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# Possible Implication of *NFKB1A* and *NKG2D* Genes in Susceptibility to HTLV-1-Associated Myelopathy/ Tropical Spastic Paraparesis in Peruvian Patients Infected With HTLV-1

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The human T-cell lymphotropic virus type 1 (HTLV-1) is the etiological agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a progressive disease causing paraparesis of the lower limbs. Only a minority of persons infected with HTLV-1 develop HAM/TSP. Universal susceptibility factors for HAM/TSP are not known. The viral genotype is similar in asymptomatic carriers and HAM/TSP patients. High proviral load has been associated consistently with HAM/TSP, but this factor does not explain fully the presence of disease in HTLV-1-infected subjects. Most likely, host genetic factors will play an important role in HAM/TSP development. A two-stage case-control study was carried out to evaluate the association between HAM/TSP and candidate single nucleotide polymorphisms (SNPs) from 45 genes in addition to six human leukocyte antigen (HLA) alleles. Ancestry-informative markers were used to correct for population stratification. Several SNPs belonging to *NFKB1A* and *NKG2D* showed a trend of association in both stages. The fact that the direction of the association observed in the first stage was the same in the second stage suggests that *NFKB1A* and *NKG2D* may be implicated in the development of HAM/TSP. Further replication studies in independent HTLV-1 patient groups should validate further these associations. ***J. Med. Virol. 84:319–326, 2012.***
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**KEY WORDS:** human T-cell lymphotropic virus 1; tropical spastic paraparesis; tropical spastic; Peru; genetic association studies

## INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus to be discovered. HTLV-1 infects approximately 20 million people around the world [de Thé and Bomford, 1993]. Although this virus has a worldwide distribution,

Additional supporting information may be found in the online version of this article.

Abbreviations: HTLV-1, human T-lymphotropic virus 1; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; NKG2D, killer cell lectin-like receptor subfamily K, member 1; NFKB1A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; ATL, adult T-cell leukemia/lymphoma.

Conflicts of interest: None.

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some areas are considered endemic: Japan, Africa, the Caribbean basin, and South America. HTLV-1 is transmitted by three main routes: mother-to-child transmission, sharing of infected blood (drug users, transfusions), and by sexual intercourse [Proietti et al., 2005].

HTLV-1 infects preferentially CD4+ T cells and to a lesser extent CD8+ T cells [Ruscetti et al., 1983; Nagai et al., 2001]. Most of the infected individuals remain asymptomatic but between 5 and 10% develop adult T-cell Leukemia (ATL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), infective dermatitis, or are prone to opportunistic infections (e.g., strongyloidiasis, scabies) or inflammatory (e.g., uveitis, alveolitis) complications. HAM/TSP is a chronic disease characterized clinically by spastic weakness of the legs, back pain, sensory signs and symptoms, constipation, and bladder problems.

The causative factors that predispose to HAM/TSP development are not well known. Many viral and genetic factors have been examined in several populations to evaluate their association with HAM/TSP. So far, only a high proviral load remains associated consistently with HAM/TSP across populations [Nagai et al., 1998; Montanheiro et al., 2005; Sabouri et al., 2005; Adaui et al., 2006] although, exceptionally, asymptomatic carriers with high proviral load and HAM/TSP patients with low proviral load have been described [Montanheiro et al., 2005].

The fact that overlapping values of proviral load exist between HAM/TSP patients and asymptomatic carriers, and the fact that HTLV-1 strains with identical genomic sequences are associated with two clinically different disease outcomes such as ATL and HAM/TSP suggest that HTLV-1 infection alone is not sufficient to cause HAM/TSP. A multifactorial etiology is proposed for HAM/TSP, with host genetic, viral, and environmental factors as contributors to susceptibility. As such, the identification of genetic determinants of HAM/TSP susceptibility constitutes a major challenge.

The purpose of this study was to determine whether certain human genetic factors are associated with HAM/TSP in Peruvian subjects infected with HTLV-1, using a two-stage candidate gene association study. The selected genes were related to immune functions including genes involved in innate immunity, genes encoding cytokines, chemokines, human leukocyte antigens (HLA), and genes involved in cell adhesion.

## METHODS

### Study Participants

All patients belong to the HTLV-1 cohort of the Institute of Tropical Medicine Alexander von Humboldt in Lima, Peru. They were either recruited passively at the Institute, or referred from blood banks and other hospitals. The diagnosis of HTLV-1 infection was based on ELISA results using the Ortho and/or Murex assays (Ortho-Clinical Diagnostics, Amersham, UK; Murex Biotech Limited, Dartford, UK), which were confirmed by Inno-lia HTLVI/II Score (Innogenetics, Ghent, Belgium). All patients infected with HTLV-1 underwent clinical examination. HAM/TSP status was determined by one or two experts according to international guidelines [Osame, 1990; De Castro-Costa et al., 2006]. Patients with undefined neurological manifestations, or with other disease manifestations associated with HTLV-1 and HTLV-II were excluded from the study. The origin of the patients was defined as Andean (mostly Quechua ancestry) if both parents or all grandparents were born in the Andes, or as Mestizo (mostly European ancestry) if at least one parent was not born in the Andes. Patients with known Asian or African ancestry were excluded from the study. All patients were unrelated.

A case–control study was undertaken in two stages in order to avoid false positive findings. In the first stage we tested samples from 56 HAM/TSP patients and 114 asymptomatic carriers. With this number of samples the study has 80% power to detect susceptibility variants with relative risks of 2.1 or higher. In the second stage, 85 HAM/TSP patients and 146 asymptomatic carriers were evaluated. The study protocol was approved by the Institutional Research Ethics Committee of the Universidad Peruana Cayetano Heredia and a written informed consent was obtained from each participant.

### Candidate Genes

The candidate genes for the analysis were selected based on published information according to the following criteria:

(i) Genes associated previously with HAM/TSP protection or risk in other populations (e.g., *TNF-α*, *IFN-γ*, *SDF-1*, *HLA-A02*, *HLA-Cw08*).
(ii) Genes associated with control of viral load or disease progression in other viral diseases (*CX3CR1*, *DC-SIGN*, and *PD-1*).
(iii) Genes with different expression levels between HAM/TSP patients and asymptomatic carriers (e.g., *Foxp3*, *Granzyme A*, *Granzyme H*, *Granulysin*, *IL6*, *NKG2D*, *NKG7*, and *Perforin*).
(iv) Genes involved in the NF-kB pathway (e.g., *NEMO* and *NFKBIA*).
(v) Genes involved in self-reactive immune responses (e.g., *PD-1*) or autoimmune diseases (e.g., *CXCL10*).
(vi) Genes encoding cytokines (e.g., *IL2* and *IL10*) and their receptors (*IL2R*).
(vii) Genes encoding cellular adhesion molecules (e.g., *ICAM-1* and *VCAM*).

Relevant references for the selection of each gene are given in Supplementary Table S1.

## SNP Selection

Within the selected genes and based on literature, SNPs associated previously with other viral or inflammatory diseases or to HAM/TSP in other populations (Japan, Brazil, Iran, and Jamaica), were selected for examination in this study. Some selected SNPs were located within the gene, whereas others were located in the promoter region. For instance, for some genes expressed differentially between HAM/TSP and asymptomatic carriers, functional SNPs located in the promotor region were selected (e.g., *Metalloproteinase2*-1306).

At least one SNP was selected for each gene. When no candidate SNPs were available for a candidate gene, the HapMap catalogue was used to select tagSNPs [The HapMap Consortium, 2005], using the Tagger option of the Haploview software [Barrett et al., 2005] and the European-derived HapMap panel as the reference panel. Service et al. [2007] and González-Neira et al. [2006] showed that tagSNPs defined in the CEU reference panel are also efficient for populations from the Americas. The six HLA alleles, the 45 candidate genes, the rs number of the 94 selected SNPs, and the literature that was used to select the genes/SNPs are listed in Supplementary Table SI.

For SNPs showing a trend of association in both stages of the present study, fine mapping was performed by analyzing additional tagSNPs in the regions adjacent to both sides of the associated SNPs.

## Ancestry-Informative Markers

Population stratification can lead to spurious associations between a phenotype and a marker locus, or indeed may mask true associations. To reduce this possibility, SNPs with documented differences in allele frequency between populations were selected. Such SNPs are known as ancestry-informative markers (AIMs) and can be used to correct for population stratification [Enoch et al., 2006]. Thirty-seven ancestry-informative markers with large differences in allele frequencies ($\Delta > 0.67$) between native Americans and European Americans [Mao et al., 2007] were selected. The ancestry-informative markers were distributed across the genome and unlinked to the selected candidate genes. The rs number, the chromosome number, and chromosome position of the analyzed AIMs are shown in Supplementary Table SII.

## Genotyping

DNA was extracted from EDTA-treated blood samples using the genomic prep Blood DNA Isolation Kit (Amersham Biosciences, Amersham, UK). For HLA typing, sequence-specific PCR primers were used as described previously [Bunce et al., 1995]. SNP genotyping was performed by Kbiosciences (Hoddesdon, Hertfordshire, UK) using KASPar® assays, which use a modification of allele specific PCR combined with Fluorescence Resonance Energy Transfer (FRET) to determine the alleles at a specific locus.

## Proviral Load

The DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the QIAamp DNA minikit (Qiagen, Hilden, Germany). The proviral load was determined using a SYBR Green-based real-time quantitative PCR on an iCycler Thermal Cycler (BioRad, Hercules, CA) as described elsewhere [Adaui et al., 2006]. Human endogenous retrovirus 3 was used as reference gene. The proviral load was expressed as the number of HTLV-1 copies per $10^4$ PBMCs.

## Statistical Analysis

***Quality control.*** Samples and SNPs with more than 10% missing genotypes and non-polymorphic SNPs were excluded from the study. A test for deviation of Hardy–Weinberg equilibrium was performed and SNPs were excluded if $P < 0.001$.

***Detecting and correcting for population stratification.*** To evaluate whether the ancestry-informative markers show differences in allele frequency between cases and controls, a Chi square ($\chi^2$) test was performed and Quantile–Quantile plots (Q–Q plots) were constructed which allow to compare the distribution of observed and expected $\chi^2$ values. If the results are in agreement with the null hypothesis (i.e., no differences in allele frequency of ancestry-informative markers between cases and controls and therefore no differences in admixture proportion), the points should fall approximately along the reference line. A deviation from the reference line might be indicative of the presence of population stratification between cases and controls. An identical approach was used for a comparison between Andeans and Mestizos. Principal component analysis was conducted on the ancestry-informative markers using the EIGENSOFT software [Price et al., 2006] and the first three principal components were used to correct for population stratification.

***Association analysis.*** A univariate analysis was carried out first. A Chi square ($\chi^2$) or Fisher exact test for categorical variables and a Mann–Whitney U-test for continuous variables were used when appropriate. Logistic regression analysis was performed to test for association between disease status and genotype under the assumption of an additive genetic model. Age, gender, proviral load, and the first three principal components from the principal component analysis on the ancestry-informative markers were used as covariates to adjust each association. Replication analysis was performed in an independent data set. Due to the low power because of the moderate sample size and the exploratory nature of the study, replication in an independent data set was considered more important than correcting for multiple testing. Therefore, a liberal threshold was used in the first stage. Those SNPs displaying a trend of association in the first stage (*P*-

value ≤ 0.1) were evaluated in the second stage. The statistical software package R was used for all analyses.

## RESULTS

### Patient Characteristics

The characteristics of the participants are summarized in Table I. In both stages there were more women among HAM/TSP patients than among asymptomatic carriers ($P < 0.01$; Table I). HAM/TSP patients were older than asymptomatic carriers ($P < 0.001$; both stages; Table I). Overall, the proviral load (expressed as the copy number of HTLV-1 proviral DNA per $10^4$ PBMCs) was significantly higher in HAM/TSP patients than in asymptomatic carriers ($P < 0.001$; Table I). Regarding ethnicity, a marginal difference was observed in the first stage ($P = 0.044$; Table I), while no statistical difference was observed in the second stage ($P = 0.29$; Table I), although in both stages, a slight excess of Andean origin was noted among HAM/TSP patients (Table I).

### Population Structure

The Q–Q plots for Andeans and Mestizos show a higher deviation from the reference line in both stages (Fig. 1a,b). This fact suggests differences in admixture proportion between Andean and Mestizos. The differences observed in the Q–Q plot evaluating ethnic origin suggest that the number of ancestry-informative markers analyzed in this study is sufficient to detect differences in admixture proportion. For cases and controls, the Q–Q plots show a closer match with the reference line in both stages, suggesting that no significant differences in admixture proportion exist between cases and controls (Fig. 1c,d).

### First Stage

After genotyping, quality control was performed resulting in the exclusion of 12 samples with more than 10% missing genotypes, 10 non-polymorphic SNPs (Supplementary Table SIII), and 2 SNPs with more than 10% missing genotypes. None of the SNPs deviated from Hardy–Weinberg equilibrium.

Initially, univariate analysis was performed to investigate the relationship between each genotype and disease. The complete list of analyzed candidate SNPs and their *P*-values resulting from the univariate analysis are listed in Supplementary Table SI.

To correct for possible confounding variables, a multivariate analysis was also performed. Age, gender, and the three first principal components obtained using EIGENSOFT based on the ancestry-informative markers, were included as covariates in a logistic regression model. After analysis, two HLA alleles and ten SNPs belonging to seven genes showed a trend of association ($P < 0.1$, Supplementary Table I). When proviral load was included as covariate in the logistic regression model, two HLA alleles and twelve SNPs

TABLE I. Descriptive Characteristics of HAM/TSP Patients and Asymptomatic Carriers Infected With HTLV-1

| | Stage 1 | | | Stage 2 | | |
|---|---|---|---|---|---|---|
| | HAM/TSP (N = 56) n (%) | ACs (N = 114) n (%) | *P*-value | HAM/TSP (N = 85) n (%) | ACs (N = 146) n (%) | *P*-value |
| Male gender[a] | 10 (18) | 63 (55) | <0.001 | 15 (18) | 54 (37) | 0.003 |
| Age in years, median (Q1–Q3) | 52.5 (44.8–58.3) | 44 (37–54.5) | 0.001 | 55 (43–65) | 45 (38–53) | <0.001 |
| Andean origin[a] | 42 (75) | 66 (58) | 0.044 | 52 (61) | 79 (54) | 0.29 |
| Proviral load[b], median (Q1–Q3) | 2943 (2057–4418) | 1273 (535–2567) | <0.001 | 2650 (1596–4709) | 871 (226–1971) | <0.001 |

ACs, asymptomatic carriers; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; Q1-Q3, first quartile-third quartile.
[a]Data are presented as absolute numbers and percentages (between brackets).
[b]The proviral load is expressed as the copy number of HTLV-1 per $10^4$ peripheral blood mononuclear cells.

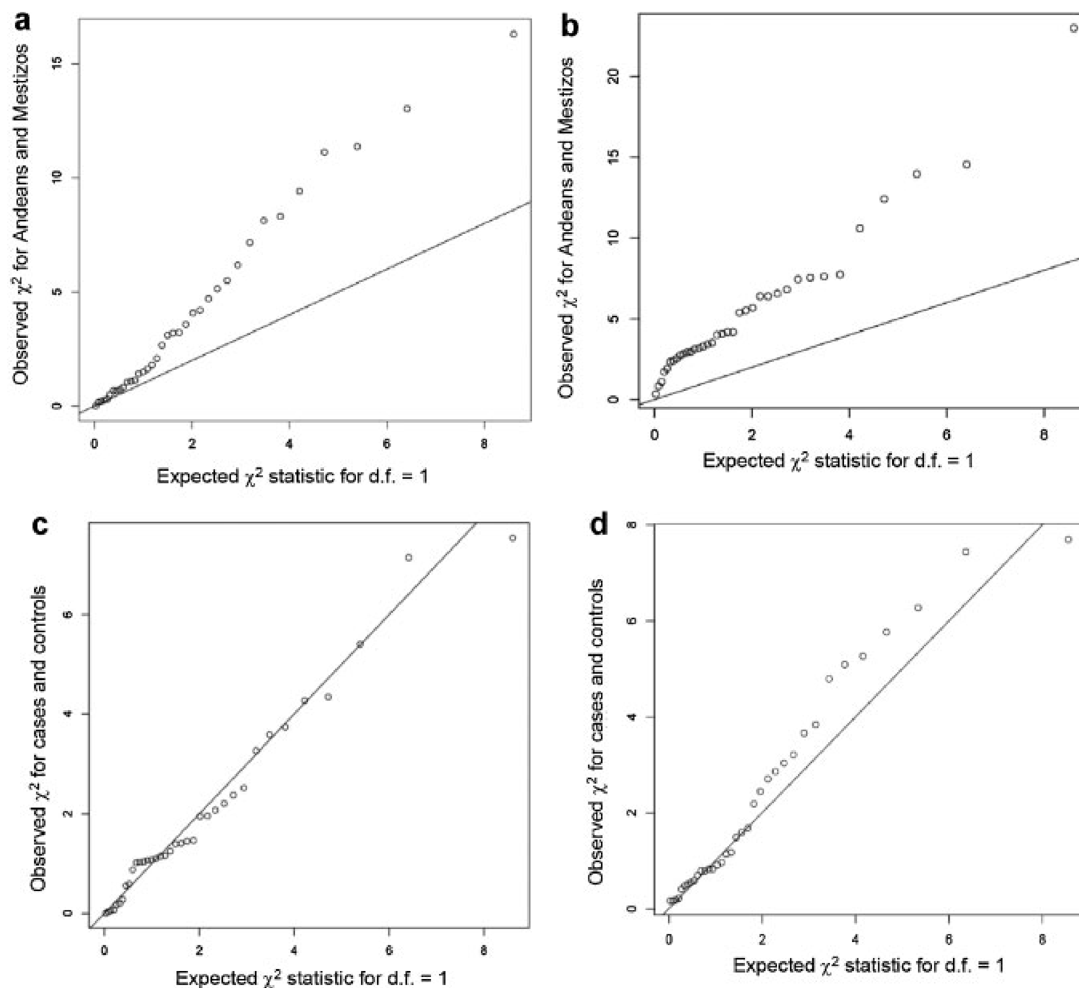


Fig. 1. Q–Q plots of expected $\chi^2$ statistic for one degree of freedom (x-axis) versus observed $\chi^2$ (y-axis) for the comparison of ancestry-informative markers frequencies between Andeans and Mestizos from the first stage (**a**), and the second stage (**b**), and for the comparison between HAM/TSP and asymptomatic carriers from the first stage (**c**) and the second stage (**d**). The significant deviation from the reference line in panels (**a**) and (**b**) suggests that the ancestry-informative markers are able to differentiate between Andeans and Mestizos. The deviation from the reference line in panel (**c**) and panel (**d**) is negligible, suggesting an absence of population stratification between cases and controls. HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis.

belonging to nine genes showed a trend of association ($P < 0.1$; Table II and Supplementary Table I). The trends observed initially for *CTLA4*_49 rs231775 and for *TLR9*_1486 rs187084 (Supplementary Table I), disappeared when proviral load was included into the model, indicating that the effect of these SNPs may be acting through proviral load. Four additional SNPs showed a trend of association when proviral load was included as a covariate, *NKG7*_2 rs3009, *MMP2*_1306, *TLR2*_16934 rs4696480, and *PD1*_19 rs2227982 ($P < 0.1$, Table II and Supplementary Table I). The two HLA alleles and the twelve SNPs found when proviral load was included in the logistic regression model were evaluated in the second stage, as the objective of the study was to identify markers associated with HAM/TSP but not acting through proviral load.

## Second Stage

To determine whether the trends of association observed in the first stage were consistent, the relevant SNPs were analyzed in the samples from the second stage. Three of the twelve evaluated SNPs showed a trend of association after logistic regression analysis including age, gender, proviral load, and the three first principal components as covariates ($P \leq 0.1$; Table II), *NFKBIA* (rs3138053 $P = 0.1$, rs2233406 $P = 0.074$), and *NKG2D* (rs1049174 $P = 0.0823$). *P*-values for the complete data set including both stages were also calculated (Table II). *P*-values $< 0.05$ were observed for SNPs belonging to *NFKBIA* (rs3138053 $P = 0.0279$, rs2233406 $P = 0.0167$) and *NKG2D* (rs1049174 $P = 0.0038$, rs12821887 $P = 0.0248$,

TABLE II. HLA Alleles and SNPs Showing a Trend of Association in the First Stage, and Their *P*-Values Observed in the First Stage, the Second Stage and When Using the Complete Data Set

| HLA/SNP | rs number | *P*-value* (first stage) | *P*-value* (second stage) | *P*-value* (complete data set) |
|---|---|---|---|---|
| HLA.B07 | | 0.0886 | 0.4548 | 0.6712 |
| HLA.Cw07 | | 0.0107 | 0.4068 | 0.2771 |
| IFNg_874 | rs2430561 | 0.0433 | 0.1333 | 0.8281 |
| MMP2_1306 | | 0.0173 | 0.3768 | 0.1954 |
| NFKBIA_2 | rs3138053 | 0.036 | 0.1096 | 0.0279 |
| NFKBIA_3 | rs2233406 | 0.0316 | 0.074 | 0.0167 |
| NKG2D_2 | rs1049174 | 0.0225 | 0.0823 | 0.0038 |
| NKG2D_3 | rs12821887 | 0.0692 | 0.2728 | 0.0248 |
| NKG2D_6 | rs12819494 | 0.0536 | 0.3553 | 0.0235 |
| NKG7_2 | rs3009 | 0.0804 | 0.9873 | 0.2758 |
| PD1_19 | rs2227982 | 0.0473 | 0.818 | 0.3103 |
| RANTES_403 | rs2107538 | 0.0895 | 0.1216 | 0.8923 |
| TGFbeta_509 | rs1800469 | 0.0214 | 0.9867 | 0.1591 |
| TLR2_16934 | rs4696480 | 0.0503 | 0.9134 | 0.3949 |

**P*-values obtained after logistic regression analysis including age, gender, proviral load, and three principal components as covariates. Genes or SNPs displaying a trend of association (*P*-value < 0.10) in the first stage were analyzed in the second stage. *P*-values for the complete data set are also given. The *P*-values are not corrected for multiple testing.

rs12819494 $P = 0.0235$), indicating that the association was in the same direction in the two stages.

### Fine Mapping

To perform a fine-mapping in the associated region, additional tag SNPs were evaluated adjacent to the SNPs initially associated. One SNP for *NFKBIA* and four SNPs for *NKG2D* were analyzed. *P*-values resulting from the analysis of these extra SNPs are given in Table III. This experiment did not result in the detection of stronger association signals or in a further delimitation of the region possibly associated with HAM/TSP. When both sample sets were combined, we found a strong association signal for two SNPs, *NKG2D* (rs11053781, $P = 0.0042$) and *NFKBIA* (rs3138045, $P = 0.0085$) (Table III). However, these associations did not survive a correction for multiple comparisons.

## DISCUSSION

A consensus exists on the fact that host genetic factors are important for the development of HAM/TSP in HTLV-1-infected patients. However, so far, no host genetic factors that are consistent across populations have been established in association with HAM/TSP. A limited set of genetic factors associated with HAM/TSP in other populations was studied; these associations could not be replicated in samples of Peruvian HAM/TSP patients and asymptomatic carriers [Talledo et al., 2010]. This fact and the possibility that other host genetic factors might be involved in the development of HAM/TSP in HTLV-1-infected patients, prompted the analysis of other gene polymorphisms as candidates for susceptibility to HAM/TSP.

Genes associated previously with HAM/TSP in other populations such as *IL6-634* in Japan and Brazil [Nishimura et al., 2002; Gadelha et al., 2008] or *Perforine 418**C/T in Iran [Rafatpanah et al., 2004] were not replicated in the patients included in the current study despite the similar sample sizes. Additionally, no significant differences were found for *IL10-592*, which is in agreement with the results obtained in Brazil [Gadelha et al., 2008] and in contrast to the association found in Japan by Sabouri et al. [2004].

Similar to a previous report by our group, no trends of association between HAM/TSP and *HLA-A*02* or *HLA-Cw*08* were observed in the first stage of our study [Talledo et al., 2010]. In addition, *HLA-A*24* and *HLA-DRB1*01* were not associated with HAM/TSP. *HLA-B*07* and *HLA-Cw*07* showed a trend for association with HAM/TSP in the first stage, but were not replicated in the second stage.

Population stratification can lead to false positive and false negative findings. Therefore, ancestry-informative markers were used to correct for population stratification. The minor deviation from the reference line observed between cases and controls in the Q–Q plots in both stages suggest that population

TABLE III. Fine Mapping Using Additional TagSNPs Within *NFKBIA* and *NKG2D*

| Gen/SNP | rs number | *P*-value* (first stage) | *P*-value* (second stage) | *P*-value* (complete data set) |
|---|---|---|---|---|
| NFKBIA_6 | rs3138045 | 0.0078 | 0.1078 | 0.0085 |
| NKG2D_8 | rs10772271 | 0.2755 | 0.0699 | 0.0299 |
| NKG2D_9 | rs10845123 | 0.1148 | 0.8732 | 0.4702 |
| NKG2D_10 | rs12231827 | 0.0108 | 0.3992 | 0.2756 |
| NKG2D_11 | rs11053781 | 0.0318 | 0.0803 | 0.0042 |

**P*-values obtained after logistic regression analysis including age, gender, proviral load, and three principal components as covariates. *P*-values for the complete data set are also given. The *P*-values are not corrected for multiple testing.

stratification might not be a problem in our study. However, to exclude completely a possible influence of population stratification, the first three principal components of an EIGENSOFT analysis were used as covariates in the logistic regression model.

Although no correction for multiple testing was performed due to the exploratory nature of the study, the *P*-values observed for some SNPs of *NFKBIA* and *NKG2D* suggest that these genes may influence susceptibility to HAM/TSP. These findings need to be confirmed in a larger number of patients infected with HTLV-1 and the importance of these genes in HAM/TSP development across populations should also be evaluated in populations different from the Peruvian.

NKG2D is a C-type lectin-like receptor constitutively expressed on all human natural killer (NK) cells, CD8+ T cells, and γδ T cells. NKG2D engagement stimulates the secretion of cytokines and release of cytolytic granules. The expression of *NKG2D* in CD8+ cells from AC or HAM/TSP patients with low proviral load was high compared to the expression observed in AC or HAM/TSP patients with high proviral load [Vine et al., 2004], suggesting an inverse correlation between *NKG2D* expression and proviral load. Our findings are in agreement with those observed by Vine et al. [2004]. We conclude that NK cells might play an important role in the host defence against HTLV-1 infection.

Tax protein is a potent transcriptional activator of HTLV-1 genes as well as specific cellular genes. It is known, for example, that tax stimulates the NF-kB pathway [Sun, 1999]. NF-kB is sequestered tightly in the cytoplasmic compartment due to its interaction with members of the IkB family of inhibitory proteins such as NFKBIA. The SNPs of *NFKBIA* which showed a trend of association with HAM/TSP susceptibility in this study are localized in the promoter region of the gene. Possibly, these SNPs are involved in the modulation of *NFKBIA* expression. A plausible hypothesis may be that the susceptibility alleles lead to a decreased expression of the inhibitory gene *NFKBIA*, resulting in a decreased inhibition of NF-kB. This may in turn lead to NF-kB activation and an enhanced transcription of a large number of immunorelevant genes as a consequence. As such, *NFKBIA* may play a crucial role in the pathogenesis of HTLV-1 infection. However, functional expression analysis studies are needed to confirm this hypothesis.

The primary objective of the present study was to identify markers associated with HAM/TSP that act independently of proviral load, as these are the most valuable for prognosis. However, proviral load itself has been shown to be under (partial) host genetic control [Jeffery et al., 1999].

For several alleles belonging to different genes we could not detect an association with HAM/TSP. Possibly, the effect sizes of these factors are very small and the sample size in the current study may not have sufficient power to detect the effect of these alleles on HAM/TSP development. Given that no large cohorts, comparable with those used in genetic association studies of malaria, tuberculosis, or HIV, are available to attain sufficient statistical power, it is essential to conduct a study by different centres involved in the evaluation of patients infected with HTLV-1 in several countries to find genes associated significantly with HAM/TSP susceptibility across diverse populations.

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