Journal of Medical Virology 82:460–466 (2010)

Evaluation of Host Genetic and Viral Factors as Surrogate Markers for HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis in Peruvian HTLV-1-Infected Patients

Michael Talledo,[1,2]* Giovanni López,[1] Jeroen R. Huyghe,[2] Kristien Verdonck,[1,3] Vanessa Adaui,[1] Elsa González,[1,4] Iván Best,[1] Daniel Clark,[1,5] Guido Vanham,[3,6] Eduardo Gotuzzo,[1,4] Guy Van Camp,[2] and Lut Van Laer[2]

[1]*Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru*
[2]*Department of Medical Genetics, University of Antwerp, Antwerp, Belgium*
[3]*Virology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium*
[4]*Faculty of Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru*
[5]*Research and Development Laboratories, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru*
[6]*Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium*

Human T-lymphotropic virus 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a complication that affects up to 5% of HTLV-1-infected individuals. Several host genetic and viral factors have been associated with the risk of HAM/TSP. The aim of this study was to evaluate the performance of a prognostic model for HAM/TSP developed in Japan in a Peruvian population of 71 HAM/TSP patients and 94 asymptomatic carriers (ACs). This model included age, proviral load (PVL), the presence of *HLA-A*02* and *HLA-Cw*08* alleles, *SDF-1* +*801*, and *TNF*-α −863 polymorphisms, and viral subgroup. We describe frequencies for the four host genetic markers and demonstrate the presence of the HTLV-1 *tax* B subgroup in Peru. Using cross-validation, we show that the predictive ability of the prognostic model, as characterized by the area under the receiver-operating characteristic curve (AUC), does not differ from a model containing PVL only (both AUC = 0.74). We found some suggestive evidence of a protective effect of the *HLA-A*02* allele but failed to replicate the associations with the other three genetic markers and with viral subgroup. A logistic model containing PVL, age, gender, and *HLA-A*02* provided the best predictive ability in the Peruvian cohort (AUC = 0.79). ***J. Med. Virol. 82:460–466, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: human T-lymphotropic virus 1; paraparesis; tropical spastic; genetic predisposition to disease; Peru

INTRODUCTION

Human T-lymphotropic virus 1 (HTLV-1) infects an estimated 15–20 million people worldwide [de The, 1993]. The infection is particularly frequent in Japan, Melanesia, Central Australia, Iran, Central and West Africa, the Caribbean, and South America [Proietti et al., 2005]. In South America, Peru shows one of the highest prevalence rates, with an estimated 1–2% of the adult population being infected [Sanchez-Palacios et al., 2003; Alarcon et al., 2006].

Although most HTLV-1-infected people remain lifelong asymptomatic, approximately 5–10% will develop severe diseases such as adult T-cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [Verdonck et al., 2007]. ATL is a neoplastic disease of CD4+ T lymphocytes, subclassified in acute, lymphoma, chronic, and

Grant sponsor: Universidad Peruana Cayetano Heredia [internal research award (Fondo Concursable)]; Grant sponsor: Directorate-General for Development Cooperation of the Belgian Government [framework agreement with the Institute of Tropical Medicine of Antwerp; through the Flemish Interuniversity Council (VLIR)].

Lut Van Laer's present address is Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium.

*Correspondence to: Michael Talledo, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Avenida Honorio Delgado 430, Lima 31, Peru.
E-mail: 11936@upch.edu.pe

Accepted 28 August 2009
DOI 10.1002/jmv.21675
Published online in Wiley InterScience (www.interscience.wiley.com)

smoldering types [Tsukasaki et al., 2009]. HAM/TSP manifests itself as a progressive paraparesis of the legs, often accompanied by back pain, bladder disorders, and constipation [Verdonck et al., 2007].

It is not well understood why some HTLV-1-infected people develop disease and others do not. Disease outcome most likely depends on an interaction between viral, immune, and host genetic factors [Bangham, 2003]. In this regard, high proviral load (PVL) and viral *tax* A subgroup have been associated with the presence of HAM/TSP [Kira et al., 1991; Nagai et al., 1998; Furukawa et al., 2000]. In a study in Kagoshima, Japan, the host genetic factors *HLA-A*02* and *HLA-Cw*08* were independently associated with both lower PVL and protection against HAM/TSP [Jeffery et al., 1999, 2000], while the *SDF-1* +801A allele was linked to HAM/TSP protection and the *TNF*-α −863A allele to increased risk of HAM/TSP [Vine et al., 2002]. Using these factors, a logistic regression equation was developed to estimate the odds of HAM/TSP in this Japanese population [Vine et al., 2002]. Among the predictors included in this prognostic model, the association of high PVL with HAM/TSP was confirmed in a Peruvian sample set as well as in other settings [Adaui et al., 2006; Best et al., 2006]. However, PVL shows an extensive overlap between HAM/TSP patients and asymptomatic carriers (ACs) as previously reported [Nagai et al., 1998] and does not fully explain why some people will develop HAM/TSP [Adaui et al., 2006].

Besides PVL, no other viral and host genetic factors have been tested for association with HAM/TSP in Peruvian HTLV-1-infected patients. The present study aimed to assess the usefulness of the aforementioned factors as surrogate markers for HAM/TSP in Peru. We determined the frequency of the *HLA-A*02* and *HLA-Cw*08* alleles, *SDF-1* +801 and *TNF*-α −863 polymorphisms, and viral *tax* A and B subgroups in a Peruvian study population of 71 HAM/TSP patients and 94 ACs. We subsequently investigated the contribution of these factors to the risk of having HAM/TSP in this cohort and evaluated the predictive ability of the prognostic model developed by Vine et al. [2002].

METHODS

Subjects

We included 71 HAM/TSP patients and 94 ACs. They were participants of the HTLV-1 cohort at the "Instituto de Medicina Tropical Alexander von Humboldt" (Lima, Peru). For HAM/TSP diagnosis, internationally accepted clinical criteria were considered [Osame, 1990]. All subjects were genetically unrelated. Origin was defined as Andean (Quechua) if the place of birth of both parents was in the Andean highlands, whereas it was considered Mestizo, if the place of birth of one or both parents was not in the highlands. Patients with known African or Asian ancestry were excluded from the study. Informed consent was obtained from all patients. The study protocol was approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia.

DNA Extraction

For real-time quantitation, the DNA was extracted from peripheral blood mononuclear cells (PBMC) using the QIAamp DNA minikit (Qiagen, Hilden, Germany). For genotyping purposes, DNA was extracted from EDTA-treated blood samples using the genomic prep Blood DNA Isolation Kit (Amersham Biosciences UK Limited, Buckinghamshire, England).

Quantitation of HTLV-1 Provirus

PVL was measured as reported previously [Adaui et al., 2006]. Briefly, we used a SYBR Green-based real-time quantitative PCR on an iCycler Thermal Cycler (Bio-Rad), with human endogenous retrovirus 3 as reference gene. The PVL was expressed as the number of HTLV-1 copies per 10^4 PBMC.

The performance of our PVL assay was further confirmed by an external validation performed by the group of Prof. Anne-Mieke Vandamme (Rega Institute for Medical Research, Katholieke Universiteit Leuven-Belgium). Twenty-two blinded samples were analyzed using Taqman technology, resulting in a Pearson correlation coefficient of 0.87 ($r^2 = 0.87$).

Genotyping Methods

To detect *HLA* types *A*02* and *Cw*08*, we used sequence-specific PCR primers as described elsewhere [Bunce et al., 1995]. *TNF*-α −863 and *SDF-1* +801 (rs1801157) single-nucleotide polymorphism (SNP) genotyping was performed by Kbiosciences (http://www.kbioscience.co.uk).

HTLV-1 *tax* Subgroup Determination

The HTLV-1 *tax* gene subgroup (*tax* A or B) was determined by restriction fragment length polymorphism (RFLP) analysis of PCR products (amplifying the *tax* gene) with the enzyme *Acc*II, as previously reported [Furukawa et al., 2000]. The digested PCR products were analyzed by electrophoresis on 2% agarose gels.

Admixture Proportion

To investigate potential population stratification problems, 36 Ancestry Informative Markers (AIMs), with large differences in allele frequency between Native American and European populations [Mao et al., 2007], were analyzed in 125 samples (53 HAM/TSP and 72 ACs). Admixture proportions for each individual were subsequently estimated using STRUCTURE software [Falush et al., 2003; Pritchard et al., 2000].

Statistical Analysis

Pearson's chi-square or Fisher's exact test (for categorical variables) and Mann–Whitney *U*-test (for

continuous variables) were used when appropriate. Logistic regression analysis was performed to test if the host genetic and viral factors analyzed were significant after correction for age, gender, and PVL. Three predictive logistic regression equations were obtained by using (1) PVL alone, (2) backward elimination on a model containing all variables analyzed in this study, and (3) the factors included in the model developed by Vine et al. [2002]. Assessing the predictive ability of these models by reclassifying the training set to obtain an estimate of the misclassification rate would lead to estimates that are biased upwards due to inevitable overfitting. Therefore, the predictive ability of the different logistic regression models was evaluated by means of 10-fold cross-validation. In this procedure, the data are randomly split into 10 parts. Each part is omitted in turn from the data and the model is fitted to the other nine parts. Each time, the predictions for the omitted part (test set) are obtained using the model fitted to the other nine parts (training set). We subsequently drew receiver-operating characteristic (ROC) curves to compare the performance of the three formulas in discriminating HAM/TSP patients from ACs. The area under the curve (AUC) was used to estimate the accuracy of the prediction. To reduce fluctuations due to chance divisions of the data, the cross-validation procedure was repeated 20 times and results were averaged. R-software was used for all calculations using the packages MASS, SNPassoc [González et al., 2007] and ROCR [Sing et al., 2005].

RESULTS

Characteristics of HTLV-1-Infected Patients

The median age of HAM/TSP patients (52 years; range: 17–78 years) was significantly higher than that of ACs (44 years; range: 13–77 years, $P = 0.004$; Table I). There were more women among HAM/TSP patients (79%) than among ACs (49%, $P < 0.001$; Table I). The median PVL, expressed as the copy number of HTLV-1 proviral DNA per 10^4 PBMC, was significantly higher for HAM/TSP patients (2,602; first quartile (Q1)–third quartile (Q3) = 1,586–3,943) compared to ACs (1,101; Q1–Q3 = 374–2,115, $P < 0.001$; Table I).

Regarding the origin of the participants, 66% of HAM/TSP patients and 53% of ACs were Andean ($P = 0.09$). When analyzing 36 AIMs using STRUCTURE, we observed no differences in admixture proportions between HAM/TSP patients and ACs (Mann–Whitney U-test, $P > 0.05$). In addition, a quantile–quantile plot (data not shown) revealed that the distribution of observed χ^2 values obtained from testing the association between AIMs and HAM/TSP status, did not deviate from the expected distribution under the null hypothesis. In contrast, significant differences in admixture proportions were observed between Mestizos and Andeans (Mann–Withney U-test, $P < 0.001$) and the distribution of observed χ^2 values obtained from testing the association of AIMs and ethnicity deviated from the expected distribution under the null hypothesis. These results indicate that the number of AIMs we selected was appropriate and that confounding due to population stratification was of minor concern in our study.

Comparison of Genotypes and Allele Frequency Between HAM/TSP Patients and ACs

The presence of *HLA-A*02* and *HLA-Cw*08* genotypes has been associated with lower PVL and with a diminished risk to have HAM/TSP in Japan [Jeffery et al., 1999, 2000]. To examine their link with the presence of HAM/TSP in Peruvian individuals, *HLA-A*02* and *HLA-Cw*08* were genotyped in HTLV-1-infected ACs and HAM/TSP patients. Although *HLA-A*02* and *HLA-Cw*08* were more frequent in ACs than HAM/TSP patients, univariate analysis did not reveal any statistically significant difference in the frequency of these markers between ACs and HAM/TSP patients ($P > 0.05$; Table II). The effect of *HLA-A*02* and *HLA-Cw*08* on PVL was tested. Median PVL was higher for *HLA-A*02*-positive patients compared to *HLA-A*02*-negative patients but this difference was not significant (1,717 copies per 10^4 PBMC compared to 1,444 copies per 10^4 PBMC; $P = 0.19$; Table III). In an analysis stratified according to clinical status, no significant differences in PVL were observed among *HLA-A*02*-positive and *HLA-A*02*-negative patients ($P > 0.05$; Table III). No difference in median PVL was observed between *HLA-Cw*08*-positive and *HLA-Cw*08*-negative subjects (1,105 copies per 10^4 PBMC compared to 1,776 copies per 10^4 PBMC, $P = 0.07$; Table III). In relation to the clinical status, no significant differences were observed in PVL among *HLA-Cw*08*-positive and *HLA-Cw*08*-negative patients ($P > 0.05$; Table III).

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

	ACs (n = 94)	HAM/TSP (n = 71)	*P*-value
Male gender[a]	48 (51)	15 (21)	<0.001
Age in years, median	44	52	0.004
Andean origin[a]	50 (53)	47 (66)	0.09
PVL,[b] median (Q1–Q3)	1,101 (374–2,115)	2,602 (1,586–3,943)	<0.001

ACs, asymptomatic carriers; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; PVL, proviral load; 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.

TABLE II. Genotype/Allele Frequencies in Peruvian HTLV-1-Infected ACs and HAM/TSP Patients

Gene	Genotype	ACs (%) (n = 94)	HAM/TSP (%) (n = 71)	*P*-value	*P*-value corrected[a]
*HLA-A*02*		79 (84)	51 (72)	0.06	0.04
*HLA-Cw*08*		17 (18)	10 (14)	0.5	0.83
TNF-α −863	AA	0 (0)	0 (0)	0.85	0.98
	AC	11 (12)	9 (13)		
	CC	83 (88)	62 (87)		
	A	11 (6)	9 (6)	0.85	
	C	177 (94)	133 (94)		
SDF-1 +801	AA	9 (10)	5 (7)	0.64	0.56
	AG	31 (33)	28 (39)		
	GG	54 (57)	38 (54)		
	A	49 (26)	38 (27)	0.89	
	G	139 (74)	104 (73)		
HTLV-1 subgroup	B	0 (0)	2 (3)	0.2	0.99

ACs, asymptomatic carriers; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis.
Data are presented as absolute numbers and percentages (between brackets).
[a]Corrected by age, gender, and proviral load.

TNF-α −863 and *SDF-1* +801 genotypes were determined in ACs and HAM/TSP patients. Both markers were in Hardy–Weinberg equilibrium ($P = 0.4$ and 0.3, respectively), and no differences in the distribution of the genotypes or alleles were observed between ACs and HAM/TSP patients for both markers (Table II). The *TNF*-α −863A allele, previously associated with risk of HAM/TSP, or the *SDF-1* +801AA and *SDF-1* +801GA genotypes, previously associated with protection against HAM/TSP, both in the Japanese cohort [Vine et al., 2002], were not significant in the Peruvian sample set ($P = 0.8$, 0.5, and 0.6, respectively). The hypothesis that the *TNF*-α −863A allele increases the risk to develop HAM/TSP in patients with PVL equal or higher than 3 copies/100 PBMC [Vine et al., 2002] was not confirmed in our study. No significant results were obtained by univariate and multivariate analysis ($P > 0.05$).

tax Viral Subgroup

Furukawa et al. [2000] found that *tax* subgroup A increased the risk of developing HAM/TSP. In our study only two subjects were infected with HTLV-1 *tax* B subgroup; all other subjects (98.8%) were infected with HTLV-1 *tax* A subgroup and no significant differences were established for *tax* viral subgroup between ACs and HAM/TSP ($P = 0.2$; Table II).

Multivariate Logistic Regression Analysis

Logistic regression analysis was performed to determine the effects of *HLA-A*02*, *HLA-Cw*08*, *SDF-1* +801, *TNF*-α −863, and viral subgroup on HAM/TSP while adjusting for the effects of age, gender, and PVL. Only *HLA-A*02* showed an association with a corrected *P*-value <0.05 ($P = 0.04$; Table II). However, this value did not remain significant after correction for multiple testing.

Three different logistic regression models were evaluated using cross-validation (see the Methods Section). The AUC values of the ROC curves were used to compare the performance of these equations in HAM/TSP outcome prediction. No appreciable difference was observed between the equation developed by Vine (AUC = 0.739) using age, PVL, *TNF* −863A/C, *SDF* +801G/A, *HLA-A*02*, *HLA-Cw*08*, tax viral subgroup [Vine et al., 2002], and the equation using PVL alone (AUC = 0.732). The model obtained using backward elimination which includes age, gender, PVL, and *HLA-A*02* (Table IV) showed better discriminative accuracy (AUC = 0.799) compared to either the equation using PVL alone (AUC = 0.732) or the model developed by Vine (AUC = 0.739) [Vine et al., 2002] (Fig. 1).

Although *HLA-A*02* remains included in the model obtained by backward elimination ($P = 0.04$, uncorrected for multiple testing), its inclusion in the model

TABLE III. Proviral Load per *HLA* Type for All HTLV-1-Infected ACs and HAM/TSP Patients and Stratified by Disease Status

	All subjects		ACs		HAM/TSP	
HLA allele	Median PVL (*N*)	*P*-value	Median PVL (*N*)	*P*-value	Median PVL (*N*)	*P*-value
*HLA-A*02* pos	1,717 (130)	0.19	1,182 (79)	0.10	2,884 (51)	0.06
*HLA-A*02* neg	1,444 (35)		611 (15)		1,950 (20)	
*HLA-Cw*08* pos	1,105 (27)	0.07	907 (17)	0.21	2,232 (10)	0.38
*HLA-Cw*08* neg	1,776 (138)		1,248 (77)		2,602 (61)	

ACs, asymptomatic carriers; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis.
Data are presented as median of HTLV-1 copy number per 10^4 PBMCs and number of subjects (between brackets).

TABLE IV. Odds Ratios, Confidence Intervals, and *P*-Values for the Final Logistic Regression Model Obtained by Backward Elimination

Factor	OR	95% CI	*P*-value
Age[a]	1.41	1.12–1.7	0.02
Gender (male)	0.31	0.14–0.67	0.003
PVL[b]	2.79	1.69–4.61	—[c]
*HLA-A*02*	0.37	0.14–0.94	0.04

OR, odds ratio; PVL, proviral load; CI, confidence interval.
[a]OR associated with a 10 years increase in age.
[b]OR associated with an increase of 1,000 HTLV-1 copies/10^4 PBMC.
[c]*P*-values for PVL and PVL^2 were 0.0001 and 0.011, respectively.

does not improve the predictive accuracy of the model noticeably (AUC = 0.799 compared to AUC = 0.794 for the model without *HLA-A*02*).

DISCUSSION

In this study, we evaluated the usefulness of *HLA-A*02*, *HLA-Cw*08*, *TNF-α* −863, *SDF-1* +801, and *tax* viral subgroup as surrogate markers for the presence of HAM/TSP in Peruvian HTLV-1-infected subjects. These factors have previously been implicated in susceptibility to HAM/TSP in Kagoshima, Japan and a prognostic model using these factors has been proposed by Vine et al. [2002]. Until now, the association between host genetic or viral factors other than PVL and HAM/TSP in a Peruvian HTLV-1-infected population had not been investigated.

It has been proposed that *HLA-A*02* presents an immunodominant peptide (Tax 11–19) from HTLV-1 to CD8+ cells, thereby improving the efficiency of CD8+ to kill HTLV-1-infected cells [Sakai et al., 2001]. The possession of either *HLA-A*02* or *HLA-Cw*08* was associated with a reduction of both HTLV-1 PVL and the risk of developing HAM/TSP in a study from Kagoshima Japan [Jeffery et al., 1999, 2000]. The phenotype frequencies for *HLA-A*02* (78.8%) and *HLA-Cw*08* (16.4%) obtained in this study were similar to the phenotype frequencies reported previously in subjects originating from Arequipa, Peru (*HLA-A*02* = 79.1% and *HLA-Cw*08* = 15.3%) [de Pablo et al., 2000]. The *HLA-A*02* type is more frequent in Peruvian compared to Iranian (31.1%) or Japanese (37.9%) [Sabouri et al., 2005] HTLV-1-infected subjects. In our Peruvian study population, univariate analysis did not show any significant difference in either PVL or risk of HAM/TSP between *HLA-A*02* or *HLA-Cw*08*

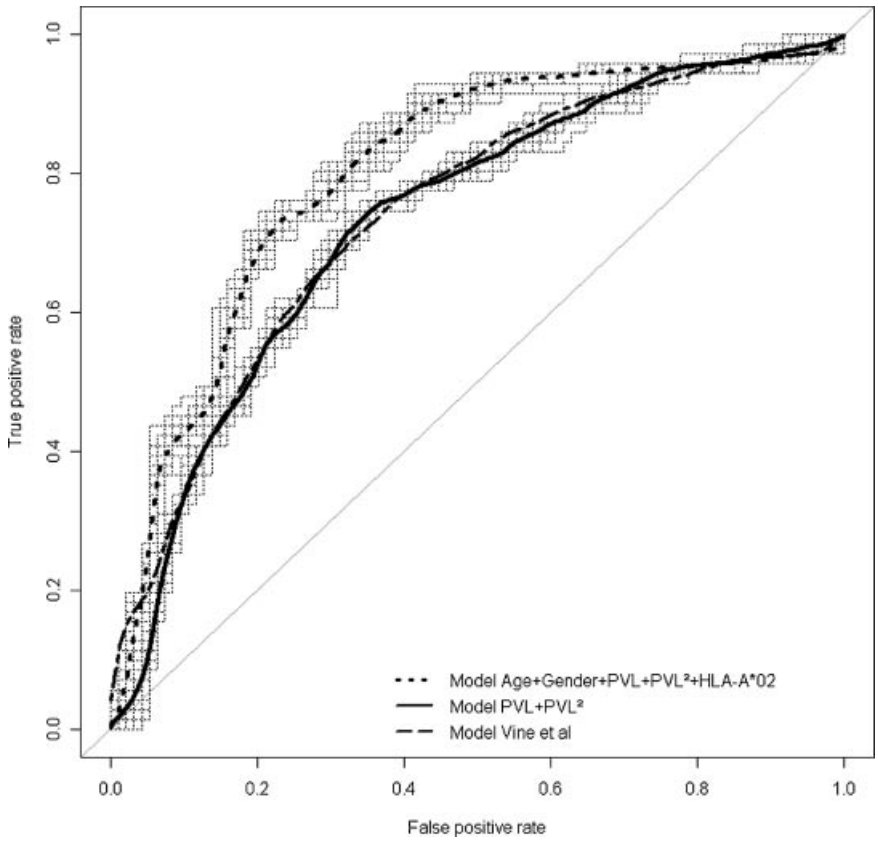

Fig. 1. Receiver-operating characteristic (ROC) curve of the model containing proviral load, the model developed by Vine et al. and the model described in this study. The model obtained in this study using backward elimination includes age + gender + PVL + PVL^2 + HLAA2. The PVL model includes PVL + PVL^2. The model by Vine et al. includes age + age^2 + PVL + PVL^2 + TNF863.A + TNF863.A*PVL + TNF863.A*PVL^2 + SDF.GA + SDF.AA + HLAA2 + HLACw08 + *tax* viral subgroup. ROC curves were obtained using 10-fold cross-validation (CV). Bold lines are the averages over 20 CV runs.

positives and negatives. *HLA-A*02*-positive patients showed a trend towards an increased PVL. The same trend was previously described in a study from Iran [Sabouri et al., 2005]. Intriguingly, at the same time we obtained some suggestive evidence from our multivariate analysis that for a fixed PVL, *HLA-A*02* confers protection to HAM/TSP (OR = 0.37; 95% confidence interval (CI) = [0.14–0.94]; *P*-value = 0.04). The lack of association of the *HLA-A*02* type with low PVL and the only marginal evidence for HAM/TSP protection in the Peruvian cohort is consistent with the hypothesis that *HLA-A*02* is associated with HAM/TSP protection only in *tax* subgroup-B-infected subjects [Sabouri et al., 2005]. Our results are in concordance with a previous report from Masshad, Iran [Sabouri et al., 2005], except for the suggestive association with HAM/TSP protection shown by *HLA-A*02* after correction for age, gender, and PVL.

TNF-α production is of clinical importance because of its inflammatory effects and its possible contribution to tissue damage of the central nervous system [Umehara et al., 1994]. Several studies reported contradictory results concerning TNF-α production and *TNF*-α −863 alleles [Higuchi et al., 1998; Skoog et al., 1999; Udalova et al., 2000]. The presence of the *TNF*-α −863A allele has been associated with HTLV-1-associated uveitis [Seki et al., 1999] and with increased odds of developing HAM/TSP in HTLV-1-infected subjects with a high PVL (≥3 proviral copies/100 PBMC) [Vine et al., 2002]. However, this interaction was not observed in our study. Our result is in agreement with a study from our group [Best et al., 2006] and a study from Brazil [Santos et al., 2004], in which no significant differences were found in the spontaneous production of TNF-α between HAM/TSP patients and ACs. The association of *SDF-1* +801 with HAM/TSP risk among HTLV-1-infected subjects in Kagoshima [Vine et al., 2002] was also not replicated in the Peruvian cohort.

The discrepancies between our and previous reports [Jeffery et al., 1999, 2000; Furukawa et al., 2000; Nose et al., 2006] may be due to the fact that the host genetic factors studied might not be involved in the etiology of HAM/TSP across all populations. In this aspect, it is worthwhile noting that our study was in agreement with an Iranian study [Sabouri et al., 2005]. However, another plausible explanation for the inconsistencies between our study and the study by Vine et al. [2002] may be that in the latter study no corrections for multiple comparisons were applied and that because of this reason some of the reported associations may in fact be false positives. Indeed, also Yashiki et al. [2001] failed to replicate the associations between HLA-A*02 and HLA-Cw*08 alleles and HAM/TSP in an independent group of patients from Kagoshima, Japan. When association results cannot be replicated, this might indicate that these variants do not influence disease susceptibility [Hattersley and McCarthy, 2005].

Concerning the *tax* viral subgroup, almost all patients from the Peruvian cohort were infected with HTLV-1 *tax* subgroup A, corresponding to the cosmopolitan subtype A as reported by Van Dooren et al. [1999]. The *tax* subgroup B was observed in only 1.2% of the patients and we did not detect significant differences between the *tax* viral subgroup of HAM/TSP patients and ACs. An identical result was reported previously for Iranian HTLV-1-infected patients [Sabouri et al., 2005].

Although we recognize that our study is limited in sample size, it does not appear to be underpowered when testing for associations between *HLA-A*02*, *HLA-Cw*08*, *TNF*-α −863A, *SDF-1* +801 polymorphisms, *tax* viral subgroup, and HAM/TSP. It is unlikely that our negative results are due to type II errors since the sample size used in our study was larger than the sample size used in the first stage of the discovery of the association between *HLA-A*02* and protection against HAM/TSP in the Japanese setting (50 HAM/TSP and 56 ACs) [Jeffery et al., 1999]. In fact, their replication set from London consisted of only 15 HAM/TSP patients and 14 ACs.

We have shown that, in the Peruvian cohort, the prognostic model developed by Vine et al. [2002] has the same discriminative accuracy to distinguish between HAM/TSP patients and ACs as a model containing only PVL. In addition, we noted that Vine et al. [2002] evaluated their prognostic model using their training set, a procedure that inevitably results in an over-optimistic estimation of the predictive ability due to overfitting. Furthermore, our data did not provide sufficient evidence for associations between *HLA-A*02*, *HLA-Cw*08*, *TNF*-α −863, *SDF-1* +801, and *tax* viral subgroup to HAM/TSP susceptibility in the Peruvian setting.

In conclusion, the differences observed in HAM/TSP susceptibility between Peruvian and Japanese HTLV-1-infected subjects may be due to possible spurious association in the Japanese population, genetic heterogeneity, gene–gene and gene–environment interactions, and diverging HTLV-1 variants between these populations as important determinants of HAM/TSP. Different factors from the ones evaluated in this study may determine risk of or protection to HAM/TSP disease in Peruvian HTLV-1-infected subjects. As such, efforts towards detecting universal host genetic and viral factors associated with HAM/TSP different from PVL need to be made. Such efforts may ultimately lead to a better understanding of the pathological mechanisms as well as to predictive testing for HAM/TSP.

ACKNOWLEDGMENTS

We thank the patients and the staff of the HTLV-1 study group of the Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia for their contributions and Dr. Anne-Mieke Vandamme for the external validation of the proviral load assay.

REFERENCES

Adaui V, Verdonck K, Best I, González E, Tipismana M, Arévalo J, Vanham G, Campos M, Zimic M, Gotuzzo E. 2006. SYBR

Green-based quantitation of human T-lymphotropic virus type 1 proviral load in Peruvian patients with neurological disease and asymptomatic carriers: Influence of clinical status, sex, and familial relatedness. J Neurovirol 12:456–465.

Alarcon J, Friedman HB, Montano SM, Zunt JR, Holmes KK, Quinnan GV, Jr. 2006. High endemicity of human T-cell lymphotropic virus type 1 among pregnant women in Peru. J Acquir Immune Defic Syndr 42:604–609.

Bangham CR. 2003. The immune control and cell-to-cell spread of human T-lymphotropic virus type 1. J Gen Virol 84:3177–3189.

Best I, Adaui V, Verdonck K, Gonzalez E, Tipismana M, Clark D, Gotuzzo E, Vanham G. 2006. Proviral load and immune markers associated with human T-lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Peru. Clin Exp Immunol 146:226–233.

Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, Welsh KI. 1995. Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 46:355–367.

de Pablo R, Beraun Y, Nieto A, Calzada JE, Rementería MC, Sanz L, López-Nevot MA, Martín J. 2000. HLA class I and class II allele distribution in the Peruvian population. Tissue Antigens 56:507–514.

de The G. 1993. The history of HTLV-I. Leuk Res 17.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Furukawa Y, Yamashita M, Usuku K, Izumo S, Nakagawa M, Osame M. 2000. Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type I in the tax gene and their association with different risks for HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 182.

González JR, Armengol L, Solé X, Guinó E, Mercader JM, Estivill X, Moreno V. 2007. SNPassoc: An R package to perform whole genome association studies. Bioinformatics 23:644–645.

Hattersley AT, McCarthy M. 2005. What makes a good genetic association study? Lancet 366:1315–1323.

Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, Itoh K. 1998. Polymorphism of the 59-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 51:605–612.

Jeffery KJ, Usuku K, Hall SE, Matsumoto W, Taylor GP, Procter J, Bunce M, Ogg GS, Welsh KI, et al. 1999. HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. Proc Natl Acad Sci USA 96: 3848–3853.

Jeffery KJ, Siddiqui A, Bunce M, Lloyd AL, Vine AM, Witkover AD, Izumo S, Usuku K, Welsh KI, et al. 2000. The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. J Immunol 165:7278–7284.

Kira J, Koyanagi Y, Yamada T, Itoyama Y, Goto I, Yamamoto N, Sasaki H, Sakaki Y. 1991. Increased HTLV-I proviral DNA in HTLV-I-associated myelopathy: A quantitative polymerase chain reaction study. Ann Neurol 29:194–201.

Mao X, Bigham A, Mei R, Gutierrez G, Weiss K, Brutsaert T, Leon-Velarde F, Moore L, Vargas E, McKeigue P, Shriver M, Parra E. 2007. A genomewide admixture mapping panel for Hispanic/Latino populations. Am J Hum Genet 80:1171–1178.

Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Moritoyo T, Hashiguchi S, Ichinose M, Bangham CR, et al. 1998. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: High proviral load strongly predisposes to HAM/TSP. J Neurovirol 4:586–593.

Nose H, Saito M, Usuku K, Sabouri AH, Matsuzaki T, Kubota R, Eiraku N, Furukawa Y, Izumo S, et al. 2006. Clinical symptoms and the odds of human T-cell lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in healthy virus carriers: Application of best-fit logistic regression equation based on host genotype, age, and provirus load. J Neurovirol 12:171–177.

Osame M. 1990. Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In: Blattner WA, editor. Human retrovirology. New York: Raven Press. pp 191–197.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Proietti F, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL. 2005. Global epidemiology of HTLV-I infection and associated diseases. Oncogene 24:6058–6068.

Sabouri AH, Saito M, Usuku K, Bajestan SN, Mahmoudi M, Forughipour M, Sabouri Z, Abbaspour Z, Goharjoo ME, et al. 2005. Differences in viral and host genetic risk factors for development of human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis between Iranian and Japanese HTLV-1-infected individuals. J Gen Virol 86:773–781.

Sakai JA, Nagai M, Brennan MB, Mora CA, Jacobson S. 2001. In vitro spontaneous lymphoproliferation in patients with human T-cell lymphotropic virus type I-associated neurologic disease: Predominant expansion of CD8+ T cells. Blood 98:1506–1511.

Sanchez-Palacios C, Gotuzzo E, Vandamme AM, Maldonado Y. 2003. Seroprevalence and risk factors for human T-cell lymphotropic virus (HTLV-I) infection among ethnically and geographically diverse Peruvian women. Int J Infect Dis 7:132–137.

Santos SB, Porto A, Muniz AL, de Jesus AR, Magalhães E, Melo A, Dutra WO, Gollob KJ, Carvalho EM. 2004. Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers. BMC Infect Dis 2:4–7.

Seki N, Yamaguchi K, Yamada A, Kamizono S, Sugita S, Taguchi C, Matsuoka M, Matsumoto H, Nishizaka S, et al. 1999. Polymorphism of the 5'-flanking region of the tumor necrosis factor (TNF)-alpha gene and susceptibility to human T-cell lymphotropic virus type I (HTLV-I) uveitis. J Infect Dis 180:880–883.

Sing T, Sander O, Beerenwinkel N, Lengauer T. 2005. ROCR: Visualizing classifier performance in R. Bioinformatics 21:3940–3941.

Skoog T, van't Hooft FM, Kallin B, Jovinge S, Boquist S, Nilsson J, Eriksson P, Hamsten A. 1999. A common functional polymorphism (C3A substitution at position −863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. Hum Mol Genet 8:1443–1449.

Tsukasaki K, Hermine O, Bazarbachi A, Ratner L, Ramos JC, Harrington W, Jr., O'Mahony D, Janik JE, Bittencourt AL, et al. 2009. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: A proposal from an international consensus meeting. J Clin Oncol 27:453–459.

Udalova IA, Richardson A, Denys A, Smith C, Ackerman H, Foxwell B, Kwiatkowski D. 2000. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promoter region. Mol Cell Biol 20:9113–9119.

Umehara F, Izumo S, Ronquillo AT, Matsumuro K, Sato E, Osame M. 1994. Cytokine expression in the spinal cord lesions in HTLV-I-associated myelopathy. J Neuropathol Exp Neurol 53:72–77.

Van Dooren S, Gotuzzo E, Salemi M, Watts D, Audenaert E, Duwe S, Ellerbrok H, Grassmann R, Hagelberg E, et al. 1999. Evidence for a post-Columbian introduction of human T-cell lymphotropic virus [type I] [corrected] in Latin America. J Gen Virol 80:2695–2708.

Verdonck K, Gonzalez E, Van Dooren S, Vandamme AM, Vanham G, Gotuzzo E. 2007. Human T-lymphotropic virus 1: Recent knowledge about an ancient infection. Lancet Infect Dis 7:266–281.

Vine AM, Witkover A, Lloyd AL, Jeffery KJ, Siddiqui A, Marshall SE, Bunce M, Eiraku N, Izumo S, et al. 2002. Polygenic control of human T lymphotropic virus type I (HTLV-I) provirus load and the risk of HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 186:932–939.

Yashiki S, Fujiyoshi T, Arima N, Osame M, Yoshinaga M, Nagata Y, Tara M, Nomura K, Utsunomiya A, et al. 2001. HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801 alleles predispose to adult T cell leukemia: The limited recognition of HTLV type 1 tax peptide anchor motifs and epitopes to generate anti-HTLV type 1 tax CD8(+) cytotoxic T lymphocytes. AIDS Res Hum Retroviruses 17:1047–1061.