Polygenic Control of Human T Lymphotropic Virus Type I (HTLV-I) Provirus Load and the Risk of HTLV-I–Associated Myelopathy/Tropical Spastic Paraparesis

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Human T lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) is one outcome of infection with HTLV-I. A population association study of 229 patients with HAM/TSP and 202 healthy carriers of HTLV-I in southern Japan showed that this outcome of HTLV-I infection and the HTLV-I provirus load are under polygenic control. Of 58 polymorphic sites studied in 39 non-HLA candidate gene loci, 3 new host genetic factors that influenced the risk of HAM/TSP or the provirus load of HTLV-I were identified. The promoter *TNF −863A* allele predisposed to HAM/TSP, whereas *SDF-1 +801A* 3′UTR, and *IL-15 191C* alleles conferred protection. Knowledge of HTLV-I–infected individuals' ages, sex, provirus load, HTLV-I subgroup, and genotypes at the loci *HLA-A, HLA-C, SDF-1,* and *TNF-α* allowed for the correct identification of 88% of cases of HAM/TSP in this Japanese cohort.

Human T lymphotropic virus type I (HTLV-I), a member of the Oncovirus family, is the etiological agent of 2 diverse diseases: the neurological disorder HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1, 2] and adult T cell leukemia/lymphoma [3]. The outcome of HTLV-I infection depends on both host genetic and viral factors. At best, an individual may exhibit a life-long asymptomatic infection; at worst, either an inflammatory disease or rapidly fatal leukemia may ensue. Here, we provide evidence for the involvement of host genetic and viral subgroup factors [4] in HAM/TSP in a region of southern Japan (Kagoshima) where HTLV-I is endemic, using an association study and appropriate statistical analyses to predict disease outcome and provirus load.

Although different virus strains (denoted HTLV-I subgroups) can influence the risk of developing HAM/TSP [4], the impact of HTLV-I viral sequence variation in determining the risk of developing HAM/TSP in Kagoshima is limited, and no sequence variant of HTLV-I is uniquely associated with the disease [5]. These observations strongly suggest that viral factors alone are not sufficient to predict whether an HTLV-I–infected individual will develop HAM/TSP. We therefore hypothesized that host genetic factors were also important in determining the outcome of HTLV-I infection.

The cytotoxic T lymphocyte (CTL) response to HTLV-I in patients with HAM/TSP is very vigorous, usually chronically activated, and predominantly directed at the viral transactivator protein Tax. Healthy carriers (HCs) of HTLV-I also demonstrate a strong anti-Tax CTL response to the virus that differs little from that seen in patients with HAM/TSP in terms of its antigen specificity or lytic activity [6–8]. Although the provirus load of HTLV-I is typically 10–100-fold greater in patients with HAM/TSP than in HCs, the frequency of HTLV-I–specific CTLs in patients with HAM/TSP, compared with that seen in HCs, is more variable, with reports of 1–4-fold [8, 9] and 40–280-fold [10] higher HTLV-I–specific precursor CTLs in patients with HAM/TSP. Further differences between patients with HAM/TSP and HCs become apparent when the selection pressure exerted on the Tax protein by the CTLs is taken into account; this selection was more stringent in HCs than in patients with HAM/TSP [11, 12]. We have therefore suggested that CTLs play a pivotal role in limiting HTLV-I replication in vivo [13, 14] and that the CTL response is more effective in HCs, who maintain a lower median provirus load [15], than in patients with HAM/TSP [16]. This hypothesis can be extended to suggest that the disparity between individuals in the outcome of HTLV-I infection is due to genetically determined differences in the efficiency with which anti-Tax CTL limit HTLV-I rep-

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Informed written consent was obtained from patients and healthy carriers, and human experimentation guidelines of Kagoshima University were followed in the conduct of clinical research.

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Table 1. Single-nucleotide polymorphisms (SNPs) genotyped according to staged experimental design.

Stage of study	Loci	SNPs	Outcome
Total no. of genes studied	39	58	
SNP allele frequency			
In 20 patients with HAM/TSP and in 20 HCs, <0.1	14	22	Eliminated from study
In 20 patients with HAM/TSP and in 20 HCs, >0.1	25	36	Retained
Retained SNPs showing no significant effect[a]			
In 100 patients with HAM/TSP and in 100 HCs	18	27	Eliminated from study
Retained SNPs showing a significant effect[a]			
In 100 patients with HAM/TSP and in 100 HCs	7	9[b]	Entire cohort (229 patients with HAM/TSP and 202 HCs) genotyped[c]

NOTE. HAM/TSP, human T cell leukemia virus type I–associated myelopathy/tropical spastic paraparesis; HCs, healthy carriers.

[a] $P < .05$ for HAM/TSP risk or provirus load.

[b] The 9 SNPs genotyped in the entire cohort were from the following genes: *ICAM-1* (1 SNP), *IFN-α17* (1 SNP), *TNF-α* (3 SNPs), *LMP7* (1 SNP), *IL–2* (1 SNP), *SDF-1β* 3′UTR (1 SNP), and *IL-15* (1 SNP).

[c] Single-factor and multifactor analyses were performed.

lication. Here, we consider the question of which host factors and which factors in the HTLV-I genotype dictate who remains healthy and who develops HAM/TSP.

Until recently, the only factors known to be associated with a higher risk of HAM/TSP were having a high provirus load or being female. We hypothesized that polymorphisms in host genes involved in the immune response to the HTLV-I virus affect both the provirus load and the risk of developing HAM/TSP. With the completion of the Human Genome Project, thousands of single-nucleotide polymorphisms (SNPs) have been identified and are increasingly used as markers for polygenic disease loci in natural populations [17]. Recent data from our laboratory have demonstrated the importance of host HLA genotype in determining the outcome of HTLV-I infection; specifically, *HLA-A*02* and *HLA-Cw*08* each independently halved the odds of developing HAM/TSP in residents of Kyushu, Japan [9, 18].

We now report the results of a candidate gene study comprising 58 polymorphic sites from 39 non-HLA gene loci in the same Japanese HTLV-I–infected population. We chose a candidate gene approach for 3 reasons. First, we had strong reasons to select certain genes as candidates on the basis of prior published information on the immune response to HTLV-I, the expression of cytokines by HTLV-I–infected cells, and the pathological features of the myelopathy. Second, the candidate gene approach has already been shown to be successful in identifying markers in other infectious diseases [19, 20]. Third, the frequency of multiplex families with HAM/TSP in the study population was too low to permit a family-based study, and the high mean age at the onset of HAM/TSP made transmission/disequilibrium studies impracticable.

We show here that a promoter polymorphism in the cytokine gene *TNF-α −863A* increased an individual's risk of developing HAM/TSP. Furthermore, this polymorphism exerted its effect selectively in individuals with a high provirus load of HTLV-I. We also provide evidence for a role of polymorphisms in *SDF-1* and *IL-15* in determining the risk of HAM/TSP. Finally, we show that the protective effects associated with 2 class I HLA alleles *(HLA-A*02* and *Cw*08)* were significant predictors of HTLV-I provirus load in HCs but not in patients with HAM/TSP. We conclude that HCs maintain effective immune control of HTLV-I replication but that this immune control is ineffective in patients with HAM/TSP.

Materials and Methods

Selection of candidate genes. A list of immune response candidate genes was categorized into 6 main groups. The candidate genes were prioritized according to existing evidence for an associated difference in protein function or evidence of association with another infectious disease. These 6 groups were as follows:

1. HLA class I and class II [9, 18];
2. Cytokines and their receptors, tumor necrosis factor (TNF)–α in particular, because of its reported association with HTLV-I uveitis [21];
3. Cell adhesion molecules (e.g., intercellular adhesion molecule–1);
4. Other factors involved in the immune response (e.g., chemokines and factors involved in antigen presentation and processing);
5. Factors involved in lymphocyte penetration into tissue (e.g., matrix metalloproteinases); and
6. Factors involved in Tax-induced T cell activation (e.g., NF-κB).

Study population. We used a standard population association case-control study. The study cohort consisted of 229 patients with HAM/TSP receiving care at the Third Department of Internal Medicine, Kagoshima University (Kagoshima, Japan) and 202 HCs of HTLV-I randomly selected from the same geographical location, as described elsewhere [9, 18]. All individuals screened were of Japanese descent and resided within Kagoshima prefecture, Kyushu, Japan. The diagnosis of HAM/TSP was made in accordance with World Health Organization criteria [22]. Genomic DNA was extracted from peripheral blood mononuclear cells using a QIAamp blood kit (Qiagen), according to the manufacturer's instructions, prior to genotyping.

Genotyping methods for non-HLA candidate genes. Initially, for each candidate gene, we sequenced 50 ng of genomic DNA across each SNP site from each of 20 patients with HAM/TSP and 20 HCs, randomly chosen from the study cohort, using d-

Table 2. Single-factor analysis of single-nucleotide polymorphisms (SNPs) tested in human T cell leukemia virus type I (HTLV-I) immunogenetic study in Kagoshima, Japan.

	Allele frequency				Genotype frequency		
Gene, locus, no. typed	Patients with HAM/TSP	HCs	P[a]	Genotype	Patients with HAM/TSP	HCs	P[a]
IFN-α17	T 0.39	T 0.37	.5803	TT	0.18	0.13	.2793
T+1453C	C 0.61	C 0.63		TC	0.41	0.47	
In 203 patients with HAM/TSP and in 185 HCs				CC	0.41	0.40	
LMP7	C 0.89	C 0.84	**.0286**[b]	CC	0.80	0.72	.0560
C+145A	A 0.11	A 0.16		CA	0.18	0.25	
In 218 patients with HAM/TSP and in 191 HCs				AA	0.02	0.03	
IL-2	G 0.50	G 0.46	.3309	GG	0.25	0.18	.1886
G+166T	T 0.50	T 0.54		GT	0.50	0.57	
In 226 patients with HAM/TSP and in 197 HCs				TT	0.25	0.25	
ICAM-1	A 0.94	A 0.93	.5576	AA	0.89	0.86	.5270
Kilifi	T 0.06	T 0.07		AT	0.10	0.13	
In 220 patients with HAM/TSP and in 196 HCs				TT	0.01	0.01	
TNF-α	T 0.83	T 0.86	.2372	TT	0.69	0.75	.3785
T –1031C	C 0.17	C 0.14		TC	0.28	0.23	
In 221 patients with HAM/TSP and in 199 HCs				CC	0.03	0.02	
TNF-α	C 0.84	C 0.87	.2407	CC	0.70	0.76	.3785
C –863A	A 0.16	A 0.13		CA	0.28	0.21	
In 227 patients with HAM/TSP and in 200 HCs				AA	0.02	0.02	
TNF-α	C 0.78	C 0.81	.4154	CC	0.60	0.68	**.0296**[c] (χ^2 = 7.39)
C−857T	T 0.22	T 0.19		CT	0.37	0.26	
In 227 patients with HAM/TSP and in 199 HCs				TT	0.03	0.06	
IL-15	T 0.94	T 0.93	.5792	TT	0.89	0.85	**.0323**[c] (χ^2 = 6.87)
T+191C	C 0.06	C 0.07		TC	0.09	0.15	
In 227 patients with HAM/TSP and in 197 HCs				CC	0.02	0.00	
SDF-1β	G 0.69	G 0.59	**.0021** (χ^2 = 9.49)	GG	0.48	0.38	**.0031**[c] (χ^2= 11.54)
G+801A	A 0.31	A 0.41		GA	0.43	0.42	
In 229 patients with HAM/TSP and in 202 HCs				AA	0.09	0.20	

NOTE. Boldface indicates significant P values at the 5% level. All genes tested were in Hardy Weinberg Equilibrium. HAM/TSP, HTLV-I–associated myelopathy/tropical spastic paraparesis; HC, healthy carrier; ICAM, intracellular adhesion molecule; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

[a] χ^2 test.

[b] LMP7 C145A was shown by logistic regression analysis not to be a significant independent predictor of HAM/TSP disease risk or to affect provirus load after the other factors had been taken into account.

[c] Uncorrected P values: these values are no longer significant after correction for multiple comparisons. See Results for a discussion of each individual polymorphism.

Rhodamine terminator chemistry (ABI 377; Applied Biosystems) to assess whether the polymorphism under test was present at an informative frequency in this population. When the frequency of the rarer SNP allele was ⩾0.1, we went on to genotype 100 patients with HAM/TSP and 100 HCs, either by DNA sequencing or by polymerase chain reaction (PCR) with allele-specific primers (table 1). Oligonucleotide primer sequences designed for each SNP and the protocol used for each assay are available from the authors on request. When there was a significant association between genotype or allele frequency (AF) and the risk of developing HAM/TSP, we completed the genotyping on the remaining 129 patients with HAM/TSP and 102 HCs. Occasionally, the genomic DNA sample available for an individual failed to amplify some SNP regions being tested. This reduces the sample size in certain situations (see tables 2, 3, 4, 5, and 6), depending on the genetic factors under consideration. The total numbers of individuals typed for each SNP are presented in the first column of table 2.

HLA typing. The results of the molecular genotyping of class I and class II HLA loci in this cohort have been reported elsewhere [9, 18].

HTLV-I genotyping. Two subgroups (A and B) of the cosmopolitan genotype of HTLV-I are present in Kagoshima, Japan [4]. Molecular typing of the HTLV-I *tax* gene was done as described elsewhere [4] to identify the HTLV-I subgroup present in each infected subject.

Provirus load measurement. The provirus load in peripheral blood mononuclear cells (PBMC) was measured using real-time PCR with an ABI 7700 sequence detection system (Applied Biosystems). All samples were amplified and analyzed in triplicate, as described elsewhere [15].

Statistical methods. The χ^2 and Fisher's exact tests (Instat GraphPad Software) were used to examine associations between HAM/TSP and single gene factors [23]. General linear model (GLM) analysis [24], which is a general form of multiple regression (of which ordinary multiple regression, analysis of variance, and analysis of covariance [ANCOVA] are familiar examples [23, 24]), was used to identify which factors were predictors of provirus load, either in patients with HAM/TSP alone, HCs alone, or all subjects in the study. We analyzed the effects of genetic factors and, in some cases, age and provirus load as well. GLM analysis (Minitab data analysis software; Minitab) also allows for calculation of the fraction of the observed variation in provirus load that can be attributed to each of the factors under consideration and provides a best-fit equation that can be used to predict provirus load in terms of these factors. Worked examples are given in the notes to tables 4, 5, and 6.

Logistic regression analysis (Minitab data analysis software;

Table 3. Best-fit logistic regression equation for the risk of human T lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) in the Kagoshima HTLV-I infected cohort ($n = 402$).

Factor, condition	ln (odds of HAM/TSP)[a]	Odds ratio *(P)*
Constant	−1.716	
Age	−(0.145 × age) + (0.003 × age^2)	—[b]
Provirus load	+ (0.460 × load) + (0.487 × load^2)	—[b]
TNF −863A$^+$	+3.057 − (4.616 × load) + (1.476 × load^2)	—[b]
SDF-1 +801GA	−0.808	0.45 (.042)
SDF-1 +801AA	−1.689	0.18 (.003)
*HLA-A*02$^+$*	−0.638	0.53 (.043)[c]
*HLA-Cw*08$^+$*	−0.894	0.41 (.046)[c]
HTLV-I subgroup B	−1.587	0.20 (.017)

NOTE. Worked example: an HTLV-I–infected individual in Kagoshima, 60 years old, with a $\log_{10}$ (provirus load) of 2.5 with the genotype *TNF −863A$^+$, SDF-1 +801AA, HLA-A*02$^-$, HLA-Cw*08$^+$*, HTLV-I subgroup B has a predicted ln odds of HAM/TSP of $-1.716-(0.145\times 60)+(0.003\times 60^2)+(0.46\times 2.5)+(0.487\times 2.5^2)+3.057-(4.616\times 2.5)+(1.476\times 2.5^2)-1.689-0.894-1.587=-1.864$. That is, this HTLV-I–infected individual's odds of developing HAM/TSP = exp(−1.864) = 0.155. Notice, as in this example, that for *TNF-863A$^+$* individuals, the table specifies that one must account for 2 pairs of terms involving provirus load.

[a] The natural logarithm of an individual's odds of HAM/TSP in the cohort is calculated as the sum of the components in the central column, contingent on the factors indicated in the left-hand column. Load denotes $\log_{10}$ (proviral copy no.)/10^4 peripheral blood mononuclear cells; age is given in years. HTLV-I subgroups are either A or B [4]. The odds ratio (OR) of developing HAM/TSP conferred by each respective genotype is shown in the right-hand column. This equation correctly classifies 88.0% of patients with HAM/TSP in this Japanese study cohort. The prevalence rate (R) of HAM/TSP in HTLV-I infected individuals of a given genotype may be calculated as $R = H\times OR/(1+OR)$, where H is the prevalence of HAM/TSP in the HTLV-I–infected population and OR is OR of developing HAM/TSP associated with that genotype. For example, the prevalence of HAM/TSP in *HLA-A*02$^+$* individuals in Kagoshima ≈ 0.01 (0.53/1.53) ≈ 0.3%, taking H in Kagoshima ≈ 1%.

[b] ORs for the continuous variables (age and load) are omitted since their quadratic terms cause the ORs to vary over age and load. Similarly, an OR for *TNF −863A* is not given as its interaction term with provirus load causes the OR to vary over load; see figure 1 for more discussion of this variation.

[c] The HLA class I alleles *A*02* and Cw*08 exert their strong effects on the outcome of HTLV-I infection primarily through an effect on provirus load [9, 18]. The 1-tailed *P* values given here relate to the additional effects of *A*02* and Cw*08 after taking into account their effect on load.

Minitab) was used to quantify the contribution of each factor to the odds of possessing HAM/TSP in the study cohort [25]. This analysis provides an equation (table 3) that can be used to predict the odds that an HTLV-I–infected individual of specified genotype, age, and provirus load in Kagoshima has HAM/TSP. In this multifactor analysis of this Japanese cohort (table 3), the natural logarithm of an individual's odds of possessing HAM/TSP is obtained by summation of the components in the central column (i.e., a constant, an age component, the value of an individual's provirus load, and the addition or subtraction of additional values according to genotype). A worked example is given as a note to table 3. This best-fit model (table 3) includes variables that are significant and omits those that do not make an appreciable contribution to predictive ability [23–25]. The factor "sex" does not appear in table 3 since its effect does not remain significant once provirus load is taken into account. For the same reason, *IL-15 T191C* does not appear here.

Logistic regression provides a rigorous method of identifying factors that act independently to influence the risk of HAM/TSP. *P* values in this multifactor analysis indicate the significance of a given effect once all the other factors in the model are accounted for; the value may therefore differ from that obtained in a single-factor analysis. The greatest reliance can be placed on the factors that remain significant in the multifactor analysis, because the potential confounding effects of other factors have been taken into account.

Results

In total, 58 SNP sites in 39 gene loci were studied (table 1). Fourteen gene loci (22 SNPs) had an SNP AF of <0.1 and were not studied further. The majority of gene loci (25 loci [36 SNPs]) had an SNP AF ⩾0.1 after initial genotyping of 100 patients with HAM/TSP and 100 HCs. Of these, 18 loci (27 SNPs) showed no difference in AF between patients with HAM/TSP and HCs. The remaining 7 loci (9 SNPs) were genotyped in all subjects (229 patients with HAM/TSP and 202 HCs), and the AF and genotype frequency data are presented in table 2. Data on all 58 SNP sites analyzed are available on our web site (http://www.wfi.med.ic.ac.uk/). SNP analysis of 3 of the candidate genes studied *(TNF, SDF,* and *IL-15)* showed an influence on the risk of HAM/TSP or the provirus load of HTLV-I.

TNF promoter SNP genotype. We studied 9 TNF-α promoter SNPs: of these, 6, including the widely studied SNPs at nt −238 and −308, were not informative in this cohort (AF, <0.1) (figure 1). The remaining 3 SNPs (nt −1031, −863, and −857) were all informative (AF, >0.1). Single-factor χ^2 statistical analysis showed a significant association (2-tailed $P =$

Table 4. Best-fit general linear model to estimate provirus load in the Kagoshima cohort ($n = 411$).

Factor, condition	$\log_{10}$ (provirus load)[a]
Constant	1.338
Subject	
Healthy carrier	0.0063 × age
Patient with HAM/TSP	1.916 − 0.0088 × age
IL-15 + 191C$^+$[b]	−0.286
*HLA-A*02$^+$*[c]	−0.206
*HLA-Cw*08$^+$*[d]	−0.231

NOTE. An individual's $\log_{10}$ (provirus load) is calculated as the sum of the components in the right-hand column of the table, according to genotype. Age is given in years. This equation explains a large proportion (40.6%) of the wide variation in $\log_{10}$ (provirus load) observed in the cohort. The interaction between age and disease status has a *P* value of .018. Since we consider the interaction between age and disease, it is not appropriate to give separate *P* values for age and disease status. Worked example: a 60-year-old patient with human T cell leukemia virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) with the genotype *IL-15 +191C$^+$, HLA-A*02$^-$, HLA-Cw*08$^+$* has a predicted $\log_{10}$ provirus load of $1.338+1.916-(0.0088\times 60)-0.286-0.231=2.209$.

[a] HTLV-I provirus load is given as $\log_{10}$ (no. of proviral copies/10^4 peripheral blood mononuclear cells).

[b] $P = .018$.

[c] $P = .013$.

[d] $P = .033$.

Table 5. Best-fit general linear model to estimate provirus load in patients with human T lymphotropic virus type I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) ($n = 215$).

Factor, condition	$\log_{10}$ (provirus load)[a]
Constant	3.189
Age[b]	$-0.0089 \times$ age
Male[c]	−0.223
*HLA⁻DRB1*0101⁺*[d]	−0.195
*HLA-B*54⁺*[e]	0.216

NOTE. This equation explains 9.2% of the observed variation in $\log_{10}$ (provirus load) in this group. The 2 class I HLA alleles *A*02* and *Cw*08* were not significant predictors of provirus load in patients with HAM/TSP. We included the *DRB1*0101* term despite borderline significance because of strong previous evidence for its importance [9, 18]. Worked example: a 60-year-old male patient with HAM/TSP with the genotype *HLA-DRB1*0101⁻, HLA-B*54⁺* has a predicted $\log_{10}$ provirus load of $3.189 - (0.0089 \times 60) - 0.223 + 0.216 = 2.648$.

[a] HTLV-I provirus load is given as $\log_{10}$(no. of proviral copies/10^4 peripheral blood mononuclear cells).

[b] $P = .012$.

[c] $P = .015$.

[d] $P = .075$.

[e] $P = .022$.

.0296 [uncorrected]; $\chi^2 = 7.39$ [2 *df*]; 2-tailed $P > .05$ [corrected]) with disease for the *TNF −857T* allele (table 2). However, we previously reported that this association is attributable to *HLA-B*54,* which is in strong linkage disequilibrium with *TNF −857T* in this cohort [18]. A similar conclusion has also been reached by Hamaguchi et al. [26] and by Seki et al. [27] in studies of Japanese subjects with other disorders that demonstrate an inflammatory component, namely type 1 diabetes and rheumatoid arthritis.

We wished to test the specific hypothesis that *TNF −863A* was associated with HAM/TSP, because Seki et al. [21] had reported an association between this allele and HTLV-I–associated uveitis in a different Japanese population. Owing to this a priori evidence for a role of the *TNF −863A* allele in HTLV-I–associated inflammatory diseases, we omitted Bonferroni's correction in the case of this SNP.

Logistic regression analysis of the 3 informative TNF-α promoter SNPs in this cohort *(TNF −1031, TNF −863,* and *TNF −857)* indicated that the TNF −863 SNP alone was indeed a predictor of HAM/TSP, whereas TNF −1031 and TNF −857 were not, after taking into account the other significant independent predictors identified in this study (age, provirus load, SDF-1 genotype, *HLA-A*02⁺, HLA-Cw*08⁺,* and HTLV-I subgroup; table 3, *left-hand column*). Furthermore, the logistic regression analysis revealed a statistically significant interaction with provirus load (table 3, *middle column*). The form of this interaction is illustrated in figure 1: the *TNF −863A* genotype increased the odds of HAM/TSP selectively in individuals with a provirus load of HTLV-I of ~3 copies/100 PBMC or greater ($\log_{10}$ provirus load, ⩾2.5; 2-tailed $P = .009$, Fisher's exact test; odds ratio [OR], 9.7; 95% confidence interval [CI], 1.3–74.). The existence of a threshold provirus load of ~1%–3% PBMC, above which the odds of possessing HAM/TSP rapidly increase with further increases in provirus load, is consistent with our previous observations [15]. It is also consistent with a mathematical model we have proposed [28] to reconcile the apparently conflicting roles of HTLV-I–specific cytotoxic T lymphocytes (see Discussion).

SDF-1β *SNP.* There was a significant association (table 2) between the odds of possessing HAM/TSP and the *SDF-1β +801A* variant on single factor χ^2 analysis at both the allele level (2-tailed $P = .0021$ [uncorrected]; $\chi^2 = 9.49$ [1 *df*]; OR, 0.64; 95% CI, 0.48–0.84; 2-tailed $P > .05$ [corrected]) and the genotype level ($\chi^2 = 11.54$ [2 *df*]; $.001 < P < .01$ [uncorrected]; $P > .05$ [corrected]). $P > .05$ after correction for the large number of comparisons was made ($n = 58$). However, logistic regression (table 3) confirmed that the *SDF-1 801A* allele was a significant independent predictor of HAM/TSP risk. Moreover, there was an effect of gene dosage: the OR of developing HAM/TSP for an *SDF-1β 801AA* homozygote (*AA* vs. *GG:* OR, 0.18; $P = .003$; table 3) was less than half the OR of a heterozygote (*GA* vs. *GG:* OR, 0.45; $P = .042$; table 3) in the multifactorial analysis. There was no effect of this polymorphism on provirus load.

Influence of IL-15 SNP *genotype.* There was a significant association between the odds of possessing HAM/TSP and genotype at the *IL-15 T+191C* SNP using single-factor χ^2 analysis (2-tailed $P = .0323$ [uncorrected]; $\chi^2 = 6.87$ [2 *df*]; table 2; $P > .05$ [corrected]). In logistic regression analyses, *IL-15* genotype did not remain significant once provirus load was accounted for. This is consistent with the notion that the effect of this polymorphism is exerted solely through an effect on provirus load. Accordingly, *IL-15* does not appear in the model

Table 6. Best-fit general linear model to estimate provirus load in healthy human T lymphotropic virus type I (HTLV-I) carriers ($n = 202$).

Factor, condition	$\log_{10}$ (provirus load)[a]
Constant	1.608
*HLA-A*02⁺*[b]	−0.311
*HLA-Cw*08⁺*[c]	−0.327

NOTE. This equation explains 5.2% of the observed variation in $\log_{10}$ (proviral) load in the carriers. Worked example: an asymptomatic carrier of HTLV-I with the genotype *HLA-A*02⁺, HLA-Cw*08⁺* has a predicted $\log_{10}$ provirus load of $1.608 - 0.311 - 0.327 = 0.97$.

[a] HTLV-I provirus load is given as $\log_{10}$ (no. of proviral copies/10^4 peripheral blood mononuclear cells).

[b] $P = .019$.

[c] $P = .043$.

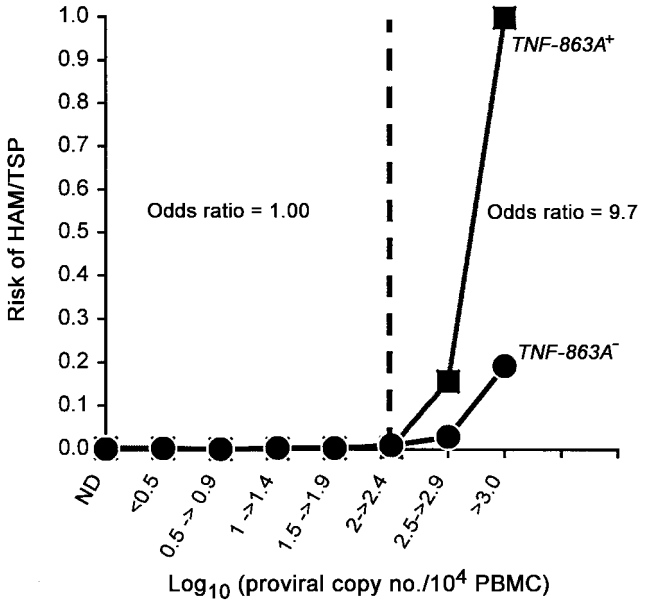

Figure 1. Interaction between tumor necrosis factor (TNF) promoter genotype and provirus load. The *TNF −863A* allele increased the risk of human T cell leukemia virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) selectively in subjects with a high provirus load of HTLV-I. A single figure for the odds ratio (OR) of HAM/TSP conferred by the *TNF −863* genotype cannot be given, because the OR varied continuously with provirus load (see table 3). However, as a simple demonstration of the strength of this effect, we compared the odds of HAM/TSP between subjects with a low provirus load and those with a high provirus load. In subjects (n = 237) with a low provirus load ($\log_{10}$ provirus load <2.5 or <3 proviral copies/100 peripheral blood mononuclear cells [PBMC]), the *TNF −863A* allele had no influence on the risk of HAM/TSP (OR, 1.00). However, in subjects (n = 171) with a high provirus load (⩾3 copies/100 PBMC), the *TNF −863A* allele was associated with a significantly increased risk of HAM/TSP (2-tailed P = .009, Fisher's exact test; OR, 9.7; 95% confidence interval, 1.3–74.1). Bayes' theorem of conditional probabilities was used to calculate the risk of HAM/TSP at a given provirus load and given TNF −863 genotype [9, 18]. *"P(HAM|L)"* denotes the risk of HAM/TSP at a given provirus load. By Bayes' theorem,

$$P(HAM|L) = \frac{P(HAM) \times P(L|HAM)}{P(HAM) \times P(L|HAM) + P(HC) \times P(L|HC)}.$$

presented in table 3. The effect of *IL-15* alone, across all individuals, showed that the *IL-15 +191C* allele was significantly associated with a lower provirus load of HTLV-I in the study cohort (P = .005, ANCOVA; P = .0083, Mann-Whitney U test; table 4).

Of the remaining 6 SNPs (5 loci) genotyped in the whole study cohort, only 1 other SNP *(LMP7 C+145A)* was associated with a significant difference in the risk of possessing HAM/TSP (without correction for multiple comparisons). However, logistic regression analysis showed that this SNP did not remain a significant predictor of HAM/TSP risk after the other factors (table 3) had been taken into account. Therefore, we do not consider these factors further in this paper.

GLMs for provirus load. GLMs were used to derive best-fit equations that predict provirus load in (1) the entire HTLV-I–infected cohort (table 4), (2) the patients with HAM/TSP alone (table 5), and (3) HCs alone (table 6). Of interest, the only 2 significant predictors of provirus load among the HCs (table 6) were the 2 protective class I HLA alleles, *HLA-A*02* and *HLA-Cw*08*. However, neither of these alleles was a significant determinant of provirus load in patients with HAM/TSP (table 5). This observation is consistent with our previous conclusion [9, 18] that the CTL response to HTLV-I is effective only in HCs of HTLV-I (see Discussion).

Discussion

We recently showed that the class I HLA genotype plays a significant part in determining both the risk of HAM/TSP and the provirus load. We reported elsewhere [9, 18] a dominant protective effect of 2 class I HLA alleles in reducing the risk of developing HAM/TSP in Kagoshima and concluded that HTLV-I–specific CTLs play an important role in limiting HTLV-I replication in vivo. Following genotyping of further loci in the present study, the observation that *HLA-A*02* and *HLA-Cw*08* are significant determinants of provirus load in HCs (table 6) but not in patients with HAM/TSP (table 5) remains true: the factors in tables 5 and 6 are mutually exclusive. This observation extends our initial proposal [9, 18] that the HLA class I–restricted CTL response to HTLV-I is more efficient in HCs [14, 16]. Although Tax-specific CTLs appear to be crucial to this immune control, other factors that influence the immune response (specifically, T cell function) and/or lymphocyte migration to sites of inflammation or infection (e.g., cytokines, chemokines, and adhesion molecules) may also modify the outcome of infection. Therefore, we examined non-HLA candidate genes for potential involvement in HTLV-I outcome.

The present study of 58 SNP sites in candidate genes suggests that the *TNF −863A* promoter allele has a role in determining the risk of HAM/TSP (figure 1 and table 3) in this Japanese population, which is consistent with the observations of Seki et al. [21], in which *TNF −863A* was associated with HTLV-I–associated uveitis. We show that the effect of the *TNF −863A* allele on the risk of HAM/TSP depended strongly on the provirus load of HTLV-I; possession of the *TNF −863A* allele was associated with an increased risk of HAM/TSP only when an individual's provirus load was high (⩾3 proviral copies/100 PBMC).

The observed interaction between the TNF promoter genotype and provirus load allows us to suggest a reason for the existence of an apparent threshold provirus load, above which

the risk of developing HAM/TSP rapidly increases [15] (figure 1). There is evidence [29] that the concentration of antigen required to stimulate a $CD8^+$ T cell to produce cytokines is greater than the concentration required to induce CTL killing of a target cell. It is, therefore, possible that, in asymptomatic carriers, efficient, abundant CTLs exist in equilibrium with a low concentration of HTLV-I antigens, whereas, in patients with HAM/TSP, a similar frequency of specific CTLs coexist with a substantially higher concentration of antigen [28]. The abundance of antigen in patients with HAM/TSP might, therefore, exceed the threshold required to stimulate the $CD8^+$ T cells to produce inflammatory cytokines, such as interferon (IFN) and TNF-α.

According to this argument [28], the anti–HTLV-I CTLs exert a beneficial effect in asymptomatic carriers, through lysis of HTLV-I–infected cells. In patients with HAM/TSP, on the other hand, the HTLV-I–specific CTLs secrete inflammatory substances such as TNF-α or IFN-γ [30, 31], although they continue to lyse HTLV-I–infected cells [32].

The association between HAM/TSP risk and the *TNF −863A* genotype is likely to be a real effect for the following reasons: first, the same allele has been associated with an increased risk of HTLV-I uveitis [21]; and, second, *TNF −863A* has also been reported to carry an increased risk of other inflammatory diseases in Japanese populations, such as Crohn disease [33, 34], ulcerative colitis [33], and thyroid associated ophthalmopathy [35]. Of interest, it has recently been shown that the *TNF −863A* allele abolishes binding to the TNF promoter of the inhibitory p50 subunit of NF-κB [36]. This polymorphism is therefore likely to carry functional consequences in vivo. We are currently examining the implications of this by using DNA expression microarrays.

The chemokine SDF has particular potential importance since it is 1 of only 2 chemokines that have been shown to be able to attract resting lymphocytes as well as activated ones [37]. The observed gene dosage effect, in which stronger protection was associated with 2 copies of the *SDF −801A* allele than with a single copy, argues in favour of a true physiological effect (table 3). HTLV-I Tax has also been shown to induce SDF-1 expression [38]. The logistic regression analysis indicates that the SDF-1 genotype remains a significant independent predictor of HAM/TSP even after the other risk factors (table 3) have been taken into consideration. Unlike the previously reported association between the *SDF G+801A* SNP and HIV-1 disease progression [39], we propose that the mechanism of its protection is different in HTLV-I infection, because SDF-1 and its receptor CXCR4 play no known role in HTLV-I entry or fusion.

Waldmann et al. [40, 41] have proposed that *IL–15* plays a part in the pathogenesis of HTLV-I–associated diseases and in maintaining the high frequency of Tax-specific $CD8^+$ T cells in HTLV-I infection [42]. IL-15 has been shown to promote the maintenance of both CTLs [43] and NK cells [44]; a strong CTL response would in turn reduce the provirus load and the risk of HTLV-I–associated inflammatory diseases. We present here evidence of an association between the *IL-15 +191C* allele and a reduction in HTLV-I provirus load in both asymptomatic carriers and patients with HAM/TSP. It remains possible, as in other association studies, that the role of this IL-15 SNP is due to functional polymorphisms in linkage disequilibrium with this SNP. Although the prevalence of HAM/TSP is greater among females than in males in this population, as in other HTLV-I–infected populations, the effect of sex on the odds of HAM/TSP did not remain significant after provirus load was taken into account.

In conclusion, the goal of this candidate gene study was to identify polymorphic genetic markers that can be used to predict an HTLV-I–infected individual's risk of HAM/TSP in our study cohort. We have demonstrated a significant interaction between host TNF 5′ promoter −863 SNP genotype and the HTLV-I provirus load in determining the risk of HAM/TSP in HTLV-I–infected individuals in Kagoshima. Furthermore, we provide evidence that polymorphisms in SDF-1 and IL-15 also influenced the outcome of HTLV-I infection in this Japanese cohort. Since there are differences both in the host genetic composition (HLA-alleles and non–HLA SNP allele frequencies) and the prevalent HTLV-I variant (cosmopolitan A or B) between endemically HTLV-I– infected populations, it is likely that individual gene effects will vary in their magnitude and statistical significance. However, it is very unlikely that the fundamental principles that govern the immune control of HTLV-I infection will differ—in particular, the principle that an efficient CTL response to HTLV-I is beneficial. Our findings suggest that selective antiretroviral therapy of individuals with a high HTLV-I provirus load ($\geq$3 copies/100 PBMC) will reduce the risk of developing HAM/TSP and that therapeutic agents designed to reduce the effects of proinflammatory cytokines may provide clinical benefit.

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