between serum concentrations and recoded genotype data. All associations were adjusted for age, race and sex. Descriptive statistical analysis and genotype recoding was performed using SPSS19 (SPSS Inc.) and Sequence Variation Suite 7 (Golden Helix Inc.) respectively. Genetic analysis was done using R software (http://www.r-project.org). A two-sided P-value of ≤ 0.05 was considered significant.

Results

The descriptive characteristics of patients with SLE or RA and control subjects are shown in Table 1. Patients with SLE were younger and more likely to be female than patients with RA. Sixty-eight percent of patients with SLE, 88% of patients with RA and 79% of control subjects were Caucasian. In patients with RA, the median DAS28 was 4 (IQR 3-5) and in patients with SLE the median SLEDAI was 4 (0–6). As we have reported previously, concentrations of inflammatory mediators were higher in patients with RA or SLE than control subjects (9–12).[14, 15] TNFα concentrations were higher in patients with SLE (median 4.8, IQR [3.2–7.9] pg/ml) and RA (5.5 [2.8–11.0] pg/ml) than controls (2.7 [2.0–3.9] pg/ml). Serum IL-6 concentrations were higher in patients with SLE (6.2 [2.6–26.5] pg/ml) and RA (13.8 [4.4–43.0] pg/ml) than controls (2.6 [1.0–9.6] pg/ml), and were higher in RA than SLE (all $P<0.001$).

Preliminary examination of the data indicated that nominally significant candidate polymorphism associations were sometimes shared among sample groups. Shared associations were also observed between IL-6 and TNFα. Multiple markers from some genes were associated with inflammatory mediator concentrations. Association results for both IL-6 and TNFα for all SNP markers are provided in Supplementary Table 2. The top rank-order associations (adjusted for age, race and sex, $P\leq0.01$) for SLE, RA and controls (CTL) are shown in Table 2 for IL-6 and Table 3 for TNFα. In Tables 2 and 3, P-values ≤0.01 and coefficients in the 95th percentile are shown in bold-face type.

Genetic associations noted include TNFα with *TNFSF4* and *CSF2* in SLE, and *PTPN2*, *DRB1* and *NOD2* in RA, and several, including *IRF5*, in the controls. Those observed for IL-6 include *IL10*, *TYK2*, *CD40L* and *CTLA4* in SLE, and *DRB1*, *NOD2*, *SELE*, and *IL17A* in RA, and *PTPN22* and *MMP7* in the controls. Associations shared between serum TNFα and IL-6 concentration within a disease group included *DRB1*, *NOD2*, *PTPN2* and *CSF1*, all in RA. Fewer significant associations are seen for TNFα than for IL-6.

Some genetic associations appear to be shared between groups, notably *IRF5* (rs10488631) for TNFα in SLE (P=0.02) and controls (P=0.003), *PTPN22* (rs10488631, rs4731535) for IL-6 in SLE (P=0.03, 0.04) and controls (P=0.01 for each), *NOD2* (rs5743291) for TNFα in RA (P=0.01) and controls (P=0.02), and *MMP13* (rs6679677, rs2476601) for TNFα in RA (P=0.05 for each) and controls (P=0.01 for each). None of the associations withstand correction for multiple testing using Bonferroni adjustment, and only rs3021304, rs748855 and rs2076059 for IL-6 in RA withstand False Discovery Rate correction at 20%.

We have included β coefficients as a measure of magnitude of effect and as possible support of probable marker association in Tables 2 and 3. The largest coefficients (those in the 95th percentile shown in bold-face type) support some of the top-rank associations, including *TNSF4*, *IL10*, *CD40L*, *PTPN2*, *NOD2*, *CSF1*, *DRB1*, *IRF5*, *PTPN22*, and *MMP7*, but not others. Global measurement of haplotype association including all SNP within each gene (HaploStats in R) adjusted for age, race and sex provides support for some of the top-rank gene associations listed in Table 2 for IL-6, including *IL10* (P=0.01) and *CD40L* (P=0.03) in SLE, and *IL17A* (P=0.01), *SELE* (P=0.04) and *DRB1* (P=0.02) in RA, and also for TNFα

including *MMP12* (P=0.03) in controls. *PTPN2* is represented by a single SNP (rs2542151) and was not included in global haplotype estimation.

Discussion

Our major finding is that serum concentrations of IL-6 and TNFα, principal mediators of inflammation known to be elevated in patients with SLE or RA, are associated with polymorphisms in genes involved in regulation of inflammatory pathways; these genes are sometimes established susceptibility genes from genome wide association studies (GWAS) in autoimmune disorders. Such associations are seen not only in patients with SLE or RA but also, to some extent, in controls free of inflammatory disease.

Genetic associations with inflammatory mediators noted in our study include *DRB1*, *PTPN2*, *NOD2*, *SELE*, *IL17A* and *CSF1* in RA, and *IL10*, *CD40L*, *TYK2*, *CTLA4*, *CSF2* and *TNSF4* in SLE, as well as *PTPN22*, *IRF5* and several *MMP* genes in the controls. Associations shared between TNFα and IL-6 concentrations are seen primarily in the RA group. Associations shared to varying extent between disease and control groups include *IRF5*, *NOD2*, *PTPN22* and *MMP13*. The limited size of our study permits only speculative conclusions regarding the contribution of particular genes in the control of serum inflammatory mediator concentration, but the observations that some of these associations are seen in more than one group and that biologically plausible associations are seen for the disease groups is interesting and may serve as a basis for candidate replications.

It will be important to follow these observations as larger studies for which both inflammation measurements and genetic data become available. To date, no such studies have been reported for autoimmune disease, but inflammatory markers in serum, including TNFα and IL-6, have been examined in relation to genetic markers for cardiovascular disease risk in the general population.[22]

Genetic regulation of chronic inflammation in RA

The HLA-DRB1 locus, particularly the variants comprising the shared epitope (SE), has been recognized as critical in RA etiology and pathology, [23, 24] so that the association of *DRB1* markers with both TNFα and IL-6 in RA but not SLE or control groups is consistent with known pathophysiology. Given the established central role of *DRB1* in modulation of disease expression, including the classification into pathological subsets,[25] it is possible that *DRB1* is associated with elevated TNFα and IL-6 either by a direct effect upon the innate immune response or by an indirect association through other variables correlated with increased cytokine levels, for example RA severity or disease duration. It should be noted that we did not measure SE alleles directly, but the *DRB1* markers we used capture the genetic effect attributed to the SE variation through linkage disequilibrium with tagged SNP markers[26]. Moreover, a tagged SNP strategy measures the genetic effect of additional SNP variants nearby which more completely and definitively explain RA susceptibility at the HLA locus.[23]

The nonreceptor-type protein tyrosine phosphatases (PTPN) are important negative regulators of inflammatory activation and participants in immune cell signaling

pathways.[27, 28] PTPN genes are essential in maintaining immune cellular homeostasis, and their association with several autoimmune diseases exhibiting chronic inflammation is both intuitively and mechanistically satisfying. *PTPN2* is an established susceptibility gene in type 1 diabetes and Crohn's disease and these associations have more recently been extended to other autoimmune disorders including RA.[3, 7, 29] The proposed mechanisms for pathological involvement in autoimmunity and innate immunity are now becoming evident.[7, 29–32] In particular, *PTPN2* has a direct role in negative regulation of IL-6 and TNFα expression and signal transduction.[33] Mice deficient in PTPN2 exhibit high serum concentrations of TNFα and IL-6,[34, 35] suggesting that *PTPN2* is essential in maintaining signaling control of both inflammatory mediators and limiting chronic systemic inflammation. The single SNP (rs2542151) examined here is associated with both serum IL-6 and TNFα in RA.

NOD2 has been validated as a susceptibility gene in Crohn's disease and other inflammatory bowel disease, but has not yet been associated with RA.[3, 7, 29] It is one of the genes we examined which is directly involved in innate immune pathways,[5, 36] so that its association with elevated levels of inflammatory mediators in RA is interesting, particularly since both *PTPN2* and *NOD2* are important risk factors in the pathology of Crohn's disease and *PTPN2* has recently been shown to be necessary in control of toll-like receptor responses through the NOD2 ligand.[29–31, 36]

IL17A, *SELE* and *CSF1* are other genes for which we have observed associations, but the supporting evidence for their involvement in control of inflammatory mediator concentration in RA serum is less direct.[24, 37, 38] In particular, there is little support for association from GWAS in RA case-control cohorts, and most of the support comes from investigations of inflammatory mechanisms.[5, 24]

Regulation of inflammatory mediators in SLE

As in RA, several of the genetic associations we observe in SLE have been implicated as susceptibility genes in case-control studies. Thus, our finding of association of *TNFSF4* with TNFα serum concentration is consistent with SLE disease pathology.[6, 39, 40] Likewise are the associations of *IL10*, *TYK2*, *CTLA4* and *CD40L* with IL-6 concentration in SLE.[3, 7, 8, 39, 41–44] We observe a larger number of significant associations for IL-6 than TNFα in SLE, as we did for RA, but there is no apparent overlap between the genetic associations seen in SLE for TNFα and IL-6 as in RA.

Genetic control of TNFα and IL-6 in inflammatory disease-free controls

One of the more interesting findings of this study is that some of these autoimmune susceptibility genes are associated with IL-6 or TNFα concentrations in inflammatory disease-free controls. Associations observed in the controls include *IRF5*, *PTPN22* and several matrix metaloproteinase (MMP) genes (*MMP7, MMP12, MMP13*). Shared associations noted between disease and control groups included *IRF5*, *NOD2*, *MMP13*, and *PTPN22*. Occasionally, associations are shared between disease and control groups with the association in disease being attenuated compared to controls, suggesting possible dysregulation of the normal regulatory mechanisms affecting TNFα and IL-6 in the disease

state.[4, 7] Thus, although it is well-established that serum concentrations of the inflammatory mediators TNFα and IL-6 are elevated in SLE and RA, mediator concentration may be more tightly regulated in inflammatory disease-free controls by the very markers identified with risk in case-control studies.

The interferon regulatory factor 5 gene (*IRF5*) is strongly implicated in SLE susceptibility and is involved in the direct regulation of type I interferon subclass concentrations, both at the site of pathology as well as in serum.[3, 45] The interferon (IFN) signature of expressed genes is considered a useful prognostic biomarker for lupus, although signatures for IRF5 differ from the canonical IRF3/7 IFN induced expression pattern, and disease risk associated with *IRF5* has been established in other autoimmune disorders in which the role of IFN is less clear.[3, 7, 45] These observations argue for a role for *IRF5* beyond induction of IFN in dysregulation of chronic inflammation and autoimmunity,[46, 47] and the association of *IRF5* with TNFα observed here in the control group, attenuation in SLE, and lack of association in RA suggests a disruption of control leading to chronic inflammation.[48] *IRF5* acts as a master transcription factor downstream in the TLR pathway to induce proinflammatory cytokines including TNFα and IL-6.[49, 50] In *Irf5* knockout mice, induction of both TNFα and IL-6 is severely limited.[49, 50]

PTPN22 has been validated as a susceptibility gene, like *IRF5*, in several autoimmune diseases including SLE and is the most dominant genetic risk factor aside from the *HLA* locus for the development of autoimmunity.[3, 51, 52] As a nonreceptor-type protein tyrosine phosphatase gene like *PTPN2*, *PTPN22* is essential in the maintenance of signaling quiescence, and it is possible that dysregulation could result in loss of immunological tolerance or an exacerbation of inflammation.[7, 52] It is interesting that *PTPN22* association is observed here for IL-6 but not TNFα and is more pronounced in controls than in SLE.

The role of MMP genes in inflammation extends well beyond extracellular matrix organization and tissue injury to more diverse pathologic functions in disease, particularly in chronic inflammatory disease.[53] There is substantial evidence to support the role of MMP genes in maintaining a balance between tissue maintenance and remodeling, and so their association in regulation of serum IL-6 and TNFα, specifically in the control group, is not surprising, particularly since these genes are not supported in GWAS for autoimmune susceptibility.

Our study has several limitations, the most important of which is limited power to detect genetic associations of modest contribution to risk with multiple testing adjustments. This is somewhat overcome by examining association with well-established biomarkers of disease pathology, thus limiting the heterogeneity of phenotype, by focusing on a candidate gene approach, and by measuring serum biomarkers in healthy control populations as well as SLE and RA patients, thus allowing comparisons between groups. The association of some genes in more than one group argues that these associations are not simply due to chance. However, none of the associations observed withstand rigorous correction, such as Bonferroni correction for multiple comparisons, and it will be of interest to see if these associations are confirmed, either in larger studies with greater power or in more limited replication studies with fewer tested variables, where both serum biomarker and genetic

marker data are collected. We did not check anticitrullinated protein antibodies, so we could not explore if the relationship between the reported genetic variants and the cytokine concentrations was modified by ACPA positivity. In addition, our study was focused on IL-6 and TNF-α; thus, other important cytokines in the pathogenesis of SLE, such as interferon[54, 55] were not evaluated. The study was cross-sectional in design so cause and effect inferences cannot be drawn, and conclusions beyond suggestion of pathological mechanism will require more appropriately designed studies. Finally, given the known effect of positive interference in immunodetection assays for some serum samples containing rheumatoid factor, the results of IL-6 or TNFα associations seen in RA could be affected.

In conclusion, this study suggests that several genes are involved in regulating serum TNFα or IL-6 elevation in RA and SLE. Associations noted included genes clearly implicated in disease pathology such as *IL10*, *TYK2*, *CD40L*, *CTLA4* and *TNFSF4* in SLE and *DRB1*, *PTPN2*, and *IL17A* in RA, but also included some associations with genes whose pathological relevance to these diseases is less clear, particularly *NOD2* and *CSF1* in RA. There are also some genes clearly implicated in autoimmune susceptibility and pathology whose associations seen here are more apparent in the controls (*IRF5*, *PTPN22*, and *MMP13*). Susceptibility genes may affect inflammatory responses with variable effect on pathology, suggesting that differential genetic regulation of the principal mediators of chronic inflammation occurs in autoimmune disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the Study Population

	SLE (n=117)	RA (n=164)	CTL (n=172)
Age (Years**)**	**40 ± 12**	**54 ± 12**	**47 ± 13**
Sex (% Female**)**	**91%**	**68%**	**74%**
Race (% Caucasian**)**	**68.4%**	**88.4%**	**78.7%**
SLEDAI Damage Index	**4 [0–6]**	-	-
SLICC	**1 [0–3]**	-	-
DAS28	-	**4 [3–5]**	-
Duration (Years**)**	**9 ± 8**	**10 ± 11**	-
TNF-α (pg/ml)	**4.8 [3.2–7.9]**	**5.5 [2.8–11.0]**	**2.7 [2.0–3.9]**
	6.4 ± 6.1	**20.9 ± 50.8**	**3.3 ±2.9**
IL-6 (pg/ml)	**6.2 [2.6–26.5]**	**13.8 [4.4–43.0]**	**2.6 [1.0–9.6]**
	20.6 ± 32.8	**51.9 ± 160.4**	**10.4 ± 19.4**

Data are shown as mean ± SD, median [IQR] or percentages (%). The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (29) and the Systemic Lupus International Collaborating Clinics (SLICC) (30) scores are measures of disease activity and damage respectively. DAS28 (31) is a measure of RA severity.

Table 2

Association Between Serum IL-6 Concentration and Genetic Markers.

A). Rank Order Associations with IL-6 in SLE

	SLE		RA	CTL
SLE top-rank P-value	**(P-value)**	**(β)**	**(P-value)**	**(P-value)**
rs3024498(*IL10*)	**0.001**	**2.7**	0.5	0.4
rs1800890(*IL10*)	**0.001**	**2.1**	0.8	0.3
rs231726(*CTLA4*)	**0.004**	0.4	0.09	0.7
rs3092936(*CD40L*)	**0.004**	**2.5**	0.4	0.4
rs280523(*TYK2*)	**0.006**	0.3	0.6	0.8
rs9514827(*TNFSF13B*)	**0.009**	**1.8**	0.4	0.5
rs1799964(*TNFA*)	**0.009**	0.4	0.1	0.3
rs1800630(*TNFA*)	**0.01**	0.4	0.09	0.8
rs5930973(*CD40L*)	**0.01**	0.1	0.6	0.09
rs738807(*MIF*)	**0.01**	**2.2**	0.9	0.3
rs2488457(*PTPN22*)	**0.01**	**2.0**	0.8	0.3

B). Rank Order Associations with IL-6 in RA

RA top-rank P-value	**(P-value)**	**(P-value)**	**(β)**	**(P-value)**
rs3021304 (*DRB1*)	0.8	**0.0003**	0.5	0.08
rs748855 (*NOD2*)	0.7	**0.0009**	**1.8**	0.7
rs2076059 (*SELE*)	0.6	**0.001**	0.5	0.9
rs1024610 (*CCL2*)	0.9	**0.003**	0.5	0.3
rs3819025 (*IL17A*)	0.5	**0.004**	0.2	0.1
rs660895 (*DRB1*)	0.8	**0.004**	**1.7**	0.3
rs2542151 (*PTPN2*)	0.9	**0.004**	**1.8**	0.1
rs3738760 (*CSF1*)	0.7	**0.007**	**1.9**	0.1
rs10493936 (*VCAM1*)	0.4	**0.007**	0.5	0.1
rs6131(*SELP*)	0.8	**0.01**	**1.8**	0.4
rs333970(*CSF1*)	0.9	**0.01**	**1.6**	0.09
rs3093040(*CSF1*)	0.2	**0.01**	0.6	0.4
rs3087243(*CTLA4*)	0.1	**0.01**	0.6	0.5
rs602875(*DRB1*)	0.6	**0.01**	0.5	0.3
rs10489270(*TNFSF4*)	0.7	**0.01**	0.4	0.1
rs2066843(*NOD2*)	0.1	**0.01**	0.6	0.6

C). Rank Order Associations with IL-6 in Controls

CTL top-rank P-value	**(P-value)**	**(P-value)**	**(P-value)**	**(β)**
rs12285347(*MMP7*)	0.4	0.2	**0.004**	**1.6**
rs17098318(*MMP7*)	0.09	0.5	**0.01**	0.6
rs10502002(*MMP7*)	0.1	0.1	**0.01**	0.2
rs6679677(*PTPN22*)	**0.03**	0.7	**0.01**	**2.4**

C). Rank Order Associations with IL-6 in Controls				
CTL top-rank P-value	**(P-value)**	**(P-value)**	**(P-value)**	**(β)**
rs2476601(*PTPN22*)	**0.04**	0.6	**0.01**	**2.4**

Top-ranked associations with β-coefficients for A) SLE, B) RA, and C) CTL are shown. Number of samples (n) with both genotype and IL-6data for systemic lupus erythematosus (SLE, n=110), rheumatoid arthritis (RA, n=141) and inflammatory disease-free control (CTL, n=149) vary between groups. IL-6 data was not available for nine control samples. P-values ≤0.01 and β-coefficients in the 95th percentile are shown in bold-face type.

Table 3

Association Between Serum TNF Concentration and Genetic Markers.

A). Rank Order Associations with TNF in SLE

	SLE		RA	CTL
SLE top rank P-value	**(P-value)**	**(β)**	**(P-value)**	**(P-value)**
rs3850641(*TNFSF4*)	**0.01**	**1.3**	0.8	0.2
rs25882(*CSF2*)	**0.01**	0.7	0.1	0.8
rs8110090(*TGFB1*)	**0.01**	0.6	0.2	0.3

B). Rank Order Associations with TNF in RA

RA top-rank P-value	**(P-value)**	**(P-value)**	**(β)**	**(P-value)**
rs3021304(*DRB1*)	0.6	**0.001**	0.5	0.06
rs2542151(*PTPN2*)	0.5	**0.001**	**1.8**	0.8
rs3783546(*IL1A*)	0.3	**0.01**	0.6	0.9
rs5743291(*NOD2*)	0.3	**0.01**	**2.1**	**0.02**
rs3738760(*CSF1*)	0.1	**0.01**	**1.7**	0.8

C). Rank Order Associations with TNF in Controls

CTL top-rank P-value	**(P-value)**	**(P-value)**	**(P-value)**	**(β)**
rs10488631(*IRF5*)	**0.02**	0.8	**0.003**	**1.2**
rs4731535(*IRF5*)	0.1	0.5	**0.004**	0.3
rs12808148(*MMP12*)	0.06	0.9	**0.007**	**1.2**
rs2252070(*MMP13*)	0.5	**0.05**	**0.01**	0.8
rs833060(*VEGFA*)	0.3	0.3	**0.01**	**1.1**
rs9972960(*CCL3*)	0.7	0.7	**0.01**	**1.1**
rs346604(*TNFSF11*)	0.7	0.5	**0.01**	0.7
rs2243828(*MPO*)	0.3	0.7	**0.01**	**1.1**
rs1884444(*IL23R*)	0.5	0.6	**0.01**	0.8

Top-ranked associations with βcoefficients for A) SLE, B) RA, and C) CTL are shown. Number of samples (n) with both genotype and TNF-α data for systemic lupus erythematosus (SLE, n=110), rheumatoid arthritis (RA, n=141) and inflammatory disease-free control (CTL, n=158) vary between groups. P-values ≤0.01 and β-coefficients in the 95th percentile are shown in boldface type.