TNFAIP3 Gene Polymorphisms Are Associated with Response to TNF Blockade in Psoriasis

Trilokraj Tejasvi1, Philip E. Stuart1, Vinod Chandran2, John J. Voorhees1, Dafna D. Gladman2, Proton Rahman3, James T. Elder1,4, and Rajan P. Nair1
1Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI
2University Health Network, Toronto Western Hospital, Toronto, Ontario, Canada
3Memorial University, St. John’s, Newfoundland, Canada
4Ann Arbor Veterans Affairs Medical Center, Ann Arbor, MI

Abstract

The TNFAIP3 gene has been associated with psoriasis, rheumatoid arthritis, type 1 diabetes mellitus, systemic lupus erythematosus and celiac disease. TNFAIP3 encodes A20, a TNF-α-inducible zinc finger protein thought to limit NF-κB mediated immune responses. In this study we report association of response of psoriasis to TNF blockers with two TNFAIP3 SNPs (rs2230926 in exon 3 and rs610604 in intron 6) and their haplotypes. Treatment response was self-evaluated using a 0–5 visual analog scale in 433 psoriasis patients who received TNF blockers. Confirmation was sought in 199 psoriasis and psoriatic arthritis patients from Toronto who were followed prospectively. Response variables were dichotomized separately in the two cohorts, yielding similar proportions of good responses. While significant associations were observed only for the Michigan cohort, fixed-effects meta-analysis retained significant association between dosage of the G allele of rs610604 and good combined response to all TNF blockers (OR = 1.50, pcorr = 0.050) and etanercept (OR = 1.64, pcorr = 0.016). The rs2230926 T–rs610604 G haplotype was similarly associated. By demonstrating an association with therapeutic response, these results provide a clinically relevant functional correlate to the recently described genetic association between psoriasis and TNFAIP3.

Keywords

psoriasis; TNFAIP3; tumor necrosis factor; pharmacogenetics
Psoriasis is a common immune mediated disorder of the skin, nails, scalp and joints, with a multifactorial genetic basis. Recent association studies have identified 25 susceptibility loci reaching genome-wide significance for psoriasis (Ellinghaus et al., 2010; Huffmeier et al., 2010; Nair et al., 2009; Strange et al., 2010; Stuart et al., 2010; Sun et al., 2010). The HLA-C region on chromosome 6p21.3 emerged as the strongest associated locus in all populations studied, accompanied by several genes involved in the immune and inflammatory pathways (Elder et al., 2010). Biological drugs targeting tumor necrosis factor-α (TNF-α, encoded by the TNF gene which happens to be located 303 kb centromeric of HLA-C), have been highly successful in treating severe psoriasis vulgaris (PsV), as well as the subset of approximately 25% of PsV patients (Gottlieb et al., 2003; Leonardi et al., 2003; Papp et al., 2005) diagnosed with psoriatic arthritis (PsA). Nevertheless, there is marked heterogeneity in treatment response to these medications. Multiple studies evaluating the antipsoriatic efficacy of anti-TNF agents have demonstrated efficacy of ≥75% reduction in Psoriasis Area Severity Index (PASI, a composite score that measures erythema, induration and scaling weighted for the body surface involved) in 45–70% patients depending on the dosage and duration of treatment (Sobell et al., 2009).

Compared with traditional systemic therapies for psoriasis such as phototherapy and methotrexate, treatment with the anti-TNF drugs poses a considerable financial burden. A year of biologic therapy can cost between US $10,000–$25,000 based on the dosage and treatment regimen prescribed (Cordoro and Feldman, 2007) while methotrexate and narrow-band ultraviolet B (NB-UVB) phototherapy would cost $623 and $3,692 respectively (Pearce et al., 2006). Given these costs, accurate predictors of therapeutic response to anti-TNF agents would be of great value in making decisions on treatment options.

Previous studies addressing genetic predictors of therapeutic response have investigated promoter region polymorphisms in the TNF gene in rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn’s disease and ulcerative colitis (Kooloos et al., 2007; Maxwell et al., 2008; Musone et al., 2008; O’Rielly et al., 2009; Pierik et al., 2006; Seitz et al., 2007). We carried out a survey-based evaluation of TNF promoter region polymorphisms in 343 psoriasis patients treated with TNF antagonists, and found an association with the TNF-238 G/A polymorphism (OR=2.03, p=0.044) (Tejasvi et al., 2008). However, this association was only nominally significant, prompting evaluation of additional patients as well as additional potential genetic loci.

TNFAIP3 was originally identified as a TNF inducible gene which, at least in some settings, functions as a negative feedback inhibitor of TNF signaling (Opipari et al., 1990; Werner et al., 2008). Recent genome-wide association studies (GWAS) have revealed associations between TNFAIP3 and psoriasis (Nair et al., 2009; Strange et al., 2010), rheumatoid arthritis (RA) (Plenge et al., 2007; Thomson et al., 2007), type 1 diabetes mellitus (Fung et al., 2009), systemic lupus erythematosus (SLE) (Graham et al., 2008), celiac disease (Trynka et al., 2009), and chronic sinusitis (Cormier et al., 2009). Its protein product, A20, functions as a dual enzyme: initially it removes Lys63-linked ubiquitin chains from RIP1, an essential mediator of the proximal TNFR signaling complex. Later, it functions as E3 ligase, leading...
to polyubiquitinylation of RIP1 via Lys48 that targets RIP1 for proteasomal degradation, resulting in termination of TNF-induced NF-κB signaling (Liu et al., 2005; Wertz et al., 2004). A20 can also remove K63-linked polyubiquitin chains from TRAF6 and RIP2, inhibiting NF-kB activation by TLR4 and NOD2, respectively (Vereecke et al., 2009). A20 also blocks NF-kB activation induced by TAK1 overexpression, which signals downstream of RIP1, RIP2 and TRAF6 (Zetoune et al., 2001). It can also target TRAF2 for lysosomal degradation, independent of its ubiquitin modifying properties (Li et al., 2009). Thus A20 acts at multiple steps in the NF-kB signaling pathway (Vereecke et al., 2009).

A20 binds to ABIN-1, a protein encoded by the TNIP1 gene, which, interestingly, is also associated with psoriasis (Nair et al., 2009) and SLE (Gateva et al., 2009; Han et al., 2009). This interaction also temporally limits NF-κB activation (Heyninck et al., 1999), at least in part by binding of ABIN-1 to NEMO/IKK-γ (Mauro et al., 2006).

In a genome-wide association study (GWAS) of response to TNF blockers in rheumatoid arthritis (RA), 89 patients were analyzed using 283,348 single nucleotide polymorphisms (SNPs), yielding nine signals reaching nominal significance (p < 0.05) (Liu et al., 2008). In a very recent GWAS, a total of 1286 anti-TNF-treated patients were analyzed in a 3-stage design (Plant et al., 2011). No loci yielded statistical evidence reaching genome-wide significance, emphasizing the need for much larger cohorts for unbiased estimation of pharmacogenetic responses to TNF antagonists and indeed to other biologics and immunomodulators in general.

A recent pharmacogenomic study looking into the profile of early gene expression profile among responders and non-responders to anti-TNF therapy in RA revealed early downregulation of expression of TNFAIP3 levels secondary to TNF neutralization in responders (Koczan et al., 2008). However, to date there are no published pharmacogenetic studies of the relationship between the TNFAIP3 locus and responsiveness to anti-TNF agents in psoriasis. In this study, we evaluate the role of two polymorphisms in the TNFAIP3 gene as predictors of clinical response to TNF blockade in patients with psoriasis and/or PsA from two study sites.

RESULTS

We studied 433 psoriasis patients from Michigan and 199 patients from Toronto who received treatment with any of three different TNF blockers – etanercept, infliximab and adalimumab. We compared the two samples for 19 demographic and phenotypic variables (Table 1). Highly significant differences were observed between the means of three variables—the Toronto sample had more PsA patients (93.0% vs. 58.3%, p = 2.9 × 10⁻²⁰), while the Michigan sample had a higher mean age (51.4 yrs vs. 46.0 yrs, p = 8.6 × 10⁻⁷) and a higher percentage of patients with a family history of psoriasis (60.6% vs. 42.9 %, p = 4.5 × 10⁻⁵). The Michigan sample also had a later age at PsA onset (37.6 yrs vs. 33.6 yrs, p = 0.0028). A nominally significant difference (p = 0.019) in the percentage of patients with inflammatory bowel disease did not remain so after correction for multiple testing. No significant difference between the two samples was seen in the percentage of good response to treatment with any of the anti-TNF agents, either individually or combined. Furthermore,
the frequencies of the allele imparting risk for psoriasis did not differ significantly between the two groups for either of the two analyzed TNFAIP3 SNPs.

Covariate analyses with age at onset, BMI and presence of PsA were performed in both cohorts, along with worst-ever total body surface area (TBSA) in the Michigan cohort. Age at onset was significantly and negatively associated with response to TNF blockers in the Michigan cohort (OR=0.973, p=0.00016). Although not reaching nominal significance, age at onset also trended towards negative association with all anti-TNF responses in the Toronto sample (OR=0.982 for all TNF blockers combined).

Data were analyzed for linear trend of association between drug response and genotypes and haplotypes of one disease-associated SNP in intron 6 (rs610604, MAF = 0.338) (Nair et al., 2009) and another in exon 3 of TNFAIP3 (rs2230926, MAF = 0.029). The latter was the only tested exonic SNP that was polymorphic in our sample. In the Michigan sample, association was observed between dosage of the G allele of rs610604 and good response to all TNF blockers (OR = 1.74, p_{nom} = 0.0027, p_{corr} = 0.0084)(Table 2). Combined analysis of the two samples using a fixed-effects model showed association between dosage of the G allele of rs610604 and good response to all TNF blockers combined (OR= 1.50, p_{nom}= 0.010, p_{corr} =0.050). Despite a similar OR, this association was no longer significant when the combined analysis used a random-effects model (OR= 1.39, p_{nom} = 0.22, p_{corr} =0.77) (Table 2). Age at onset of disease was included as a covariate in all association models and the nested logistic regression models testing association for each combination of SNP and drug revealed that the associations of age at onset and SNP rs610604 with drug response were independent of each other (no significant interaction).

Haplotype analysis (Table 3) revealed that dosage of the rs2230926 T–rs610604 G haplotype was associated with good response to all TNF blockers (OR = 1.82, p_{nom} = 0.0012, p_{corr} = 0.0060) in the Michigan cohort. Combined analysis of both groups under a fixed effects model showed association between the rs2230926 T–rs610604 G haplotype and response to all TNF blockers (OR = 1.55, p_{nom}= 0.0051, p_{corr} =0.031). The association of haplotype rs2230926 T–rs610604 G with response to TNF blockers in the Michigan cohort is driven mainly by rs610604; conditional haplotype testing showed that the independent contribution of the rs610604 G allele to the association is significant (p_{nom} = 0.0016) whereas the independent contribution of the rs2230926 T allele is not (p_{nom} = 0.90).

**DISCUSSION**

The TNFAIP3 gene has been shown to be associated with psoriasis in a recent genome-wide association study (Nair et al., 2009), a finding recently confirmed strongly in Caucasian (Strange et al., 2010) and suggestively in Chinese (Sun et al., 2010) populations. Here we demonstrate that polymorphisms in TNFAIP3 are associated with response to anti-TNF agents in psoriasis patients. In the Michigan sample, good response to etanercept alone and all TNF blockers combined was positively associated with dosage of the G allele of rs610604, a psoriasis susceptibility allele (Nair et al., 2009). The proportion of good responders to anti-TNF treatment was substantially larger for people carrying two copies of this allele compared to carriers of no copies, both for etanercept (90.7% vs. 70.3%, p =
0.0027) and for all TNF blockers combined (88.1% vs. 70.7%, p = 0.0075). To a lesser degree, the same holds true for heterozygous carriers of the G risk allele compared to non-carriers (79.1% vs. 70.3%, p = 0.067 for etanercept and 80.1% vs. 70.7%, p = 0.034 for all TNF blockers). We also found an association of haplotype rs2230926 T–rs610604 G with good response to etanercept or all TNF blockers (Table 3).

Here we also report significant association between age at onset and treatment response that is independent of TNFAIP3 genotype; a later age at onset is associated with poorer response to treatment. The profile of genetic risk factors, especially the role of HLA-Cw6, differs between early and late onset psoriasis (Chen et al., 2011; Gudjonsson et al., 2002), which may influence the effectiveness of TNF blockers. The use of phenotypes as predictors of response to anti-TNF agents in RA showed that age of the patient was not associated with treatment response (Eder et al., 2010) and in AS age was negatively associated with response to treatment (Arends et al., 2011). Since we did not collect age at treatment data, a direct comparison was not possible. Other covariates analyzed did not show any association with treatment response.

We did not find any association of drug response and TNFAIP3 genotype in the Toronto replication sample. This outcome could be due to limited power. For the strongest effect observed in the Michigan sample (OR of 1.83 for etanercept response and G allele dosage at rs6010604), the Toronto sample has only 43% power to detect association at a nominal significance level of 0.05 and 29% power when significance is corrected for multiple testing. Because of the winner’s curse (Goring et al., 2001), the estimated effect size in the Michigan sample is likely biased upwards, and power for a more realistic OR of 1.50 (midway between 1.83 and the lower bound of its corrected 95% confidence interval), is only 22% and 13% at a nominal and corrected significance level of 0.05, respectively. To achieve 80% power to detect an OR of 1.83 at a nominal or corrected significance of 0.05, sample size for the Toronto cohort would need to be increased 2.5- or 3.2-fold; for an OR of 1.50 80% power requires 5.5- or 6.9-fold increases in sample size.

The lack of association in the Toronto sample could also be due to heterogeneity; i.e., response to anti-TNF drugs is genuinely more poorly associated with TNFAIP3 genotype in the Toronto population than in the Michigan population. The estimated I² coefficient of heterogeneity for association, of both etanercept response and combined TNF blocker response with r610604 genotype, is greater than zero (I² = 27.5% and 54.3%, respectively), leading to a reduced significance of the combined analysis for these two associations when using a random-effects model versus fixed-effects model (Table 2). However, neither of these I² coefficients is significant (95% confidence interval = 0.0–72.0% and 0.0–88.8%, respectively), nor is the related Cochran’s Q-test of heterogeneity (p = 0.24 and 0.14, respectively). It is important to note that these results are inconclusive, since the power of heterogeneity tests is known to be low when the number of strata is small.

Other potential limitations of this study could also be responsible for the different results for the two samples. We combined skin and joint responses that were measured individually in Toronto to approximate the undifferentiated self-evaluated response for the Michigan sample. Also, the response to medication in the Michigan cohort was assessed.
retrospectively, which may introduce recall error (though not recall bias since it is unlikely that recall accuracy is differentially affected by TNFAIP3 genotype). The similar proportion of good responders in the two samples to each of the anti-TNF therapies (Table 1) provides some confidence that the two response metrics, though measured in different ways, are reasonably similar. Nevertheless, the much higher proportion of PsA in the Toronto sample could contribute to the observed lack of association if the response of the joint symptoms of people with PsA to anti-TNF therapy is more poorly correlated with TNFAIP3 genotype than is response of the skin symptoms of people with PsA or purely cutaneous psoriasis (PsC). Table S1 compares skin-only and joint-only responses in the Toronto sample. Despite limited power, this analysis yields no indication that joint symptoms are more poorly associated with TNFAIP3 genotype than are skin symptoms—for rs610604, the OR of response of joint vs. skin phenotypes is 1.17 vs. 0.77 for etanercept and 1.08 vs. 0.65 for all TNF blockers combined. Unfortunately, a similar comparison in the Michigan sample is not possible because this information was not collected. In lieu of this, Table S2 compares response to TNF blockers in the Michigan sample for people with PsA versus those with PsC. The strength of association with SNP rs610604 is approximately equal for both phenotypes, being slightly lower in PsA (OR = 1.67 vs. 1.77 for all TNF blockers combined). Hence neither sample supports the notion that response of joint symptoms to anti-TNF therapy is less well associated with TNFAIP3 genotype than is response of skin symptoms, although the small sample sizes prevent any definitive conclusions.

In conclusion, we demonstrate in this study that the G allele of SNP rs610604 located in the TNFAIP3 gene and its haplotype with the T allele of rs2230926 are markers of good response to anti-TNF agents. The results of this study using a limited sample set calls for confirmation by additional studies with substantially larger number of samples, prospective patient and physician evaluated responses, and gene and protein expression levels measured before and after therapy. Such efforts would also provide phenotype data and material needed for a GWAS of anti-TNF agents. Further association studies on TNFAIP3 and other genetically associated loci could result in a panel of polymorphisms that can be routinely typed in a clinical setting to help the physician make an informed decision on administration of anti-TNF therapy.

MATERIALS AND METHODS

Subjects

The Michigan sample included 433 patients with psoriasis who received anti-TNF agents from 2005–2009 and completed a questionnaire concerning their response to the drug therapy. The Toronto sample consisted of 199 patients with psoriasis vulgaris who received anti-TNF therapy at Toronto Western Hospital, Center for Prognosis Studies in the Rheumatic Diseases. A physician (dermatologist and/or rheumatologist) diagnosed the presence of psoriasis and PsA in all patients. Only 3 of the 199 Toronto patients did not have cutaneous lesions.

All patients were of European Caucasian descent. Informed consent was obtained from all subjects under protocols adherent to the Declaration of Helsinki Principles, which were approved by the Institutional Review Boards of the participating institutions.
Phenotypes

For the Michigan sample, response to treatment with anti-TNF drugs (etanercept, adalimumab, and infliximab) was self-evaluated using a 0–5 visual analogue scale, 0 being not effective and 5 being very effective. Responses were dichotomized as good if they scored from 3 to 5 and poor if they scored 0 to 2. For those patients treated with more than one anti-TNF drug (17.7% of the sample received two and 5.5% all three drugs), a combined response to all TNF blockers was computed as the mean of the raw 0–5 response for all drugs used by the patient; the mean response was then dichotomized to responders and non-responders using a 2.5 threshold.

In Toronto, rheumatologists evaluated the response to treatment for the same three anti-TNF drugs. Only response to the first anti-TNF agent prescribed was considered. For skin symptoms, a 50% or greater reduction from the patient’s baseline PASI was considered a good response. For joint symptoms, a good response was defined as a 50% or better decrease in the actively inflamed (swollen and/or tender) joint count or 50% or better improvement in BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) at 6 months from the initiation of treatment. The skin and joint responses of the Toronto patients were combined into a single metric for most of the analyses—if either or both responses were good, the combined skin-joint response was considered to be good. Since all Toronto patients were evaluated for response to the first anti-TNF drug, the combined response for all TNF blockers was simply the combination of all single drug responses across the sample.

Demographic data including age, sex, race, and history of psoriasis and other autoimmune diseases in patients and family members were recorded for both samples. Body mass index, fingernail involvement, age at onset and duration of psoriasis and PsA were also recorded.

Genotyping

In Ann Arbor, genomic DNA was prepared from peripheral blood mononuclear cells or Epstein-Barr virus-immortalized lymphoblastoid cell lines, as previously described (Nair et al., 1995). In Toronto, DNA was extracted from peripheral blood using a modified salting out procedure (Gentra Puregene Blood Kit, Qiagen, Valencia, CA). The genotypes were determined by Taqman assay (Applied Biosystems, Foster City, CA).

Statistical analysis

The Michigan and Toronto samples were compared for a variety of epidemiological and phenotypic variables using two-sample tests for the equality of the means. For variables expressed as a percentage, Fisher’s exact test was used. All other variables except age were first transformed using the optimal Box-Cox power transform (power of 0.5 for age at PsV onset and duration of PsV, 0.7 for age at PsA onset, 0.2 for duration of PsA, and −0.1 for BMI) in order to achieve near-normality of the residuals of the appropriate regression model. Means for age, age at onset of PsV, and age at onset of PsA were then tested with the two-sample t-test for unequal variances. Means for duration of PsV, duration of PsA, and BMI were tested with a multiple regression model that included linear (duration PsA), linear and quadratic (BMI), or linear, quadratic, and cubic (duration PsV) terms for age and that used a Huber-White heteroscedasticity consistent covariance matrix.
Data were analyzed for linear trend of association between drug response and genotypes and haplotypes of two SNPs in \textit{TNFAIP3} using logistic regression, where drug response was coded as a binary variable (0 = poor, 1 = good), and the genotype predictor variable was expressed as the dosage of the SNP allele or two SNP haplotype. Association between drug response and several covariates (worst-ever TBSA, BMI, age at onset, and PsA) was also tested with logistic regression. The Box-Tidwell procedure was applied to association testing of TBSA, BMI, and age at onset to determine whether a power transformation was necessary to achieve a linear relationship between the logit of drug response and the covariate, which is an important assumption of logistic regression. In all cases, the deviation between a model using the optimal linearizing power transformation of the covariate and a model using the untransformed covariate was nonsignificant, so untransformed covariates were used in all tests. Age at onset, the only covariate to show significant association with drug response, was included in all tests assessing association between drug response and genotypes and haplotypes of the two SNPs in \textit{TNFAIP3}. Interaction between age at onset and SNP genotype was assessed with a likelihood ratio test applied to nested logistic regression models with and without a multiplicative interaction term. Conditional haplotype-based association testing was performed by comparing the odds of disease for pairs of haplotypes that have different alleles for the SNP under scrutiny but the same allele for the other SNP.

For individual cohorts, nominal significance of association was assessed using 100,000 random permutations of the drug response variable. A special set of 100,000 restricted permutations of drug response labels was generated to determine significance that was corrected for multiple testing of eight single marker tests (4 drug treatments × 2 SNPs), twelve individual haplotype tests (4 drugs × 3 haplotypes), or four omnibus haplotype tests (4 drugs × 1 omnibus test). Responses for all SNP–drug combinations were permuted as intact vectors to maintain all dependencies among responses, and response vectors were permuted randomly within strata formed by groups of patients receiving the same combination of drugs to ensure constant sample size among all permutations in the presence of missing genotype data. The best association p-value among all tests for each iteration of the permutation procedure was saved, the saved p-values were sorted into ascending order, and the corrected p-value determined as the fractional rank within this vector of the best observed p-value across all tests.

Meta-analysis was used to test for association across both cohorts combined. For single markers, standard fixed and random-effects models were used. Asymptotic p-values were reported for nominal significance, and the restricted permutations generated for the Michigan and Toronto samples were used to determine significance corrected for multiple testing. For haplotypes, fixed-effects meta-analysis of both cohorts was formulated as a logistic regression model with sample cohort as an additional covariate, and permutations were used to assess both nominal and corrected significance. Heterogeneity of ORs between the two samples was tested with Cochran’s Q statistic and the $I^2$ heterogeneity index. All association analyses were carried out with version 1.0.7 of PLINK (Purcell \textit{et al.}, 2007).

Power calculations were carried out with version 3.1.2 of G*Power (Faul \textit{et al.}, 2007) using an exact unconditional test of allelic association, which under Hardy-Weinberg equilibrium...
is essentially equivalent to the permutational version of the logistic regression test used in this study. Risk allele frequencies and the ratio of good to poor responders in the underlying population were estimated from the Toronto sample; the expected ORs for association were estimated from the Michigan sample. A type I error rate of 0.021 was used to compute power of association tests that are corrected for multiple testing, which is equivalent to a corrected significance of 0.05 when testing single SNPs in the Toronto sample.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by NIH grants R01 AR042742 and R01 AR050511, the Ann Arbor Veterans Affairs Hospital (JTE), the Dudley and Dawn Holmes Fund, the Babcock Memorial Trust, a Canadian Institutes of Health Research New Emerging Team (NET) Grant, the Krembil Foundation, and Arthritis Society of Canada National Research Initiative Grant. We thank Sarah Laponsa, Anna Pero and Lynda Hodges for their tireless efforts in recruiting patients and data entry.

Abbreviations

GWAS genome-wide association study
MAF minor allele frequency
NF-κB nuclear factor kappa light chain enhancer of activated B cells
SNP single nucleotide polymorphism
OR odds ratio
PsV psoriasis vulgaris
PsA psoriatic arthritis
PsC cutaneous psoriasis without arthritis
TNF tumor necrosis factor-α
TNFAIP3 tumor necrosis factor alpha induced protein 3

References

Arends S, Brouwer E, van der Veer E, et al. Baseline predictors of response and discontinuation of TNF-alpha blocking therapy in ankylosing spondylitis: a prospective longitudinal observational cohort study. Arthritis Res Ther. 2011; 13:R94. [PubMed: 21689401]
Chen H, Poon A, Yeung C, et al. A genetic risk score combining ten psoriasis risk loci improves disease prediction. PLoS One. 2011; 6:e19454. [PubMed: 21559375]
Cordoro KM, Feldman SR. TNF-alpha inhibitors in dermatology. Skin Therapy Lett. 2007; 12:4–6. [PubMed: 17940711]
Cormier C, Bosse Y, Mfuna L, et al. Polymorphisms in the tumour necrosis factor alpha-induced protein 3 (TNFAIP3) gene are associated with chronic rhinosinusitis. J Otolaryngol Head Neck Surg. 2009; 38:133–141. [PubMed: 19344623]
Eder L, Chandran V, Schentag CT, et al. Time and predictors of response to tumour necrosis factor-alpha blockers in psoriatic arthritis: an analysis of a longitudinal observational cohort. Rheumatology (Oxford). 2010; 49:1361–1366. [PubMed: 20385615]
Elder JT, Bruce AT, Gudjonsson JE, et al. Molecular dissection of psoriasis: integrating genetics and biology. J Invest Dermatol. 2010; 130:1213–1226. [PubMed: 19812592]

Ellinghaus E, Ellinghaus D, Stuart PE, et al. Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. Nat Genet. 2010; 42:991–995. [PubMed: 20953188]

Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007; 39:175–191. [PubMed: 17695343]

Fung EY, Smyth DJ, Howson JM, et al. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. Genes Immun. 2009; 10:188–191. [PubMed: 19110356]

G Gateva V, Sandling JK, Hom G, et al. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL.10 as risk loci for systemic lupus erythematosus. Nat Genet. 2009; 41:1228–1233. [PubMed: 19838195]

Goring HH, Terwilliger JD, Blangero J. Large upward bias in estimation of locus-specific effects from genomewide scans. Am J Hum Genet. 2001; 69:1357–69. [PubMed: 11593451]

Gottlieb AB, Matheson RT, Lowe N, et al. A randomized trial of etanercept as monotherapy for psoriasis. Arch Dermatol. 2003; 139:1627–1632. discussion 1632. [PubMed: 14676082]

Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet. 2008; 40:1059–1061. [PubMed: 19165918]

Gudjonsson JE, Karason A, Antonsdottir AA, et al. HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. J Invest Dermatol. 2002; 118:362–365. [PubMed: 11841557]

Han JW, Zheng HF, Cui Y, et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet. 2009; 41:1234–1237. [PubMed: 19838193]

Heyninck K, De Valck D, Vanden Berghe W, et al. The zinc finger protein A20 inhibits TNF-induced NF-kappaB-dependent gene expression by interfering with an RIP- or TRAF2-mediated transactivation signal and directly binds to a novel NF-kappaB-inhibiting protein ABIN. J Cell Biol. 1999; 145:1471–1482. [PubMed: 10385526]

Huffmeier U, Uebe S, EKici AB, et al. Common variants at TRAF3IP2 are associated with susceptibility to psoriatic arthritis and psoriasis. Nat Genet. 2009; 42:996–999. [PubMed: 20953186]

Koczan D, Drynda S, Hecker M, et al. Molecular discrimination of responders and nonresponders to anti-TNF alpha therapy in rheumatoid arthritis by etanercept. Arthritis Res Ther. 2008; 10:R50. [PubMed: 18454843]

Kooloos WM, de Jong DJ, Huizinga TW, et al. Potential role of pharmacogenetics in anti-TNF treatment of rheumatoid arthritis and Crohn’s disease. Drug Discov Today. 2007; 12:125–131. [PubMed: 17275732]

Leonardi CL, Powers JL, Matheson RT, et al. Etanercept as monotherapy in patients with psoriasis. N Engl J Med. 2003; 349:2014–2022. [PubMed: 14627786]

Li L, Soetandyo N, Wang Q, et al. The zinc finger protein A20 targets TRAF2 to the lysosomes for degradation. Biochim Biophys Acta. 2009; 1793:346–353. [PubMed: 18952128]

Liu C, Batiwalla F, Li W, et al. Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. Mol Med. 2008; 14:575–581. [PubMed: 18615156]

Liu YC, Penninger J, Karin M. Immunity by ubiquitylation: a reversible process of modification. Nat Rev Immunol. 2005; 5:941–952. [PubMed: 16322747]

Mauro C, Pacifico F, Lavorciga A, et al. ABIN-1 binds to NEMO/IKKgamma and cooperates with A20 in inhibiting NF-kappaB. J Biol Chem. 2006; 281:18482–18488. [PubMed: 16684768]

Maxwell JR, Potter C, Hyrich KL, et al. Association of the tumour necrosis factor-308 variant with differential response to anti-TNF agents in the treatment of rheumatoid arthritis. Hum Mol Genet. 2008; 17:3532–3538. [PubMed: 18713756]
Musone SL, Taylor KE, Lu TT, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. Nat Genet. 2008; 40:1062–1064. [PubMed: 19165919]

Nair RP, Duffin KC, Helms C, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. Nat Genet. 2009; 41:199–204. [PubMed: 19169254]

Nair RP, Guo SW, Jenisch S, et al. Scanning chromosome 17 for psoriasis susceptibility: lack of evidence for a distal 17q locus. Hum Hered. 1995; 45:219–230. [PubMed: 7558055]

O’Rielly DD, Roslin NM, Beyene J, et al. TNF-alpha-308 G/A polymorphism and responsiveness to TNF-alpha blockade therapy in moderate to severe rheumatoid arthritis: a systematic review and meta-analysis. Pharmacogenomics J. 2009; 9:161–167. [PubMed: 19365401]

Opipari AW Jr, Boguski MS, Dixit VM. The A20 cDNA induced by tumor necrosis factor alpha encodes a novel type of zinc finger protein. J Biol Chem. 1990; 265:14705–14708. [PubMed: 2118515]

Papp KA, Tyring S, Lahfa M, et al. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. Br J Dermatol. 2005; 152:1304–1312. [PubMed: 15948997]

Pearce DJ, Nelson AA, Fleischer AB, et al. The cost-effectiveness and cost of treatment failures associated with systemic psoriasis therapies. J Dermatolog Treat. 2006; 17:29–37. [PubMed: 16467021]

Pierik M, Rutegeerts P, Vlietinck R, et al. Pharmacogenetics in inflammatory bowel disease. World J Gastroenterol. 2006; 12:3657–3667. [PubMed: 16773681]

Plant D, Bowes J, Potter C, et al. Genome-wide association study of genetic predictors of anti-tumor necrosis factor treatment efficacy in rheumatoid arthritis identifies associations with polymorphisms at seven loci. Arthritis Rheum. 2011; 63:645–653. [PubMed: 21061259]

Plenge RM, Cotsapas C, Davies L, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet. 2007; 39:1477–1482. [PubMed: 17982456]

Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

Seitz M, Wirthmueller U, Moller B, et al. The -308 tumour necrosis factor-alpha gene polymorphism predicts therapeutic response to TNFalpha-blockers in rheumatoid arthritis and spondyloarthritis patients. Rheumatology (Oxford). 2007; 46:93–96. [PubMed: 16720636]

Sobell JM, Kalb RE, Weinberg JM. Management of moderate to severe plaque psoriasis (part I): clinical update on antitumor necrosis factor agents. J Drugs Dermatol. 2009; 8:147–154. [PubMed: 19213230]

Strange A, Capon F, Spencer CC, et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. Nat Genet. 2010; 42:985–990. [PubMed: 20953190]

Stuart PE, Nair RP, Ellinghaus E, et al. Genome-wide association analysis identifies three psoriasis susceptibility loci. Nat Genet. 2010; 42:1000–1004. [PubMed: 20953189]

Sun LD, Cheng H, Wang ZX, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. Nat Genet. 2010; 42:1005–1009. [PubMed: 20953187]

Tejasvi T, Stuart PE, Nair RP, et al. Initial pharmacogenomic assessment of TNF-alpha and TRAF1 polymorphisms in psoriasis: response to TNF blockers and other systemic therapies. J Invest Dermatol. 2008; 128:S74–S74.

Thomson W, Barton A, Ke X, et al. Rheumatoid arthritis association at 6q23. Nat Genet. 2007; 39:1431–1433. [PubMed: 17982455]

Trynka G, Zhermakova A, Romanos J, et al. Coeliac disease-associated risk variants in TNFAIP3 and REL implicate altered NF-kappaB signalling. Gut. 2009; 58:1078–1083. [PubMed: 19240061]

Vereecke L, Beyaert R, van Loo G. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. Trends Immunol. 2009; 30:383–391. [PubMed: 19643665]

Werner SL, Kearns JD, Zadorozhnaya V, et al. Encoding NF-kappaB temporal control in response to TNF: distinct roles for the negative regulators IkappaBalpha and A20. Genes Dev. 2008; 22:2093–2101. [PubMed: 18676814]
Wertz IE, O’Rourke KM, Zhou H, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature. 2004; 430:694–699. [PubMed: 15258597]
Zetoune FS, Murthy AR, Shao Z, et al. A20 inhibits NF-kappa B activation downstream of multiple Map3 kinases and interacts with the I kappa B signalosome. Cytokine. 2001; 15:282–298. [PubMed: 11594795]
Table 1

Phenotypic comparison of Michigan and Toronto samples

| Variable (units)                  | Michigan sample | Toronto sample | p-value<sup>5</sup> |
|----------------------------------|-----------------|----------------|---------------------|
| Age at study entry (yrs)         | 433 51.4 13.9   | 199 46.0 12.0  | 8.6 × 10<sup>−7</sup> |
| Age at PsV onset (yrs)           | 433 26.9 15.5   | 196 26.2 13.7  | 0.75                |
| Duration of psoriasis (yrs)      | 433 24.4 15.6   | 196 19.7 12.4  | 0.055               |
| Age at PsA onset (yrs)           | 214 37.6 13.6   | 185 33.6 12.6  | 0.0028              |
| Duration of PsA (yrs)            | 214 14.8 12.3   | 185 12.3 9.3   | 0.61                |
| BMI (kg/m<sup>2</sup>)<sup>1</sup> | 431 30.5 7.2    | 192 29.1 5.9   | 0.10                |
| Males (%)                        | 433 43.2 2.4    | 199 38.7 3.5   | 0.30                |
| PsA (%)                          | 376 58.3 2.6    | 199 93.0 1.8   | 2.9 × 10<sup>−20</sup> |
| Nail involvement (%)             | 417 79.4 2.0    | 199 84.4 2.6   | 0.15                |
| IBD (%)                          | 405 3.2 0.9     | 199 2.0 1.0    | 0.60                |
| Autoimmune disease (%)           | 402 5.7 1.2     | 197 4.6 1.5    | 0.70                |
| Family history PsV (%)           | 419 60.6 2.4    | 198 42.9 3.5   | 4.5 × 10<sup>−3</sup> |
| Family history IBD (%)           | 415 11.8 1.6    | 194 5.7 1.7    | 0.019               |
| rs610604 G allele frequency (%)   | 432 37.5 1.6    | 188 38.6 2.5   | 0.75                |
| rs2230926 T allele frequency (%) | 430 97.1 0.6    | 188 96.8 0.9   | 0.86                |
| Good etanercept response (%)     | 380 77.4 2.1    | 139 83.5 3.2   | 0.15                |
| Good adalimumab response (%)     | 89 77.5 4.4     | 31 74.2 7.0    | 0.81                |
| Good infliximab response (%)     | 89 80.9 4.2     | 29 82.8 4.2    | 1.00                |
| Good TNF blocker response (%)    | 433 77.6 2.0    | 199 81.9 2.7   | 0.25                |

<sup>1</sup> Body mass index
<sup>2</sup> Irritable bowel disease
<sup>3</sup> Number of non-missing observations
<sup>4</sup> Standard deviation
<sup>5</sup> Nominal p-value for the test of the equality of the variable means for the Michigan and Toronto samples.

Tests for duration of PsV, duration of PsA, and BMI were adjusted for the effects of age. See Materials and Methods for details of testing procedure.
### Table 2

Association of genotypes for two *TNFAIP3* SNPs with a combined skin-joint response to TNF blockers for psoriasis patients in the Michigan, Toronto, and combined samples. Age at onset of disease is included as a covariate in the association model.

| SNP | Drug     | Genotype | Michigan sample | Toronto Sample | Combined analysis (Fixed/random effects) |
|-----|----------|----------|-----------------|----------------|------------------------------------------|
|     |          |          | Good response$^1$ | Poor response$^1$ | OR$^2$ | P$^3$ | Good response$^1$ | Poor response$^1$ | OR$^2$ | P$^3$ | OR$^2$ | P$^3$ |
|     |          |          |                 |                 |       |       |                 |                 |       |       |       |       |
| rs2230926 | etanercept | TT | 275 (.945) | 79 (.919) | 0.19 (0.74) | 101 (.927) | 19 (.950) | 1.53/1.53 | 0.30/0.30 | (0.92/0.89) |
|         |          | TG | 16 (.055) | 6 (.070) | 1.77 | 8 (.073) | 1 (.050) | 0.63 | 0.67 (0.99) |
|         |          | GG | 0 (.000) | 1 (.011) |  | 0 (.000) | 0 (.000) |  |
|         |          | TG | 54 (.915) | 28 (.933) |  | 20 (.952) | 5 (.833) | 1.12/1.12 | 0.89/0.89 | (1.00/1.00) |
|         |          | GG | 0 (.000) | 0 (.000) |  | 0 (.000) | 0 (.000) |  |
| rs610604 | infliximab | TT | 69 (.958) | 15 (.882) |  | 23 (.958) | 5 (.100) |  |  |  |
|         |          | TG | 3 (.042) | 2 (.118) | 3.00 | 1 (.042) | 0 (.000) | 0.00 | 1.00 (1.00) | 3.00/3.00 | 0.26/0.26 | (0.89/0.86) |
|         |          | GG | 0 (.000) | 0 (.000) |  | 0 (.000) | 0 (.000) |  |
|         |          | TT | 315 (.956) | 91 (.944) |  | 144 (.935) | 29 (.935) |  |  |  |
|         |          | TG | 18 (.044) | 5 (.055) | 1.40 | 10 (.065) | 2 (.065) | 0.90 | 0.89 (1.00) | 1.26/1.26 | 0.55/0.55 | (0.99/0.99) |
|         |          | GG | 0 (.020) | 1 (.019) |  | 0 (.000) | 0 (.000) |  |
|         | etanercept | GG | 49 (.167) | 5 (.058) |  | 14 (.128) | 2 (.100) |  |  |  |
|         |          | GT | 140 (.478) | 37 (.430) | 1.83 | 55 (.505) | 10 (.500) | 1.10 | 0.79 (1.00) | 1.64/1.57 | 0.0043/0.051 | (0.0160/0.31) |
|         |          | TT | 104 (.355) | 44 (.512) |  | 40 (.367) | 8 (.400) |  |  |  |
|         |          | GG | 3 (.052) | 1 (.033) |  | 4 (.190) | 0 (.000) |  |  |  |
|         | adalimumab | GT | 30 (.517) | 12 (.400) | 1.67 | 8 (.381) | 3 (.300) | 1.42 | 0.65 (0.99) | 1.61/1.61 | 0.19/0.19 | (0.75/0.70) |
|         |          | TT | 25 (.431) | 17 (.567) |  | 9 (.429) | 3 (.500) |  |  |  |
|         |          | GG | 11 (.153) | 2 (.118) |  | 3 (.125) | 2 (.400) |  |  |  |
|         | infliximab | GT | 35 (.486) | 7 (.412) | 1.34 | 14 (.583) | 2 (.400) | 0.40 | 0.26 (0.72) | 1.05/0.90 | 0.89/0.85 | (1.00/1.00) |
|         |          | TT | 26 (.361) | 8 (.471) |  | 7 (.292) | 1 (.200) |  |  |  |
|         |          | GG | 52 (.156) | 7 (.033) | 1.74 | 21 (.136) | 4 (.129) | 1.01 | 0.98 (1.00) | 1.50/1.39 | 0.01/0.22 | (0.50/0.76) |
| SNP | Drug | Genotype | Good response\(^1\) | Poor response\(^1\) | OR\(^2\) | P\(^3\) | Good response\(^1\) | Poor response\(^1\) | OR\(^2\) | P\(^3\) |
|-----|------|----------|----------------------|---------------------|--------|--------|----------------------|---------------------|--------|--------|
| GT  |      |          | 165 (.493)           | 41 (.483)           |        |        | 77 (.500)           | 15 (.484)           |        |        |
| TT  |      |          | 118 (.351)           | 49 (.483)           |        |        | 56 (.364)           | 12 (.387)           |        |        |

\(^1\) Frequency and proportion of genotype among good and poor responders to drug therapy

\(^2\) Odds ratio for association of drug response and TNFAIP3 SNP in a logistic regression model using SNP allele dosages and age at onset

\(^3\) Nominal and corrected p-values (latter in parentheses) for association of drug response and TNFAIP3 genotype, with age at onset covariate
Association of *TNFAIP3* haplotypes and a combined skin-joint response to TNF blockers for psoriasis patients in the Michigan, Toronto, and combined samples. Age at onset of disease is included as a covariate in the association model.

| Drug     | Haplotype | Michigan Sample freq | Michigan Sample OR | Michigan Sample P nom | Michigan Sample P corr | Toronto Sample freq | Toronto Sample OR | Toronto Sample P nom | Toronto Sample P corr | Combined analysis freq | Combined analysis OR | Combined analysis P nom | Combined analysis P corr |
|----------|-----------|----------------------|--------------------|----------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|------------------------|----------------------|--------------------------|--------------------------|
| etanercept | TG        | .409/.269            | 1.92               | 0.00077              | 0.0038                | .381/.350            | 1.10               | 0.83                 | 1.00                 | 1.71                   | 0.0017               | 0.0077                   | 0.011                    |
|          | GT        | .025/.040            | 0.55               | 0.18                 | 0.82                  | .037/.025            | 1.60               | 0.44                 | 1.00                 | 0.67                   | 0.36                 | 0.96                     |
|          | TT        | .566/.691            | 0.58               | 0.0039               | 0.023                 | .583/.625            | 0.85               | 0.71                 | 1.00                 | 0.63                   | 0.0063               | 0.039                    |
| omnibus  | —         | —                    | 0.0030             | 0.0067               | —                     | —                    | 0.81               | 0.98                 | —                    | —                      | 0.0077               | 0.023                    |
| adalimumab | TG        | .303/.232            | 1.63               | 0.26                 | 0.87                  | .381/.250            | 1.42               | 0.67                 | 1.00                 | 1.57                   | 0.23                 | 0.83                     |
|          | GT        | .032/.032            | 1.21               | 0.60                 | 1.00                  | .024/.083            | 0.42               | 0.29                 | 0.99                 | 0.87                   | 0.92                 | 1.00                     |
|          | TT        | .665/.736            | 0.63               | 0.24                 | 0.84                  | .595/.667            | 0.86               | 0.85                 | 1.00                 | 0.67                   | 0.25                 | 0.86                     |
| omnibus  | —         | —                    | 0.36               | 0.86                 | —                     | —                    | 0.68               | 0.97                 | —                    | —                      | 0.38                 | 0.82                     |
| infliximab | TG        | .391/.322            | 1.32               | 0.53                 | 0.99                  | .417/.600            | 0.40               | 0.24                 | 0.79                 | 1.02                   | 0.96                 | 1.00                     |
|          | GT        | .013/.056            | 0.21               | 0.14                 | 0.76                  | .021/.000            | ∞                  | 0.73                 | 1.00                 | 0.29                   | 0.17                 | 0.87                     |
|          | TT        | .595/.622            | 0.90               | 0.81                 | 1.00                  | .563/.400            | 2.10               | 0.40                 | 0.88                 | 1.08                   | 0.81                 | 1.00                     |
| omnibus  | —         | —                    | 0.25               | 0.74                 | —                     | —                    | 0.27               | 0.79                 | —                    | —                      | 0.41                 | 0.91                     |
| combined | TG        | .404/.279            | 1.82               | 0.0012               | 0.0060                | .386/.371            | 1.01               | 0.98                 | 1.00                 | 1.55                   | 0.0051               | 0.031                    |
|          | GT        | .025/.031            | 0.73               | 0.49                 | 0.99                  | .032/.032            | 1.11               | 0.61                 | 1.00                 | 0.81                   | 0.63                 | 1.00                     |
|          | TT        | .571/.690            | 0.59               | 0.0034               | 0.016                 | .581/.597            | 0.98               | 0.93                 | 1.00                 | 0.67                   | 0.0095               | 0.066                    |
| omnibus  | —         | —                    | 0.0055             | 0.014                | —                     | —                    | 0.96               | 1.00                 | —                    | —                      | 0.021                | 0.066                    |

1. Haplotype for SNPs rs2230926 and rs610604; omnibus refers to all haplotypes combined
2. Frequency of haplotypes in good responders and poor responders
3. Odds ratio for association of drug response and *TNFAIP3* haplotype in a logistic regression model using haplotype dosages
4. Nominal p-value for association of drug response and *TNFAIP3* haplotype
5. Corrected p-value for association of drug response and *TNFAIP3* haplotype
6. Fixed effects meta-analysis