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ORIGINAL INVESTIGATION

Linkage and association analysis of candidate genes for TB and TNFα cytokine expression: evidence for association with IFNGR1, IL-10, and TNF receptor 1 genes

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Abstract Tuberculosis (TB) is a growing public health threat globally and several studies suggest a role of host genetic susceptibility in increased TB risk. As part of a household contact study in Kampala, Uganda, we have taken a unique approach to the study of genetic susceptibility to TB by developing an intermediate phenotype model for TB susceptibility, analyzing levels of tumor necrosis factor-α (TNFα) in response to culture filtrate as the phenotype. In the present study, we analyzed candidate genes related to TNFα regulation and found that interleukin *(IL)-10*, interferon-gamma receptor 1 (*IFNGR1*), and TNFα receptor 1 (*TNFR1*) genes were linked and associated to both TB and TNFα. We also show that these associations are with progression to active disease and not susceptibility to latent infection. This is the first report of an association between TB and *TNFR1* in a human population and our findings for *IL-10* and *IFNGR1* replicate previous findings. By observing pleiotropic effects on both phenotypes, we show construct validity of our intermediate phenotype model, which enables the characterization of the role of these genetic polymorphisms on TB pathogenesis. This study further illustrates the utility of such a model for disentangling complex traits.

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Introduction

Tuberculosis (TB) is a growing public health problem globally; approximately one-third of the world's population is infected with the causal bacterium, *Mycobacterium tuberculosis* (Mtb), and the incidence of TB disease is increasing in the face of the HIV pandemic (Raviglione et al. 1995). TB is a complex trait for several reasons. Phenotype definition is not trivial because TB disease may be expressed with varying severity in a number of organ systems after a long and variable latency period. Both host and environmental factors affect the risk of infection by Mtb following exposure and progression to TB disease, and few studies have examined whether genetic susceptibility to these two stages in disease progression differ (Flores-Villanueva et al. 2005). Previous genetic studies

have also differed dramatically in TB diagnostic criteria and characterization of controls. Very few of these studies have been able to establish a link between candidate genes and TB pathogenesis. Furthermore, few studies have examined the linkage disequilibrium (LD) structure within these genes, and thus are unable to account for untyped polymorphisms that may influence TB risk.

To address the complexities of TB, we have examined expression of tumor necrosis factor-α protein (TNFα) in response to mycobacterial antigens as an intermediate phenotype for TB disease. We chose TNFα as an intermediate phenotype because it is a central cytokine in TB pathogenesis that is involved in granuloma formation, induces symptoms including fever and weight loss (Barnes et al. 1990; Roach et al. 2002), and is important in the containment of latent Mtb infection (Keane et al. 2001). In our preliminary work, we have found that TNFα expression following antigen stimulation has a high heritability that is partially attributable to a complicated major gene effect and potentially influenced by gene-environment interaction (Stein et al. 2003, 2005).

Based on our intermediate phenotype model, we identified 12 candidate genes that may have roles in TNFα regulation and expression or have been associated with TB susceptibility in either human studies or mouse models (Table 1). The TNFα gene (*TNFA*) resides in the MHC class III region on chromosome 6 and is in high LD with MHC Class I and Class II genes, which code for the various HLA subtypes. *TNFA* knockout mice die from infection by Mtb (Flynn et al. 1995). *TNFA* promoter polymorphisms have been shown to be associated with TNFα levels (Pociot et al. 1993; Wilson et al. 1997). A number of HLA polymorphisms have been associated with TB as well (Bellamy 2003). There are two receptors for TNFα, coded by *TNFR1* (also referred to as *TNFRSF1A*) and *TNFR2*, and efficient binding of TNFα depends on the self-assembly of these two receptors. Both receptors have been shown to influence TNFα levels (Peschon et al. 1998), and *TNFR1* and *TNFR2* knockout mice succumb to Mtb infection and Bacille Calmette-Guérin (BCG) infection (Peschon et al. 1998; Piguet et al. 2002). Toll-like receptors (TLRs) also play a role in TB. Mtb bacilli contain distinct ligands that activate cells via *TLR2* and *TLR4* (Means et al. 1999), which results in TNFα production by macrophages (Underhill et al. 1999). *TLR2* deficient mice produce less TNFα and lack functional granulomas (Bochud et al. 2003; Drennan et al. 2004). It has been shown that Mtb activates cells via TLR2 and TLR4 (Means et al. 1999) and that Mtb-induced TNFα production is differentially affected by a TLR4-specific antagonist (Means et al. 2001). The first TB susceptibility locus mapped in mice was *Nramp1*, which has since been renamed *Slc11a1* (Blackwell et al. 2004; Skamene et al. 1998); the human ortholog of this gene (originally named *NRAMP1*, now named *SLC11A1*) has been studied in association with TB in humans in a number of populations (Bellamy 2003; Li et al. 2006). In mice, *Slc11a1* has pleiotropic effects, including an influence on TNFα release by macrophages (Blackwell et al. 2004; Formica et al. 1994). Interleukin *(IL)-10* is an inhibitor of Th-1 cell and macrophage functions, and drug and molecular studies suggest that IL-10 activity leads to decreased TNFα production (Balcewicz-Sablinska et al. 1999; Goldman et al.

Table 1 Microsatellite markers analyzed by candidate gene

Candidate gene	Gene (Mb location from pter)	Markers	Mb location (Mb)
Tumor necrosis factor-α and human leukocyte antigen	TNFA/HLA (31.6)	D6S306	28.0
		D6S265	30.1
		D6S273	31.8
		D6S439	35.2
		TNFB	44.2
		D6S1701	47.7
Tumor necrosis factor-α receptor type-1	TNFR1 (6.3)	D12S221	4.9
		D12S825	5.9
		D12S1625	7.0
Tumor necrosis factor-α receptor type-2	TNFR2 (11.9)	D1S244	10.3
		D1S489	11.7
		D1S228	13.3
Interleukin-10	IL-10 (204.0)	D1S256	202.0
		D1S177	204.0
		D1S2692	205.1
Natural resistance-associated macrophage protein 1	SLC11A1 (219.4)	D2S301	218.1
		D2S2179	219.0
		D2S2359	221.1
Interleukin-12 sub-unit A	IL12A (161.0)	D3S1553	160.6
		D3S3580	160.9
		D3S3708	163.4
		D3S1607	158.3
Interleukin-12 sub-unit B	IL12B (158.7)	D5S487	155.6
		D5S1971	158.5
		D5S2047	160.5
Interferon-γ receptor 1	IFNGR1 (137.5)	D6S270	134.6
		D6S1587	138.4
		D6S1675	140.0
Interferon-γ receptor 2	IFNGR2 (33.7)	D21S223	32.2
		D21S2039	33.6
		D21S65	35.0
Toll-Like receptor 2	TLR2 (155.2)	D4S233	154.6
		D4S3049	155.3
		D4S2976	156.4
Toll-like receptor 4	TLR4 (115.8)	D9S154	114.7
		D9S1864	115.8
		D9S275	116.9

1996). IL-12, which is composed of two subunits coded for by *IL12A* and *IL12B*, prompts the release of TNFα by T cells (van Crevel et al. 2002). Finally, macrophages may have decreased function due to deficient receptors. Interferon-γ (IFNγ), a key cytokine in the response to Mtb, is released by T-cells; its receptors, *IFNGR1* and *IFNGR2*, are on macrophages. In this way, IFNγ may influence TNFα production (Knight and Kwiatkowski 1999). *IL-10, IL-12B*, and *IFNGR1* have been studied in human populations, but with mixed results (Bellamy 2003).

In this analysis, we took a two-stage approach to the analysis of these candidate genes. First, we conducted a positional candidate linkage analysis in each of these candidate genes. After detecting nominally significant linkage, we followed-up those genes of interest by conducting a family-based association analysis. In the association stage, we analyzed single nucleotide polymorphisms (SNPs) that characterized the LD structure of the genes of interest. This study provides additional validity to our intermediate phenotype model by demonstrating pleiotropic effects of TB susceptibility genes. Additionally, this analysis illustrates the utility of the intermediate phenotype approach to demonstrate construct validity and elucidate the impact of risk alleles on the pathogenesis of a complex disease.

Materials and methods

Subject recruitment and assessment

The families presented in this analysis are part of an ongoing household contact study (Guwattude et al. 2003) in Kawempe Division of Kampala, Uganda. Index cases and household contacts were enrolled between April 2002 and July 2003 [Phase II of the study (Stein et al. 2005)]. Index case patients were diagnosed with TB disease if their sputum contained Mtb on Ziehl–Nielsen stain or mycobacterial culture. These index cases were referred to the study through the Uganda National Tuberculosis and Leprosy Programme, public and private clinics in Kawempe Division, or by self-referral after community sensitization efforts or word of mouth from friends currently enrolled in the study. To be included in the study, both the index case and household members provided informed consent. The institutional review boards at University Hospitals of Cleveland and the Uganda Council for Science and Technology approved the study.

Upon enrollment, the index case and all household members underwent a clinical examination, including a health and symptom survey, tuberculin skin test using purified protein derivative and the Mantoux method (PPD, 5 TU, Tubersol; Connaught Laboratories, Limited, Toronto, Canada), and HIV testing using traditional ELISA methods (Cambridge BioScience). Individuals who had suspected TB also had chest radiography, sputum microscopy and cultures performed. Patients with active TB disease were given the recommended course of therapy (Blumberg et al. 2003). In addition, household members who did not have active disease but demonstrated latent Mtb infection by the tuberculin skin test were given isoniazid preventive therapy. In this paper, ''TB'' refers to active pulmonary disease, and ''Mtb infection'' refers to latent infection that has been contained and has not progressed to active disease. TNFα protein production in response to Mtb culture filtrate, the intermediate phenotype, was measured using the whole blood cytokine assay. Blood cells were stimulated with Mtb culture filtrate obtained from Colorado State University. After 18 h of incubation, TNFα concentrations in the supernatants were measured using ELISA (Endogen) (Stein et al. 2005).

Molecular methods

DNA was extracted from buffy coats using a salting procedure (Miller et al. 1988) and consequently quantified using PicoGreen (Invitrogen) and standardized to 10 ng/μL. Quality of DNA was validated using the amelogenin marker. DNA was arrayed in a 96-well format, and PCR was performed in a MJ Tetrad thermocycler.

Three microsatellite markers were typed per candidate gene region, with the exception of the *HLA/TNFA* region, which was covered with 6 microsatellite markers (Table 1) that were chosen to have high heterozygosity (>70%) and mapped within 2 Mb of the gene of interest. Genetic map locations of these markers were based on the most recent map (Kong et al. 2004); when markers were not included on this map, their locations were obtained by linear interpolation on physical map locations on the UCSC Genome Browser (http://www.genome.ucsc.edu/). The markers were multiplexed and run on an ABI 3700 capillary machine (Applied Biosystems). Internal controls were included on each gel, consisting of two CEPH controls and four replicate samples. The ABI ROX 500 standard (present in every lane) was used to estimate size of alleles. Familial relationships were verified or reclassified using all microsatellite data based on likelihood methods implemented in RELPAIR (Epstein et al. 2000); a total of 20 relationships were reclassified. Inconsistencies in the segregation of the genotypes within families were examined using MARKERINFO (S.A.G.E. 2006). Individuals demonstrating Mendelian inconsistencies at multiple markers that could not be resolved by retyping or reclassifying familial relationship were treated as missing for the purpose of this analysis. In total, 5% of the microsatellite data were treated as missing, resulting in a loss of 6% of the sibpairs. Marker allele frequencies conformed to Hardy–

Weinberg expectations. We estimated the allele frequencies for each genetic marker by simple gene counting (disregarding relationships), using FREQ (S.A.G.E. 2006).

To follow-up candidate genes that demonstrated nominal evidence for linkage to both traits (at the $\alpha = 0.05$ level), we genotyped SNPs within these genes (Table 2). Freely-available databases were used to select SNPs, including HapMap Phases I and II with Haploview software (Altshuler et al. 2005; Barrett et al. 2005), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/index.html), and SNPBrowser by Applied Biosystems. SNP assays were available as Assays-on-Demand or Assays-by-Design (Applied Biosystems). Haplotype-tagging SNPs were selected using an r^2 threshold of 0.90, restricted to those with a minor allele frequency (MAF) of at least 10% in African or African–American populations and that have been validated by at least two sources. If a haplotype-tagging SNP did not meet the MAF criterion, a nearby SNP within the same LD bin (within 5 kb) was identified to replace it. SNPs were genotyped on the TaqMan platform, and data cleaning was conducted as described above, resulting in 2.95% of genotypes being deleted due to Mendelian inconsistencies or low quality scores. SNP genotypes were tested for Hardy–Weinberg proportions using PEDSTATS (Wigginton and Abecasis 2005) in a random sample of unrelated individuals, separately in individuals affected and unaffected for TB.

Statistical analysis

Because of the multifactorial nature of TB and TNFα, we used two complementary model-free linkage analysis strategies to analyze the microsatellite data. Both single-point and multipoint analyses were conducted; for brevity, only multipoint analysis results are shown. For each relative pair, the proportion of alleles shared identical by descent (IBD) was estimated using the GENIBD program (S.A.G.E. 2006). First, both the quantitative trait TNFα and the binary trait TB (presence of active disease), were analyzed using Haseman–Elston regression (Haseman and Elston 1972), in which a measure of sibling trait similarity is regressed on the proportion of alleles shared IBD by that sibpair. There is a variety of ways to parameterize the phenotypic similarity between sibs to account for the non-independence of siblings within a sibship; we used the W4 option in SIBPAL (S.A.G.E. 2006), as it is asymptotically the most powerful (Shete et al. 2003). Recently, the Haseman–Elston method has been extended to include both full-sibs as well as half-sib pairs (S.A.G.E. 2006). Secondly, the conditional logistic model (Goddard et al. 2001; Olson 1999) was used to further analyze the binary trait TB, as is implemented in LODPAL (S.A.G.E. 2006). This affected sibpair (ASP) analysis model compares the observed IBD sharing to Mendelian expectation under the null hypothesis of no linkage. Unaffected individuals can be incorporated in the analysis by including an indicator variable covariate for discordant pair status; when the model uses this covariate, the LOD score is asymptotically distributed as $\frac{1}{2}\chi_1^2 + \frac{1}{2}\chi_2^2$ (Goddard et al. 2001). To adjust for the possible confounding effects, HIV serostatus was used as a covariate in the Haseman–Elston models, and conditional logistic regression models were run on the subset of HIV concordantly seronegative relative pairs. Since this was a focused candidate gene linkage study, we did not apply genome-wide significance thresholds (Lander and Kruglyak 1995; Witte et al. 1996), but instead followed-up loci significant at $\alpha = 0.05$ with SNP association analysis.

After finding nominal evidence for linkage to *IL-10*, *TNFR1*, and *IFNGR1*, association analysis of SNPs within these genes was conducted. Again, both TB and TNFα were examined as phenotypes of interest. In these data, the traditional transmission-disequilibrium test (TDT) is not appropriate because most of our affected individuals are parents, not offspring. Instead, we used the family-based test of association between a marker and continuous phenotype developed by George and Elston (1987), which allows for familial correlations by simultaneously estimating residual and multifactorial (polygenic, familial, and marital) variance components (Elston et al. 1992). This model has since been extended to allow for binary traits by incorporating a logit link function (Gray-McGuire 2004). This regression framework has the flexibility to allow families of any size or structure, and is implemented in ASSOC (S.A.G.E. 2006), which tests the significance of covariate coefficients using both the likelihood ratio test and the Wald test. Within this regression model, alleles are coded according to three to three possible modes of inheritance (recessive, additive, and dominant). Incorpo-

Table 2 Single nucleotide polymorphisms (SNPs) analyzed by candidate gene

Candidate gene	SNP name	Minor allele	Minor allele frequency (MAF)
IFNGR1	rs4896243	C	0.245
	rs1327474	A	0.018
	rs2234711	C	0.405
IL-10	rs1518111	A	0.406
	rs1554286	T	0.415
	rs1800872	A	0.418
TNFR1	rs4149623	T	0.392
	rs4149639	G	0.208
	rs4149622	A	0.398
	rs4149578	A	0.282

ration of covariates other than locus-specific effects was done in a stepwise fashion; since the effect sizes for the SNPs were consistent across models, only the models with both a marker and significant covariates are presented. Covariates included HIV status and a composite environmental variable that was derived to depict components of shared environment and modeled nutritional status and shared environment—frequency of contact with the index TB case, clinical characteristics of the index case, poor ventilation within the home and poverty (Stein et al. 2005). Because of the possible confounding effects of HIV infection on the association between these phenotypes and loci, we also evaluated the effects of interaction between a locus and HIV within the association models.

To examine whether the associations between SNPs and TB disease actually reflected susceptibility to latent infection, we constructed a variable to contrast these two stages of disease. For this analysis, we considered latent Mtb infection to be evidenced by a TST reading of 10 mm or greater but no clinical or microbiological evidence of TB disease (CDC 2003). The variable was coded as 1 = active TB disease and 0 = latent Mtb infection; individuals without TB disease or latent Mtb infection were treated as missing.

Haplotypes of SNPs within genes were estimated using the expectation-maximization algorithm as implemented in DECIPHER (S.A.G.E. 2006). When phase could not be resolved with 100% certainty, the phased genotypes with the highest posterior probability was selected. The most likely haplotypes were coded as indicator variables in the data; only haplotypes with greater than 10% sample frequency were considered. Haplotypes were individually included as covariates within the ASSOC models to ascertain the locus-specific effects.

Finally, to evaluate whether a SNP accounted for the observed linkage effects, we included the SNPs as covariates within the Haseman–Elston regression, coding the genotypes according to the most significant genetic model.

Results

This analysis included 398 individuals, comprising 66 pedigrees, 232 full sibling pairs, and 157 half sibling pairs. Of these individuals, 15.0% had active TB disease, 16.4% were HIV seropositive, and 74.3% had latent Mtb infection based on the tuberculin skin test. After elimination of individuals with Mendelian inconsistencies, there were 213 full sibling pairs and 120 half sibling pairs available for linkage analysis; all of these individuals had complete data for TB and TNFα.

We analyzed linkage between our two phenotypes, TNFα and TB, and microsatellite markers that map to our candidate genes of interest (Table 1). Using the Haseman–Elston regression model and adjusting for HIV status, we found that *TNFR1* and *IFNGR1* were linked to both TNFα and TB (Table 3). The most significant marker for *TNFR1* was D12S1625 for both TNFα ($p = 0.0184$) and TB ($p = 9 \times 10^{-7}$), and the most significant marker for *IFNGR1* was D6S270 for both traits (TNFα $p = 0.006$ and

Table 3 Summary of significant linkage results

Gene	Marker	Haseman–Elston *p*-values		Conditional logistic *p*-values, TB	
		TNFα	TB	All sibs	HIV negative only
TNFR1	D12S221	0.0202	2.30×10^{-6}	NS	NS
	D12S374	0.0401	2.06×10^{-5}	NS	NS
	D12S1625	0.0184	9.00×10^{-7}	NS	NS
IL-10	D1S236	0.0321	NS	0.029	NS
	D1S177	0.0788	NS	NS	NS
	D1S2692	0.0023	NS	0.021	NS
IFNGR1	D6S270	0.006	2.82×10^{-5}	0.004	0.009
	D6S1587	0.061	2.15×10^{-4}	0.003	0.002
	D6S1675	0.049	2.84×10^{-4}	0.012	0.045
TNFR2	D1S244	NS	0.0053	NS	NS
	D1S489	NS	0.0034	0.015	NS
	D1S228	NS	5.80×10^{-4}	0.008	NS
TLR4	D9S154	NS	1.60×10^{-4}	0.012	NS
	D9S1864	NS	3.70×10^{-6}	0.030	NS
	D9S1675	NS	0.0023	NS	NS
TLR2	D4S233	NS	NS	0.016	NS
	D4S3049	NS	NS	0.014	NS
	D4S2976	NS	NS	0.017	NS

NS not significant at $\alpha = 0.05$

TB $p = 2.82 \times 10^{-5}$). In addition, the *TNFR2* and *TLR4* genes also demonstrated significant linkage to TB only, and *IL-10* demonstrated significant linkage to TNFα.

The conditional logistic analysis results confirmed many of the Haseman–Elston analysis results. When including all relative pairs, significant linkage between TB disease and microsatellite markers at the $\alpha = 0.05$ threshold was attained for *IL-10, IFNGR1, TNFR2, TLR4*, and *TLR2* (Table 3). When the analysis of the TB phenotype was repeated for HIV negative pairs only, the statistical significance diminished for all genes except *IFNGR1* which remained significant. The decline in significance may be attributed to a substantial drop in sample size (entire sample included 71 relative pairs, compared to 47 concordantly HIV negative pairs, for this analysis).

Because *IFNGR1, IL-10*, and *TNFR1* were linked to both TNFα and TB status, we genotyped tagSNPs in these genes (Table 2) and analyzed them using family-based association analysis. All of these SNPs were in Hardy–Weinberg equilibrium in cases and controls ($p > 0.10$, data not shown). Using the model that allowed for multifactorial correlations, we evaluated association between both traits and all the SNPs, where genotype coding schemes were used for recessive, dominant, and additive models; the referent allele for each gene is noted (Tables 4, 5). In addition, we included as covariates HIV status and an environmental variable that we developed in a path analysis (Stein et al. 2005). In our analysis of TNFα as the phenotype, we found association with SNPs in all three genes (Table 4), with several highly significant *p*-values, especially in the *IL-10* and *TNFR1* genes. These results were significant even after applying a conservative Bonferroni correction ($\alpha^* = 0.05/10 = 0.005$). When analyzing TB as the trait, we found association with *TNFR1* and *IFNGR1*, but not *IL-10* (Table 5). Though less statistically significant than the results for TNFα, we still observed multiple SNPs within both genes demonstrating significance. All but one of these SNPs were significant using the

Table 4 Results of association analysis of TNFα as the phenotype

Gene	Marker	Referent allele	Best fitting model	β coef	Genotype *p*-value*	HIV × locus interaction[§]
IFNGR1	rs4896243	C	Dominant	–0.700	2.89×10^{-11}	0.004
	rs1327474	G	Dominant	0.044	0.814	NS
	rs2234711	T	Additive	–0.307	8.978×10^{-7}	NS
IL-10	rs1518111	A	Dominant	–0.774	1.54×10^{-13}	NS
	rs1554286	C	Recessive	0.779	6.31×10^{-14}	NS
	rs1800872	A	Dominant	–0.770	1.50×10^{-13}	NS
TNFR1	rs4149623	T	Dominant	–0.467	1.13×10^{-5}	1×10^{-7}
	rs4149639	A	Recessive	–0.435	4.15×10^{-6}	0.070
	rs4149622	A	Dominant	–0.443	3.44×10^{-5}	1×10^{-7}
	rs4149578	G	Dominant	0.410	0.010	NS

* Larger *p*-value reported, though LRT always similar in magnitude to the Wald test

[§] Non-significant (*NS*) *p*-values >0.10

Table 5 Results of association analysis of TB as the phenotype

Gene	Marker	Referent allele	Best fitting model	β coef	Genotype *p*-value*	HIV × locus interaction[§]
IFNGR1	rs4896243	C	Additive	0.467	0.002	NS
	rs1327474	G	Dominant	–1.312	0.243	NS
	rs2234711	T	Dominant	0.677	0.002	0.067
IL-10	rs1518111	A	Dominant	–0.163	0.376	NS
	rs1554286	C	Recessive	0.199	0.276	NS
	rs1800872	A	Dominant	–0.196	0.270	NS
TNFR1	rs4149623	T	Recessive	–0.784	0.003	NS
	rs4149639	A	Recessive	0.210	0.196	0.019
	rs4149622	A	Recessive	–0.794	0.002	NS
	rs4149578	G	Additive	0.293	0.030	NS

* Larger *p*-value reported, though LRT always similar in magnitude to the Wald test

[§] Non-significant (*NS*) *p*-values >0.10

above Bonferroni correction. Based on the regression coefficients from the association models for *IFNGR1*, the allele associated with increased risk for TB (through either an additive or dominant model) was associated with increased TNFα expression. The same appears to be true for *TNFR1*, though the results are less consistent. When including the SNPs within the Haseman–Elston model, we found that the most significant SNP (per gene) accounted for the whole linkage signal. The exception was the linkage analysis of TNFα and *IFNGR1*, when both rs2234711 and rs4896243 were needed to account for the linkage signal (data not shown).

To explore whether the genetic associations seen with the TB phenotype depict risk for active disease or latent Mtb infection, we conducted an analysis where we contrasted TB patients with individuals having latent Mtb infection but no evidence of disease. The associations seen with *IFNGR1* and *TNFR1* remained significant (same SNPs, $p < 0.01$, data not shown), implying that these loci influence progression from Mtb infection to active disease.

When we examined the effect of interaction between HIV status and the SNPs on both phenotypes, we found that *TNFR1* is the only gene that showed consistent evidence for a locus × HIV interaction on both phenotypes; three out of four markers had significant (at $\alpha = 0.10$) HIV × SNP interactions when TNFα was the phenotype (Table 4), and one out of four markers had a significant interaction when TB was the phenotype (Table 5). It is likely that we did not have the power to detect interactions for TB as the trait because there were only 61 HIV negative TB cases and 48 HIV positive TB cases. There was one SNP showing a significant HIV × SNP interaction for *IFNGR1*. These results are weaker because the other SNPs within *IFNGR1* did not show a significant interaction ($p > 0.10$) and the specific SNP involved in the interaction differed across phenotypes. To further examine the interaction effects, we redid the analysis of TNFα, stratifying on HIV status (Table 6). We did not conduct a stratified analysis for the TB trait because of the sample size limitations noted above. For TNFα as the trait of interest, we found the SNP-phenotype associations remained significant within strata ($p < 0.10$). Furthermore, we found the SNPs had stronger negative associations with TNFα levels in the HIV negative subgroup than in the HIV positive subgroup.

Next, we evaluated associations between haplotypes in these genes with both phenotypes. A haplotype in *IL-10* (T-A-A) was associated with protection against TB ($\beta = -0.958$, $p = 0.002$, data not shown), even though the SNPs analyzed individually did not demonstrate association. This suggests that another polymorphism within *IL-10* may convey TB risk, though these particular SNPs do not. This haplotype, consisting of all three minor alleles, increases risk for TB, and the minor alleles also appear to be associated with decreased TNFα levels (Table 4).

Discussion

Several studies have suggested that risk for developing TB disease is influenced by host genetic factors. However, many of the previous studies have been hindered by inconsistent phenotype definitions that ignore the complex nature of TB. In this study conducted in Kampala, Uganda, a country endemic for TB, we took a unique approach to evaluate candidate genes for TB by using two phenotypes. Pulmonary TB, the disease phenotype, was evaluated and diagnosed in a rigorous and consistent way throughout the study. Our study is distinctive in that the circumstances of exposure are known and accounted for in the analysis as a covariate. Furthermore, because we assess latent Mtb infection prospectively, our study has the ability to distinguish whether genetic loci predispose to latent Mtb infection or active disease. The other phenotype is an intermediate phenotype based on known components of the host immune response to Mtb. By examining linkage and association for both phenotypes in the same study sample, we were able to show construct validity and internal consistency in a single study.

We have developed expression levels of TNFα in response to Mtb culture filtrate as an intermediate phenotype (Stein et al. 2003, 2005) and, in the present study, we have examined linkage and association of candidate genes hypothesized to regulate TNFα expression. The intermediate phenotype model is useful because such traits are thought to be more powerful than binary traits because of their quantitative nature (Duggirala et al. 1997) and because they are more closely tied to the level of gene expression (Rice et al. 2001; Risch 2000).

Table 6 Association analysis stratified by HIV status with TNFα as the phenotype

Gene	Marker	β in HIV+	p-value in HIV+	β in HIV–	p-value in HIV–
IFNGR1	rs4896243	–0.431	0.074	–1.270	4.44×10^{-16}
TNFR1	rs4149623	–0.255	0.011	–1.451	4.44×10^{-16}
	rs4149639	–0.398	3.32×10^{-5}	–0.507	1.00×10^{-5}
	rs4149622	–0.223	0.016	–1.482	1.00×10^{-7}

By observing linkage and association between markers and both traits, we can delineate the role of these genes and TB pathogenesis. Our analyses for *TNFR1* and *IFNGR1* suggest that the overexpression of a proinflammatory cytokine, TNFα, is associated with disease. These results are consistent with previous studies demonstrating a positive correlation between TB severity and TNFα levels, and a corresponding decrease in TNFα levels after treatment (Bekker et al. 2000; Ribeiro-Rodrigues et al. 2002). Furthermore, our results suggest that HIV modifies the effect of *TNFR1* on TNFα expression. These results illustrate that the impact of TNFα release on TB risk clearly depends on the specific genes involved, implying that the function of these genes must be considered in the immunologic analyses. Certainly, these association results do not imply causality, but they do generate new hypotheses that warrant follow-up in functional studies. In addition, it is possible that the relationship between TNFα levels and TB depends on the stage of disease. At this time we do not have sufficient data to test this hypothesis, but it will be the focus of future research.

To our knowledge, we are the first to find an association between *TNFR1* and TNFα regulation and TB risk in a human population. It has been suggested that the soluble portion of TNFα receptors may block TNFα activity, increasing susceptibility to TB (Keane et al. 2001). Moreover, a role for *TNFR1* in TB susceptibility and TNFα protein production has been demonstrated through knockout mouse models (Peschon et al. 1998; Piguet et al. 2002). Despite this association with disease and cytokine expression, none of the tagSNPs we genotyped appear to have known functional implications. Since this is the first report finding an association between TB risk and the human *TNFR1* gene, further studies are needed to replicate the finding.

The first report of an association with *IFNGR1* was from a Maltese pedigree; this association was observed with non-tuberculous mycobacterial infection (Newport et al. 1996). Association between a SNP in *IFNGR1* and disease caused by specifically *M. tuberculosis* was recently reported in a Gambian population (Cooke et al. 2006), though previous analyses of microsatellites within *IFNGR1* were inconsistent (Awomoyi et al. 2004; Fraser et al. 2003). Our analysis independently confirms that of Cooke and colleagues who found the CC genotype at the –56 SNP (rs2234711) to be associated with increased risk for TB disease. In addition to replicating this association, we make the novel observation that this SNP is associated with TNFα levels, and that two additional SNPs within this gene are associated with both traits. Since IFNγ has a role in TNFα production (Knight and Kwiatkowski 1999), deficiencies in IFNGR1 may also affect TNFα activity, ultimately influencing TB susceptibility.

In this analysis, a haplotype in *IL-10* showed protection against TB disease. The relationship between *IL-10* SNPs and TNFα is less clear; further molecular experiments are needed to clarify the relationship between the *IL-10* gene and TNFα regulation. IL-10 is an inhibitor of Th-1 cell and macrophage functions (Janeway et al. 2001), and drug and molecular studies suggest that IL-10 activity leads to decreased TNFα production (Balcewicz-Sablinska et al. 1999; Goldman et al. 1996). There are several case-control studies examining the association between the *IL-10* gene and TB risk, but their results are inconsistent. Though a promotor SNP, –1082, has been associated with protection against TB in studies in Malawi (Fitness et al. 2004), Cambodia (Delgado et al. 2002), and Sicily (Scola et al. 2003), nonsignificant results were found in studies in Spain (López-Maderuelo et al. 2003), Hong Kong (Tso et al. 2005), Korea (Shin et al. 2005), and the Gambia (Bellamy et al. 1998). We did not genotype the –1082 SNP (rs1800896) because it was not a tagSNP. However, rs1800872 is also a promoter SNP (–592) and is in strong LD with –1082 (Kurreeman et al. 2004). Interestingly, though the Korean study (Shin et al. 2005) did not find association between TB and –1082, it did detect association between TB and –592, so our report provides replication of that findings. Apart from the disease phenotype, we found linkage and association between *IL-10* and TNFα levels. Since we used a haplotype-tagging approach to select SNPs and observed association between TB and a haplotype in *IL-10*, but not with the individual SNPs, it is possible that we are detecting association to a polymorphism in LD with our SNPs, such as –1082. Fine mapping is necessary to clarify this hypothesis.

One of the most studied genes with regard to TB risk is *SLC11A1*, which has been associated with TB across a number of populations, as shown by a recent meta-analysis (Li et al. 2006). We did not detect linkage to this gene and consequently, we did not pursue association analysis. A study conducted in a Gambian population also did not find association between *SLC11A1* and TNFα response to lipopolysaccaharide (LPS) (Awomoyi et al. 2002). On the one hand, it is possible that the association between *SLC11A1* does not exist in this Ugandan population; on the other, *SLC11A1* may have a small effect on TB risk, and small effects are less likely to be detected by linkage analysis (Risch and Merikangas 1996).

The linkage analysis results have other noteworthy findings. First, *TNFR2*, *TLR4*, and *TLR2* were linked to TB disease but not to TNFα levels. These findings suggest that these genes are related to TB susceptibility but not via TNFα regulation. Second, the Haseman–Elston and conditional logistic methods provided slightly different results for the binary trait—*TNFR1, IL-10, TNFR2* and *TLR2* were only detected by one method. The explanation for this

apparent inconsistency is that these two models are based on different, complementary models. The Haseman–Elston model contrasts concordant and discordant pairs, but when extrapolated, allows for impossible values for IBD sharing less than 0 and greater than 1. The conditional logistic model as implemented in LODPAL compares observed allele sharing IBD to that expected under Mendelian segregation alone. This model avoids the aforementioned problem of IBD sharing less 0 and greater than 1, but relies heavily on the assumption of Mendelian segregation without any selective forces acting to distort it. Sometimes one model may be better than another, but the underlying biologic reasons for this phenomenon are not understood. However, it is important to emphasize that these analyses were based on only 109 individuals with TB, so this small sample size is a limitation of our study.

In this first molecular genetic study of TB susceptibility conducted from Uganda, we took a unique approach to the characterization of a complex trait. In doing so, we found a new association between TB and *TNFR1* in a human population and replicated previous findings for *IFNGR1* and *IL-10* from different ethnic populations. These findings are consistent with the immunologic model for TB pathogenesis that Th-1-like cytokines such as IFNγ and TNFα create a microenvironment within granuloma which contains growth of Mtb and that is down-regulated by IL-10. Validation of these findings in other populations is warranted as well as finer mapping of these candidate genes, especially since previous studies have focused on a few polymorphisms per gene, and it is possible that there are polymorphisms in LD with those studied that actually convey risk.

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References

Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P (2005) A haplotype map of the human genome. Nature 437:1299–1320

Awomoyi A, Marchant A, Howson J, McAdam K, Blackwell J, Newport M (2002) Interleukin-10, Polymorphism in SLC11A1 (formerly NRAMP1), and susceptibility to tuberculosis. J Infect Dis 186:1808–1814

Awomoyi AA, Nejentsev S, Richardson A, Hull J, Koch O, Podinovskaia M, Todd JA, McAdam KPWJ, Blackwell JM, Kwiatkowski D, Newport MJ (2004) No association between interferon-γ receptor-1 gene polymorphism and pulmonary tuberculosis in a Gambian population sample. Thorax 59:291–294

Balcewicz-Sablinska M, Gan H, Remold H (1999) Interleukin 10 produced by macrophages inoculated with *Mycobacterium avium* attenuates mycobacteria-induced apoptosis by reduction of TNF-α activity. J Infect Dis 180:1230–1237

Barnes P, Fong S, Brennan P, Twomey P, Mazumder A, Modlin R (1990) Local production of tumor necrosis factor and IFN-gamma in tuberculosis pleuritis. J Immunol 145:149–154

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Bekker LG, Haslett P, Maartens G, Steyn L, Kaplan G (2000) Thalidomide-induced antigen-specific immune stimulation in patients with human immunodeficiency virus type 1 and tuberculosis. J Infect Dis 181:954–965

Bellamy R (2003) Susceptibility to mycobacterial infections: the importance of host genetics. Genes Immun 4:4–11

Bellamy R, Ruwende C, Corrah T, McAdam K, Whittle H, Hill A (1998) Assessment of the interleukin 1 gene cluster and other candidate gene polymorphisms in host susceptibility to tuberculosis. Tuberc Lung Dis 79:83–89

Blackwell J, Barton C, White J, Searle S, Baker A, Williams H, Shaw M (2004) Genomic organizaton and sequence of the human NRAMP gene: identification and mapping of a promotor region polymorphism. Mol Med 1:194–205

Blumberg H, Burman W, Chaisson R, Daley C, Etkind S, Friedman L, Fujiwara P, Grzemska M, Hopewell P, Iseman M, Jasmer R, Koppaka V, Menzies R, O'Brien R, Reves R, Reichman L, Simone R, Starke J, Vernon A, American Thoracic Society, Centers for Disease Control, Prevention, Infectious Diseases Society (2003) American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am J Respir Crit Care Med 167:603–662

Bochud P-Y, Hawn T, Aderem A (2003) Cutting edge: a toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. J Immunol 170:3451–3454

Centers for Disease Control and Prevention (2003) Treatment of tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Soceity of America. MMWR 52:1–77

Cooke GS, Campbell SJ, Sillah J, Gustafson P, Bah B, Sirugo G, Bennett S, McAdam KP, Sow O, Lienhardt C, Hill AV (2006) Polymorphism within the interferon gamma/receptor complex is associated with pulmonary tuberculosis. Am J Respir Crit Care Med 174:339–343

Delgado J, Baena A, Thim S, Goldfeld A (2002) Ethnic-specific genetic associations with pulmonary tuberculosis. J Infect Dis 186:1463–1468

Drennan M, Nicolle D, Quesniaux V, Jacobs M, Allie N, Mpagi J, Frémond C, Wagner H, Kirschning C, Ryffel B (2004) Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. Am J Pathol 164:49–57

Duggirala R, Williams J, Williams-Blangero S, Blangero J (1997) A variance components approach to dichotomous trait linkage using a threshold model. Genet Epidemiol 14:987–992

Elston RC, George VT, Severtson F (1992) The Elston–Stewart algorithm for continuous genotypes and environmental factors. Hum Hered 42:16–27

Epstein M, Duren W, Boehnke M (2000) Improved inference of relationship for pairs of individuals. Am J Hum Genet 67:1219–1231

Fitness J, Floyd S, Warndorff D, Sichali L, Malema S, Crampin A, Fine P, Hill A (2004) Large-scale candidate gene study of tuberculosis susceptibility in the Karonga district of Nothern Malawi. Am J Trop Med Hyg 71:341–349

Flores-Villanueva PO, Ruiz-Morales JA, Song CH, Flores LM, Jo EK, Montano M, Barnes PF, Selman M, Granados J (2005) A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. J Exp Med 202:1649–1658

Flynn J, Goldstein M, Chan J, Triebold K, Pfeffer K, Lowenstein C, Schreiber R, Mak T, Bloom B (1995) Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. Immunity 2:561–572

Formica S, Roach T, Blackwell J (1994) Interaction with extracellular matrix proteins influences Lsh/Ity/Bcg (candidate Nramp) gene regulation of macrophage priming/activation for tumor necrosis factor-α and nitrite release. Immunology 82:42–50

Fraser D, Bulat-Kardum L, Knezevic J, Babarovic P, Matakovic-Mileunic N, Dellacasagrande J, Matanic D, Pavelic J, Beg-Zec Z, Dembic Z (2003) Interferon-γ receptor-1 gene polymorphism in tuberculosis patients from Croatia. Scand J Immunol 57:480–484

George V, Elston RC (1987) Testing the association between polymorphic markers and quantitative traits in pedigrees. Genet Epidemiol 4:193–202

Goddard K, Witte J, Suarez B, Catalona W, Olson J (2001) Model-free linkage analysis with covariates confirms linkage of prostate cancer to chromosomes 1 and 4. Am J Hum Genet 68:1197–1206

Goldman M, Marchant A, Schandené L (1996) Endogenous interleukin-10 in inflammatory disorders: Regulatory roles and pharmacological modulation. Ann NY Acad Sci 796:282–293

Gray-McGuire C (2004) Assessment of a variance component method for binary phenotype data: model misspecification and effects of ascertainment. PhD Dissertation, Case Western Reserve University

Guwattude D, Nakakeeto M, Jones-Lopez E, Maganda A, Chiunda A, Mugerwa R, Ellner J, Bukenya G, Whalen C (2003) Tuberculosis in household contacts of infectious cases in Kampala, Uganda. Am J Epidemiol 158:887–898

Haseman J, Elston R (1972) The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3–19

Janeway C, Travers P, Walport M, Shlomchik M (2001) Immunobiology, 5th edn. Garland Publishing, New York

Keane J, Gershon S, Wise R, Mirabile-Levens E, Kaszinca J, Schwietermann W, Siegel J, Braun M (2001) Tuberculosis associated with inflixamab, a tumor necrosis factor α-neutralizing agent. N Engl J Med 345:1098–1104

Knight J, Kwiatkowski D (1999) Inherited variability of tumor necrosis factor production and susceptibility to infectious disease. Proc Assoc Am Phys 111:290–298

Kong X, Murphy K, Raj T, He C, White P, Matise T (2004) A combined linkage-physical map of the human genome. Am J Hum Genet 75:1143–1148

Kurreeman FA, Schonkeren JJ, Heijmans BT, Toes RE, Huizinga TW (2004) Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. Hum Mol Genet 13:1755–1762

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Li HT, Zhang TT, Zhou YQ, Huang QH, Huang J (2006) SLC11A1 (formerly NRAMP1) gene polymorphisms and tuberculosis susceptibility: a meta-analysis. Int J Tuberc Lung Dis 10:3–12

López-Maderuelo D, Arnalich F, Serantes R, González A, Codeoceo R, Madero R, Vazquez J, Montiel C (2003) Interferon-γ and interleukin-10 gene polymorphisms in pulmonary tuberculosis. Am J Respir Crit Care Med 167:970–975

Means T, Wang S, Lien E, Yoshimura A, Golenbock D, Fenton M (1999) Human Toll-like receptors mediate ceulluar activation by *Mycobacterium tuberculosis*. J Immunol 163:3920–3927

Means T, Jones B, Schromm A, Shurtleff B, Smith J, Keane J, Golenbock D, Vogel S, Fenton M (2001) Differential effects of a toll-like receptor antagonist on Mycobacterium tuberculosis-induced macrophage responses. J Immunol 166:4074–4082

Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, Levin M (1996) A mutation in the interferon-γ-receptor gene and susceptbility to mycobacterial infection. New Engl J Med 335:1941–1949

Olson J (1999) A general conditional-logistic model for affected-relative-pair linkage studies. Am J Hum Genet 65:1760–1769

Peschon JJ, Torrance DS, Stocking KL, Glaccum MB, Otten C, Willis CR, Charrier K, Morrissey PJ, Ware CB, Mohler KM (1998) TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J Immunol 160:943–952

Piguet PF, Da Kan C, Vesin C (2002) Rolle of tumor necrosis factor receptor 2 (TNFR2) in cerebral malaria in mice. Lab Invest 82:1155–1166

Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, Thomsen M, Nerup J, Cambon-Thomsen A (1993) Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-α and TNF-β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 23:224–231

Raviglione M, Snider D, Kochi A (1995) Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. JAMA 273:220–226

Ribeiro-Rodrigues R, Resende CT, Johnson JL, Ribeiro F, Palaci M, Sa RT, Maciel EL, Pereira Lima FE, Dettoni V, Toossi Z, Boom WH, Dietze R, Ellner JJ, Hirsch CS (2002) Sputum cytokine levels in patients with pulmonary tuberculosis as early markers of mycobacterial clearance. Clin Diagn Lab Immunol 9:818–823

Rice JP, Saccone NL, Rasmussen E (2001) Definition of the phenotype. Adv Genet 42:69–76

Risch N (2000) Searching for genetic determinants in the new millenium. Nature 405:847–856

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517

Roach DR, Bean AGD, Demangel C, France MP, Briscoe H, Britton WJ (2002) TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. J Immunol 168:4620–4627

S.A.G.E. (2006) Statistical analysis for genetic epidemiology. Case Western Reserve University, Cleveland, OH

Scola L, Crivello A, Marino V, Gioia V, Serauto A, Candore G, Colonna-Romano G, Caruso C, Lio D (2003) IL-10 and TNF-alpha polymorphisms in a sample of Sicilian patients affected by tuberculosis: implication for ageing and life span expectancy. Mech Ageing Dev 124:569–572

Shete S, Jacobs KB, Elston RC (2003) Adding further power to the Haseman and Elston method for detecting linkage in larger sibships: weighting sums and differences. Hum Hered 55:79–85

Shin HD, Park BL, Kim YH, Cheong HS, Lee IH, Park SK (2005) Common interleukin 10 polymorphism associated with decreased risk of tuberculosis. Exp Mol Med 37:128–132

Skamene E, Schurr E, Gros P (1998) Infection genomics: Nramp1 as a major determinant of natural resistance to intracellular infections. Annu Rev Med 49:275–287

Stein CM, Guwattude D, Nakakeeto M, Peters P, Elston RC, Tiwari HK, Whalen CC (2003) Heritability analysis of cytokines as intermediate phenotypes of tuberculosis. J Infect Dis 187:1679–1685

Stein CM, Nshuti L, Chiunda AB, Boom WH, Elston RC, Mugerwa RD, Iyengar SK, Whalen CC (2005) Evidence for a major gene influence on tumor necrosis factor-alpha expression in tuberculosis: path and segregation analysis. Hum Hered 60:109–118

Tso H, Ip W, Chong W, Tam C, Chiang A, Lau Y (2005) Association of interferon gamma and interleukin 10 genes with tuberculosis in Hong Kong Chinese. Genes Immun 6:358–363

Underhill DM, Ozinsky A, Smith KD, Aderem A (1999) Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci 96:14459–14463

van Crevel R, Ottenhoff THM, van der Meek JWM (2002) Innate immunity to mycobacterium tuberculosis. Clin Microbiol Rev 15:294–309

Wigginton JE, Abecasis GR (2005) PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 21:3445–3447

Wilson AG, Symons JA, McDowel TL, McDevitt HO, Duff GW (1997) Effects of a polymorphism in the human tumor necrosis factor α promotor on transcriptional activation. Proc Natl Acad Sci 94:3195–3199

Witte JS, Elston RC, Schork NJ (1996) Genetic dissection of complex traits. Nat Genet 12:355–356