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A Polymorphism in Human *CD1A* is Associated with Susceptibility to Tuberculosis

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Abstract

CD1 proteins are antigen-presenting molecules that evolved to present lipids rather than peptides to T cells. However, unlike major histocompatibility complex genes, CD1 genes show low rates of polymorphism and have not been clearly associated with human disease. We report that an intronic polymorphism in *CD1A* (rs411089) is associated with susceptibility to tuberculosis in two cohorts of Vietnamese adults (combined cohort odds ratio 1.78; 95%CI: 1.24-2.57; p=0.001). These data strengthen the hypothesis that CD1A-mediated lipid antigen presentation is important for controlling tuberculosis in humans.

Keywords

Human; Tuberculosis; Host Response; CD1A; Genetic Association; Single Nucleotide Polymorphism

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Introduction

Mycobacterium tuberculosis is a pathogen of worldwide importance, but its mechanisms of virulence are only partially understood. Infection with *M. tuberculosis* may result in several outcomes including latent infection and active disease, which can be confined to the lungs or manifest at distant sites. The most common manifestation in adults is pulmonary tuberculosis, while dissemination to the central nervous system is less common but results in greater morbidity and mortality. These myriad outcomes have been difficult to model in animal systems, and human studies are limited[1].

Several lines of evidence indicate a role for T-cell mediated immunity in controlling the clinical course of tuberculosis in humans. First, CD4+ T-cell depletion as a result of HIV co-infection has been associated with increased rates of pulmonary (primary progressive and reactivation disease) and extrapulmonary tuberculosis[2]. Second, candidate gene association studies have revealed links between MHC Class II alleles and susceptibility to tuberculosis[3]. Finally, Mendelian studies indicate that the inability to respond to IFN-γ, a cytokine produced by T-cells, is associated with susceptibility to disseminated infection after vaccination with BCG[4]. The identification of the antigens mediating protective T cell immunity is critical for the rational design of novel vaccine candidates. These studies reveal that immunogenetics can illuminate the mechanisms by which protective T cell immunity is generated.

Classically, T cells recognize foreign peptide antigens in the context of polymorphic MHC Class I or Class II molecules. However, T cells can also recognize bacterial lipids in the context of non-classical CD1 molecules[5]. Thus, the potential catalog of protective antigens extends beyond the proteome of *M. tuberculosis*. A number of CD1-restricted mycobacterial lipid antigens have been characterized, and T-cell responses to these antigens are detectable in the blood of *M. tuberculosis* infected humans[6,7]. Whether CD1-restricted T-cell responses provide protective immunity to tuberculosis remains to be determined.

CD1 proteins are evolutionarily conserved; however, unlike MHC genes, CD1 genes show low rates of genetic polymorphism and have not been clearly associated with human disease[8]. The human CD1 locus contains five genes (*CD1A, CD1B, CD1C, CD1D, and CD1E)* clustered on chromosome 1. Notably, the mouse genome contains only an ortholog of human *CD1D*, thus limiting the utility of mouse models for studying CD1 biology[9]. Human CD1A-restricted T cells recognize *M. tuberculosis* infected dendritic cells *in-vitro* and respond to the mycobacterial cell wall lipopeptide antigen, dideoxymycobactin[10,11]. Therefore, we hypothesized that CD1A might play a protective role in human tuberculosis.

We recently demonstrated that a polymorphism in the 5′ UTR of *CD1A* (rs366316) is common and associated with functional CD1A-deficiency in dendritic cells[12]. The identification of genetic markers for variation in CD1A expression and function provided us with a tool to probe for human disease association. Here, we report that an intronic *CD1A* polymorphism (rs411089) is reproducibly associated with susceptibility to tuberculosis in Vietnam. These data demonstrate the utility of population genetics studies in elucidating the role of CD1 in human disease.

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Results and Discussion

We used a case-population study design to evaluate whether haplotype-tagging SNPs in CD1A were associated with the development of active tuberculosis disease in Vietnamese adults. We genotyped rs366316, rs2269714, rs411089, and rs389293 in a discovery cohort of 352 cases and 382 controls (Table 1). Polymorphisms rs366316 and rs411089 were significantly associated with the development of tuberculosis with a genotypic model (p=0.003 and p=0.036, respectively). We next performed a recessive model analysis since we previously found that the minor homozygous genotypes of both of these SNPs were associated with functional CD1A-deficiency. The minor homozygous genotype of rs411089 was associated with an increased risk of tuberculosis with an odds ratio (OR) of 1.94 (95%CI: 1.13–3.4; p=0.011). The association between the minor homozygous genotype of rs366316 and risk of tuberculosis showed a trend toward significance (OR=1.73; 95%CI: 0.96-3.15; p=0.051). Notably, rs2269714, which codes for a non-synonymous mutation, and rs389293 were not associated with disease. Thus, *CD1A* SNPs in non-coding or regulatory regions were associated with tuberculosis in this cohort.

Next, we genotyped rs366316 and rs411089 in a validation cohort of 339 cases and 376 controls. Again, we found a significant association between rs411089 and tuberculosis (p=0.012) in a genotypic analysis; however, there was no association with rs366316 (p=0.858) (Table 1). We confirmed the association of the minor homozygous genotype of rs411089 in a recessive model analysis (OR=1.75, 95%CI: 1.06–2.92; p=0.021). In a combined cohort analysis, we found a highly significant association between rs411089 and tuberculosis (OR= 1.78, 95%CI: 1.24–2.57; p=0.001). Because of its previously documented functional importance, we also examined rs366316 in a combined cohort even though the initial association did not validate. We found a statistically significant association between rs366316 and the risk of tuberculosis (p=0.019). Because rs366316 and rs411089 are in low linkage disequilibrium in Vietnam (R^2=0.15; Figure 1), we tested for evidence of genetic interaction with a haplotype analysis but found no evidence for this (data not shown). These data identify rs411089 as a second *CD1A* locus of potential functional importance that is reproducibly associated with susceptibility to tuberculosis.

Finally, we evaluated whether rs366316 and rs411089 were associated with clinical subtypes of tuberculosis, either pulmonary or meningeal disease. Stratification by site of disease showed that rs366316 was modestly associated with pulmonary disease (p=0.032) and was not associated with meningeal tuberculosis (p=0.188). In a recessive model analysis, rs366316 showed a borderline association with pulmonary disease (OR=1.59, 95%CI 0.97–2.59; p=0.045). By contrast, rs411089 was associated with both pulmonary and meningeal tuberculosis (p=0.003 and p=0.050 respectively) in a genotypic model. In a recessive model analysis, rs411089 was also associated with both pulmonary (OR=1.91, 95%CI 1.26–2.89; p=0.001) and meningeal (OR=1.62, 95%CI 1.01–2.57; p=0.031) tuberculosis (Table 1). These data are consistent with our findings in the unstratified analysis, and demonstrate that *CD1A* SNPs are associated with both pulmonary and meningeal tuberculosis in Vietnamese adults.

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The capacity for T cells to respond to mycobacterial lipid antigens in the context of CD1 molecules was described almost twenty years ago, yet the importance of this observation for human disease pathogenesis is still not clear. We found that rs411089, an intronic SNP within *CD1A*, was associated with susceptibility to tuberculosis in two cohorts of Vietnamese adults. We also found that rs366316, which is associated with functional CD1A-deficiency in dendritic cells, was not reproducibly associated with tuberculosis in this study. These data demonstrate the utility of a population genetics approach to understanding the role of CD1-mediated lipid antigen presentation in human disease. The information gained from studies like this one could be important in the design of novel vaccines. For example, T cell immunity against lipids might be expected after administration of a whole cell vaccine but not with a protein subunit vaccine.

The findings reported here are subject to at least two limitations. First, our study design included cord blood controls, and some of these individuals will surely develop tuberculosis as adults. However, misclassification of controls would indicate that the associations reported here underestimate the true genetic risk. Second, we did not validate an association between a previously known functional variant (rs366316) and tuberculosis. Rather than indicating that there is no association between CD1A-deficiency and tuberculosis, these data could be the result of a lack of statistical power, a possibility underscored by the marginal association still present in the combined cohort analysis. The other possibility is that the immunologic mechanisms involved in pulmonary and extrapulmonary infection are very different from what we have described when studying *in-vitro* derived dendritic cells. Thus, the reproducible association between rs411089 and tuberculosis could be the consequence of an additional unknown genetic regulatory mechanism for CD1A. We do not know if rs411089 modulates transcription of *CD1A* independent of rs366316, but the positive findings reported here and low linkage disequilibrium between the two SNPs suggest this is possible. It is notable that rs411089 is strongly linked to rs858998, which is located in the CD1A promoter region and might regulate transcription independently of rs366316 (Supplementary Figure 1). If confirmed through mechanistic studies, impaired lipid antigen presentation would be a plausible biological mechanism for the association between SNPs in *CD1A* and susceptibility to tuberculosis.

Materials and Methods

Human Subjects

Details of clinical characteristics and enrollment criteria for Vietnam study subjects have been previously published[13]. Population controls were enrolled at Hung Vuong Hospital where umbilical cord blood was collected from newborns. Subjects with pulmonary tuberculosis were recruited from a network of district tuberculosis control units and defined by typical clinical symptoms in addition to sputum smear positive for acid-fast bacilli and/or culture positive for *M. tuberculosis*. Subjects with meningeal tuberculosis (TBM) were classified as definite or probable. All cases in the discovery cohort had definite TBM with clinical meningitis and either positive Ziehl-Neelsen stain for acid-fast bacilli or positive *M. tuberculosis* culture from CSF. The validation cohort included subjects with both definite (N=54) and probable (N=73) meningitis defined as clinical meningitis plus one or more of

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the following: chest radiograph consistent with active tuberculosis, acid-fast bacilli found in any specimen other than CSF, and clinical evidence of extrapulmonary tuberculosis at an additional site. All case and control participants were unrelated and greater than 95% were of the Vietnamese Kinh ethnicity.

Ethics

Written, informed consent was obtained from patients or their relatives if the patient could not provide consent. Parents provided consent for cord-blood controls. All protocols were approved by human subject review committees at Pham Ngoc Thach Hospital for Tuberculosis, the Hospital for Tropical Diseases, Health Services of Ho Chi Minh City, and Hung Vuong Hosptial in Vietnam as well as the Oxford Tropical Research Ethics Committee and the University of Washington.

Selection of Single Nucleotide Polymorphisms (SNPs) for Genotyping

We used data from the International Haplotype Mapping Project (www.hapmap.org, version 3, release 2) to select SNPs within ten kilobases of CD1A from the Han Chinese in Beijing, China (CHB) or Japanese in Tokyo, Japan (JPT). We previously demonstrated similar genome-wide haplotype frequencies between Vietnam and CHB/JPT populations[14]. There are seven SNPs with a minor allele frequency greater than 4% within the *CD1A* gene region, and all but one of these SNPs is located within regulatory or non-coding regions of the gene (Supplementary Figure 1). We genotyped six of these SNPs in 382 control subjects from Vietnam, and confirmed a similar minor allele frequency and linkage pattern when compared to the CHB/JPT HapMap populations. Haplotype-tagging SNPs with a R^2 cutoff of 0.80 for linkage disequilibrium were identified using Haploview v4.2 (www.broad.mit.edu/haploview). Thus, we were able to select only four tagged SNPs as markers for a candidate gene association study (Figure 1).

Genomic techniques

Genomic DNA was prepared from peripheral blood (Qiagen). Because of limiting quantities, genomic DNA from cases in Vietnam was amplified using Repli-G (Qiagen). Multiplex genotyping of rs411089, rs2269714, and rs389293 in the discovery cohort was performed using allele-specific primer extension on the MassARRAY (Sequenom) platform. Genotyping for rs366316 in the discovery cohort and rs366316 and rs411089 in the validation cohort was performed using TaqMan SNP Genotyping Assay (Applied Biosystems, Catalog# 4351379). In all cohorts and platforms, automatic call rates exceeded 95% and less than 3% of calls were assigned manually. Genotypes were confirmed in a subset of individuals by DNA sequencing or by genotyping on an alternate platform. All SNPs were in Hardy-Weinberg equilibrium among control subjects, effectively ruling out errors in genotyping (Table 1). We have previously demonstrated a lack of population stratification in this cohort[13,14].

Statistics

Statistical analyses were performed using Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX). Function ‘pwld’ was used to calculate R^2 measurements of

linkage disequilibrium between polymorphisms. SNPs were assessed for association with tuberculosis using "genassoc"[15].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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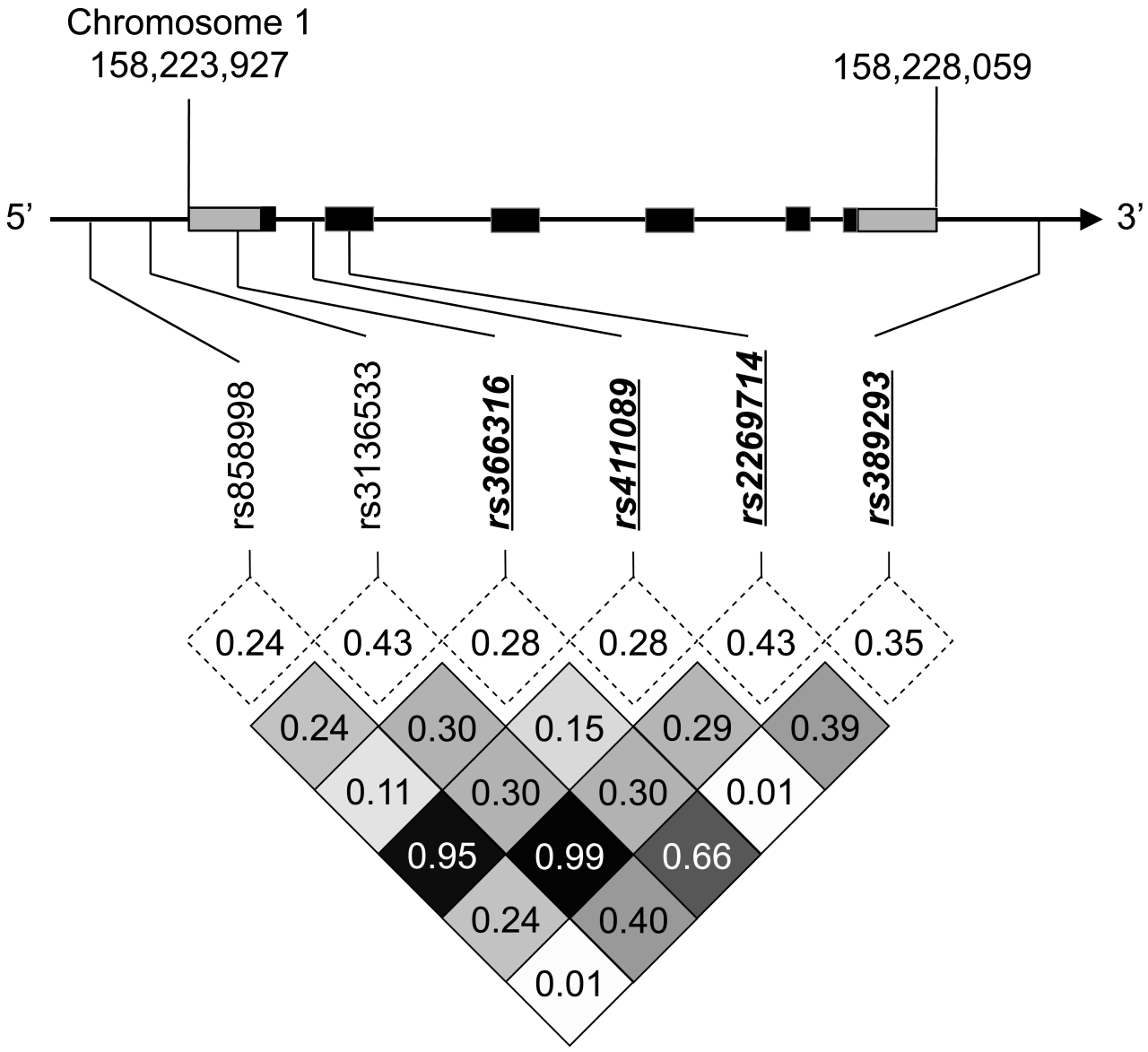

Figure 1. CD1A single nucleotide polymorphisms (SNPs) in Vietnam

(a) Linkage disequilibrium plot of SNPs in CD1A coding region ± 10kB flanking regions among cord blood controls in Vietnam. CD1A spans 4132 bases on chromosome 1 and consists of six exons (black) and two untranslated regions (gray). Minor allele frequencies (dotted box) and linkage disequilibrium as measured by R^2 values (shaded box) are indicated. Four haplotype-tagging SNPs selected for genotyping are emphasized in bold italic underline.

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Table 1
Candidate gene association study of CD1A and tuberculosis in Vietnam

Association between SNPs in CD1A and pulmonary (PTB), meningeal (TBM), or pulmonary + meningeal (TB) tuberculosis in Vietnam. SNP genotypes are shown as n(frequency) of total for common homozygous (AA), heterozygous (Aa), and minor homozygous (aa) genotypes. The p-value for deviation from Hardy-Weinberg equilibrium among cord blood controls was calculated using a χ^2 goodness-of-fit test and is indicated under "HWE." Genotypic analysis compares frequencies in a 2×3 contingency table. For the recessive model analysis, AA + Aa was compared to aa in a 2×2 contingency table. Two-sided hypothesis testing was used for all comparisons. Unadjusted p-values of significance are noted in bold italic.

SNP	Group	HWE	Genotypic Analysis					Recessive Analysis					
			AA	Aa	aa	χ^2	p-value	AA+Aa	aa	χ^2	p-value	OR	95% CI
Discovery Cohort													
rs366316	CTRL	0.20	192 (0.5)	164 (0.43)	25 (0.07)			356 (0.93)	25 (0.07)				
T → C	ALL		161 (0.58)	86 (0.31)	30 (0.11)	11.36	***0.003***	247 (0.89)	30 (0.11)	3.82	0.051	1.73	(0.96-3.15)
rs411089	CTRL	0.18	192 (0.5)	165 (0.43)	25 (0.07)			357 (0.93)	25 (0.07)				
T → C	ALL		171 (0.49)	138 (0.39)	42 (0.12)	6.64	***0.036***	309 (0.88)	42 (0.12)	6.47	***0.011***	1.94	(1.13-3.4)
rs2269714	CTRL	0.56	120 (0.32)	192 (0.51)	68 (0.18)			312 (0.82)	68 (0.18)				
C → T	ALL		116 (0.33)	168 (0.48)	68 (0.19)	0.60	0.742	284 (0.81)	68 (0.19)	0.24	0.621	1.10	(0.74-1.62)
rs389293	CTRL	0.80	159 (0.42)	176 (0.46)	46 (0.12)			335 (0.88)	46 (0.12)				
G → A	ALL		161 (0.47)	143 (0.42)	38 (0.11)	2.09	0.352	304 (0.89)	38 (0.11)	0.16	0.687	0.91	(0.56-1.47)
Validation Cohort													
rs366316	CTRL	0.91	214 (0.58)	133 (0.36)	20 (0.05)			347 (0.95)	20 (0.05)				
T → C	ALL		156 (0.59)	93 (0.35)	17 (0.06)	0.31	0.858	249 (0.94)	17 (0.06)	0.25	0.618	1.18	(0.57-2.43)
rs411089	CTRL	0.56	171 (0.47)	159 (0.44)	32 (0.09)			330 (0.91)	32 (0.09)				
T → C	ALL		161 (0.51)	110 (0.35)	46 (0.15)	8.80	***0.012***	271 (0.85)	46 (0.15)	5.35	***0.021***	1.75	(1.06-2.92)
Combined Cohort													
rs366316	CTRL	0.33	406 (0.54)	297 (0.4)	45 (0.06)			703 (0.94)	45 (0.06)				
T → C	ALL		317 (0.58)	179 (0.33)	47 (0.09)	7.90	***0.019***	496 (0.91)	47 (0.09)	3.31	0.069	1.48	(0.95-2.32)
	PTB		217 (0.57)	126 (0.33)	35 (0.09)	6.88	***0.032***	343 (0.91)	35 (0.09)	4.00	***0.045***	1.59	(0.97-2.59)
	TBM		100 (0.61)	53 (0.32)	12 (0.07)	3.35	0.188	153 (0.93)	12 (0.07)	0.36	0.546	1.23	(0.58-2.42)
rs411089	CTRL	0.27	367 (0.49)	325 (0.43)	59 (0.08)			692 (0.92)	59 (0.08)				
T → C	ALL		327 (0.5)	245 (0.37)	87 (0.13)	12.96	***0.002***	572 (0.87)	87 (0.13)	10.81	***0.001***	1.78	(1.24-2.57)
	PTB		182 (0.49)	137 (0.37)	52 (0.14)	11.96	***0.003***	319 (0.86)	52 (0.14)	10.57	***0.001***	1.91	(1.26-2.89)
	TBM		145 (0.5)	108 (0.38)	35 (0.12)	6.01	***0.050***	253 (0.88)	35 (0.12)	4.67	***0.031***	1.62	(1.01-2.57)