

# Association of Polymorphisms of the Mu Opioid Receptor Gene with the Severity of HIV Infection and Response to HIV Treatment

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**Background.** Mu opioid receptor (*OPRM1*) ligands may alter expression of chemokines and chemokine receptors involved in penetration of human immunodeficiency virus (HIV) type 1 into the cell. We suggest that *OPRM1* variants may affect the pathophysiology of HIV infection.

**Methods.** DNA samples from 1031 eligible African Americans, Hispanics, and whites from the Women's Interagency HIV Study (WIHS) who were alive as of April 2006 were analyzed. We performed regression analysis of association of 18 *OPRM1* variants with a change of viral load and CD4 cell count during 2 periods: between admission to WIHS and the start of highly active antiretroviral therapy (HAART) (interval X) and between the start of HAART and the most recent WIHS visit (interval Y), and examined the association of these variants with HIV status.

**Results.** Regardless of genotype, a significant decrease in viral load during interval X was found for each ethnicity. Whites with allele G of the functional polymorphism 118A > G (reference sequence rs1799971) showed a smaller decrease in viral load; those bearing minor alleles IVS1 + 1050A, IVS1 + 14123A, and IVS2 + 31A showed a larger decrease in viral load over interval X ( $0.01 < P < .05$ ). Hispanics with the same alleles showed a greater increase in CD4 cell count over interval Y ( $0.01 < P < .05$ ). We found an association between *OPRM1* variants and HIV status in African Americans and whites.

**Conclusions.** *OPRM1* polymorphisms may alter the severity of HIV infection before and after HAART.

After our original discovery [1] of the functionality of the 118A > G polymorphism in the gene (*OPRM1*) encoding the mu opioid receptor, variants of *OPRM1* have been extensively studied in large cohorts for their association with stress responsivity, response to pain management, affective disorders, and specific addictive disorders, including cocaine and opioid addictions and alcoholism [2–5, 6]. However, *OPRM1* variants

have not been studied in a cohort of individuals with human immunodeficiency virus (HIV) infection. *OPRM1* is involved in many physiological functions, including stress responsivity, by tonic inhibition of the hypothalamic-pituitary-adrenal (HPA) axis [7]; eating behavior; endocrine function; and cognition [2–5]. Beta-endorphin, an endogenous opioid that activates *OPRM1*, binds the 118G variant (reference sequence rs1799971) of *OPRM1* 3 times as tightly as it binds the prototype receptor [1]. Basal levels of cortisol are higher in healthy humans with 1 or 2 copies of 118G [8]. This polymorphism was shown to be functional in the treatment of alcoholism [9].

The *OPRM1* is also involved in immune response and HIV and simian immunodeficiency virus expansion [10–18]. We have studied HIV since the early 1980s [19–21]. Opioid ligands may regulate the expression of chemokines and chemokine receptors [12]. Many studies have found cross-desensitization interactions

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between opioid and chemokine receptors both in vivo and in vitro [13–15]. Both OPRM1 and delta opioid receptor (OPRD1) selective ligands can induce desensitization of CCR1, CCR2, CCR5, CXCR1, and CXCR2, but not CXCR4. Similarly, selective ligands of CCR1, CCR2, CCR5, CCR7, and CX3CR1 can desensitize both OPRM1 and OPRD1 [13–15]. Opioids selectively promote induction of proinflammatory or anti-inflammatory effects, depending on the involvement of OPRM1 or, alternatively, on the kappa opioid receptor (OPRK1), which frequently act in opposition to each other [16, 17].

Specific variants of *CCR5* and *CCR2* suppress HIV-1 transmission and cause delay in HIV-1 disease progression [22–24]. Genetic differences in major histocompatibility complex (MHC) genes (HLA in humans) have been associated with different rates of progression from HIV infection to AIDS [25–28]. We suggest that the genetic variants that contribute to depression, affective disorders, and stress responsiveness may also influence the pathophysiology of HIV infection.

In this study, we looked for an association between selected *OPRM1* polymorphisms and HIV status, using HIV-positive individuals (cases) and HIV-negative individuals (controls) recruited at Women’s Interagency HIV Study (WIHS) sites [29–32]. We also tested for the association between specific genotypes of *OPRM1* and the response to HIV treatment, including changes in the viral load and CD4 cell count, across the period from admission to WIHS to the start of HAART and the period from the start of HAART to the most recent WIHS visit for which data were available. Our findings indicate the potential involvement of the *OPRM1* in the pathophysiology of HIV infection.

## METHODS

### Study Subjects: Recruitment and Diagnostic Procedures

Characteristics of subjects included and excluded in the analysis are shown in Table 1. Unrelated African American, white, and Hispanic women were recruited by WIHS. As of 2002, 3766 subjects were enrolled in 2 cohorts, with the first recruited during 1994–2000 (most subjects were recruited during 1994–1995) and the second recruited during 2001–2002 (available at: <https://statepiaps.jhsph.edu/wihs/invest-info/dossier.pdf>; accessed 15 June 2011). Only subjects who signed an extended, updated informed consent form between 1 April 2006 and 30 September 2007 (n = 1506) were included, thus excluding those who died between WIHS entry and 2006. Subjects completed our Family Origin Questionnaire on 3 generations. Limited clinical information about subjects was provided by WIHS, including HIV status, CD4 cell count, and viral load. Physical examination, specimen collection, and interview about the history of illness, substance abuse, current medications, and medication adherence were done every 6 months.

**Table 1. Characteristics of Subjects Included or Excluded From the Study, by Human Immunodeficiency Virus (HIV) Infection Status**

Characteristic	No. of Subjects		
	HIV–	HIV+	Total
Total number of subjects	...	...	<b>1506</b>
Subjects included in this study			
Ethnicity			
African American	213	478	691
White	46	115	161
Hispanic	68	111	179
Total	<b>327</b>	<b>704</b>	<b>1031</b>
Had clinical data for 2 of 3 study points available <sup>a</sup>	...	<b>621</b>	...
Subjects excluded from this study			
Mixed ethnicity	134	287	421
Low DNA concentration	...	...	45
Seroconverted after admission to WIHS study	...	...	6
Start HAART before admission to WIHS	0	1	1
Start HAART after the most recent visit	0	1	1
Missing longitudinal data	0	1	1
Total	...	...	<b>475</b>

Abbreviations: HIV+, HIV positive; HIV–, HIV negative; WIHS, Women’s Interagency HIV Study.

<sup>a</sup> Study points were defined as admission to WIHS, initiation of highly active antiretroviral therapy, and most recent visit to WIHS for which clinical data were made available. A total number of samples that was either included or excluded from the study is shown in bold.

A total of 475 subjects were excluded because of mixed ethnicity (421 subjects; Table 1), seroconversion after admission to WIHS (6 subjects), and insufficient amount of DNA samples (45 subjects), leaving 1031 subjects for association studies involving HIV status. A subset of HIV-positive subjects with complete data across 3 study points—entrance to WIHS (hereafter, “R”), initiation of HAART (hereafter, “S”), and the most recent WIHS visit for which clinical data were made available (hereafter, “T”)—were used to compare changes in viral load and CD4 cell count during 2 intervals (Table 2). The first interval was defined as the period from R to S (hereafter, “X”), and the second interval was defined as the period from S to T (hereafter, “Y”). A different subset of HIV-positive subjects with complete data across 2 of 3 study points (R and S, or S and T) was used for analysis of the influence of genotype on changes in viral load or CD4 cell count across intervals X and Y (Table 3). Subjects who did not start HAART were excluded from viral load and CD4 cell count analyses. Study point S occurred during the period between 6 months before the start of HAART and 1 month after the initiation of HAART.

**Table 2. Ethnicity of Subjects With Human Immunodeficiency Virus Infection for Whom Viral Loads or CD4 Cell Counts From All 3 Study Points Were Available**

Ethnicity	No. of Subjects	
	Viral Load	CD4 Cell Count
African American	388	412
White	96	105
Hispanic	94	104
Total	578	621

Study points were defined as admission to the Women's Interagency HIV Study (WIHS), initiation of highly active antiretroviral therapy, and most recent visit to WIHS for which clinical data were made available.

### OPRM1 Genotyping

The structure of *OPRM1* is shown in Figure 1. DNA from peripheral blood mononuclear cell pellets obtained from the WIHS depository was isolated using the PureGene DNA purification kit (Gentra Systems, Minneapolis, MN) and used for genotyping on an ABI Prism 7900 sequence detection system according to manufacturer's protocol, with custom-synthesized oligonucleotide primers and probes specific for the polymorphism chosen (Supplementary Table 1). For each polymorphism, the results of TaqMan genotyping were confirmed by Sanger sequencing of 22 randomly selected samples, using the same primers.

### Statistical Analysis

Analysis of variance (ANOVA) with repeated measures was used to assess the change in viral load and CD4 cell counts for each ethnicity in the subset of HIV-positive subjects who had

**Table 3. Ethnicity of Subjects With Human Immunodeficiency Virus Infection for Whom Viral Loads or CD4 Cell Counts From 2 of 3 Study Points Were Available for Use in Statistical Genetics (Logistic Regression) Analysis of the Association Between *OPRM1* Polymorphisms and Change in Viral Load and CD4 Cell Count**

Ethnicity	No. of Subjects			
	Viral Load		CD4 Cell Count	
	Interval X	Interval Y	Interval X	Interval Y
African Americans	390	393	412	412
Whites	96	98	105	105
Hispanics	94	95	104	104
Total	580	586	621	621

Study points were defined as admission to the WIHS, Women's Interagency HIV Study (WIHS; R), initiation of highly active antiretroviral therapy (S), and most recent visit to WIHS for which clinical data were made available (T). Interval X was defined as the time from R to S, and interval Y was defined as the time from S to T.

data from study points R, S, and T, without stratification by genotype with data. Hardy-Weinberg equilibrium (HWE) for each marker was determined in controls, using the likelihood ratio  $\chi^2$  test, separately for each ethnicity (54 tests). For a Bonferroni-corrected value of 0.01, individual  $\chi^2$  tests are significant when  $P < .0002$ . Likelihood ratio  $\chi^2$  tests were used to determine differences in genotype frequencies among ethnicities in the group of controls only. In this test, genotypes were collapsed when the expected number in each cell was  $< 1$ .

Multiple regression analyses of viral load and CD4 cell count were carried out with cases only, using 2 approaches. In the first approach, multiple regressions were carried out, using the difference in the natural log of the viral load over interval X as the dependent variable, with the length of interval X and each genotype as independent (predictor) variables. One such analysis was carried out for each variant and each ethnicity. As viral load and CD4 cell count measurements were strongly skewed (with a long right tail), they were transformed to natural logs (1 was added to CD4 cell counts). We tested whether genotypes were associated with changes in viral load and CD4 cell counts over intervals X and Y.

In the second approach, analyses were carried out twice for each variant, once in the dominant model (genotypes AA combined with genotypes AB) and once in the recessive model (genotypes AB combined with genotypes BB). Resulting groups were used as predictor variables.

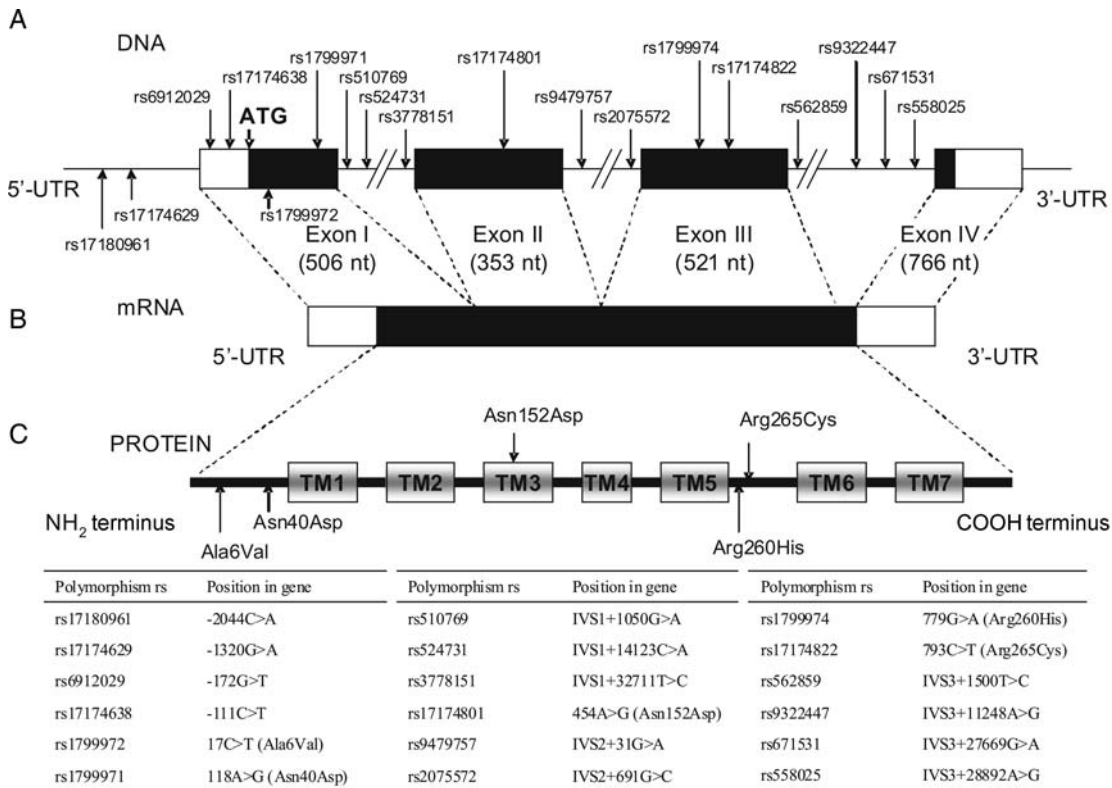
Logistic regression was performed for all cases and controls, including HIV-positive subjects who had not start HAART since admission to WIHS, to assess the association between each variant and HIV status, separately for each ethnicity and with each variant as a predictor variable, using 2 approaches. In the first approach, logistic regression was carried out for each variant, with case/control as the dependent variable and genotype code (0, 1, and 2 for genotypes AA, AB, and BB, respectively) as the independent (predictor) variable. In the second approach, the analysis was carried out twice for each variant in a fashion similar to that described above, using dominant and recessive models.

Analysis of the linkage disequilibrium (LD) of the variants studied was performed separately for each ethnicity, using Haploview 4.2 (available at: <http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview>; accessed 12 March 2011).

## RESULTS

### Analysis of Clinical Data: Overall Change of Viral Load and CD4 Cell Counts at Different Study Points, Without Stratification by *OPRM1* Genotype

In each ethnicity, there was a significant decrease in viral load between study points R and S and between study points R and T (Figure 2B). ANOVA with repeated measures across study



**Figure 1.** Structure of the *OPRM1* gene. *A*, Locations of polymorphisms. *B*, Structure of messenger RNA (mRNA). *C*, Structure of the mu opioid receptor peptide. Abbreviations: nt, nucleotides; rs, Single Nucleotide Polymorphism Database (dbSNP) reference sequence; TM, transmembrane domains; UTR, untranslated regions.

points in the subset of subjects with complete data at all 3 study points showed a significant main effect of interval in African Americans ( $F[2,274] = 28.10$ ;  $P < .00001$ ), whites ( $F[2,394] = 78.52$ ;  $P < .000001$ ), and Hispanics ( $F(2,186) = 59.48$ ;  $P < .000001$ ). Newman-Keuls post hoc tests showed that viral load fell significantly in each ethnicity during intervals X and Y (Figure 2B). The improvement (ie, reduction) of the viral load may have been due to general medical care; to monotherapy, such as azidothymidine (AZT); or to combination therapy. AZT has been shown to be effective in reducing viral load [33, 34].

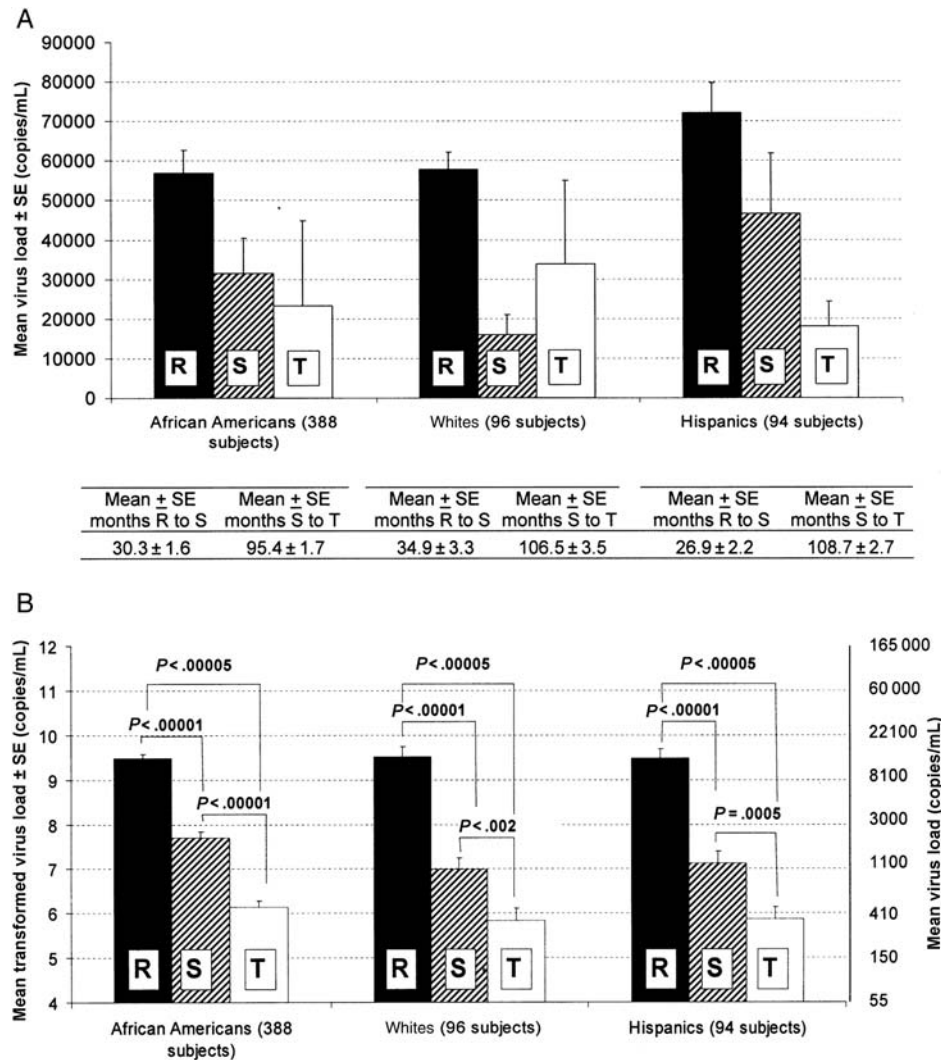
At WIHS entry, the majority of the HIV-positive subjects (70.6% of African Americans, 74.3% of whites, and 83.7% of Hispanics) had CD4 cell counts <500 cells/mL (Supplementary Table 1B). There was improvement in CD4 cell count after HAART. Significant increases in CD4 cell counts were found in African Americans ( $F[2,288] = 17.61$ ;  $P < .00005$ ) and whites ( $F[2,208] = 5.09$ ;  $P < .01$ ) but not in Hispanics ( $F[2,186] = 1.33$ ;  $P = .267$ ). Newman-Keuls post hoc tests showed an increase in CD4 cell counts in both ethnic groups only after the start of HAART (Figure 2D). Among Hispanics, there was an apparent change in CD4 cell count that had the same direction as that for African Americans and whites, but this did not reach statistical significance.

### Ethnic Differences in Allele Frequencies

In the control group, overall allele frequencies were significantly different among the 3 ethnicities in 8 variants (Supplementary Table 1C). None of the polymorphisms tested in this study showed significant deviation from HWE in controls after correction for multiple testing ( $P < .0002$ ; Supplementary Table 1D). Polymorphisms with a frequency of <0.05 in both controls and cases were excluded from analysis (Supplementary Table 1C).

### Multiple Regression Analysis of Association of Genotypes of *OPRM1* With Viral Load

By use of multiple regression analysis in approach 1 in the group of HIV-positive subjects, we found that individuals bearing the minor alleles IVS1 + 1050A, IVS1 + 14123A, and IVS2 + 31A had a greater reduction in viral load over interval X as compared to individuals bearing major alleles of these variants (Figure 3B–D and Table 4). Interestingly, individuals bearing 1 or 2 copies of variant G of polymorphism 118A > G had a smaller reduction in viral load over interval X (Figure 3A and Table 4). Hispanic subjects bearing genotype GG in IVS3 + 11248A > G (rs9322447) showed no decrease in viral load over interval Y as compared to those bearing genotypes AA and AG (Figure 3E and Table 4). Similar results were



**Figure 2.** Equivalent and natural log-transformed viral loads and CD4 cell counts, at different study points, for subjects of different ethnicities. Study points were defined as admission to the Women's Interagency HIV Study (WIHS; R), initiation of highly active antiretroviral therapy (HAART; S), and most recent visit to WIHS for which clinical data were made available (T). SE, standard error. A, Viral loads in different ethnic groups, by study point and interval between study points. B, Transformed virus loads for statistical analysis. C, CD4 cell counts in different ethnic groups, by study point and interval between study points. D, Transformed CD4 cell counts (+1) for statistical analysis.

found using the dominant-recessive model of analysis (approach 2; Table 4).

#### Multiple Regression Analysis of Association of Genotypes of OPRM1 With CD4 Cell Count

Multiple regression analysis in approach 1 in the HIV-positive group revealed no significant effect of the genotype on the change of CD4 cell count over interval X in any ethnic group studied. We found that Hispanic subjects bearing 1 or 2 copies of the minor alleles IVS1 + 1050A, IVS1 + 14123A, and IVS2 + 31A had a greater increase (ie, improvement) of CD4 cell count over interval Y (Figure 4A–C and Table 4).

In approach 2, no significant effect of any polymorphism studied on the change of the CD4 cell count over interval X was found in any ethnic group. In Hispanics, a significant effect of the genotypes of IVS1 + 1050G > A and IVS1 + 14123C > A on the change of the CD4 cell count over interval Y was found using the dominant model ( $P = .0292$  and  $P = .0170$ , respectively; Table 4) and an effect of IVS2 + 31G > A on the change of the CD4 cell count over the same period in the recessive model ( $P = .0493$ ), similar to findings from approach 1. In whites, a significant effect of the genotypes of IVS1 + 14123C > A and IVS1 + 32711T > C on the change of the CD4 cell count over interval Y was found

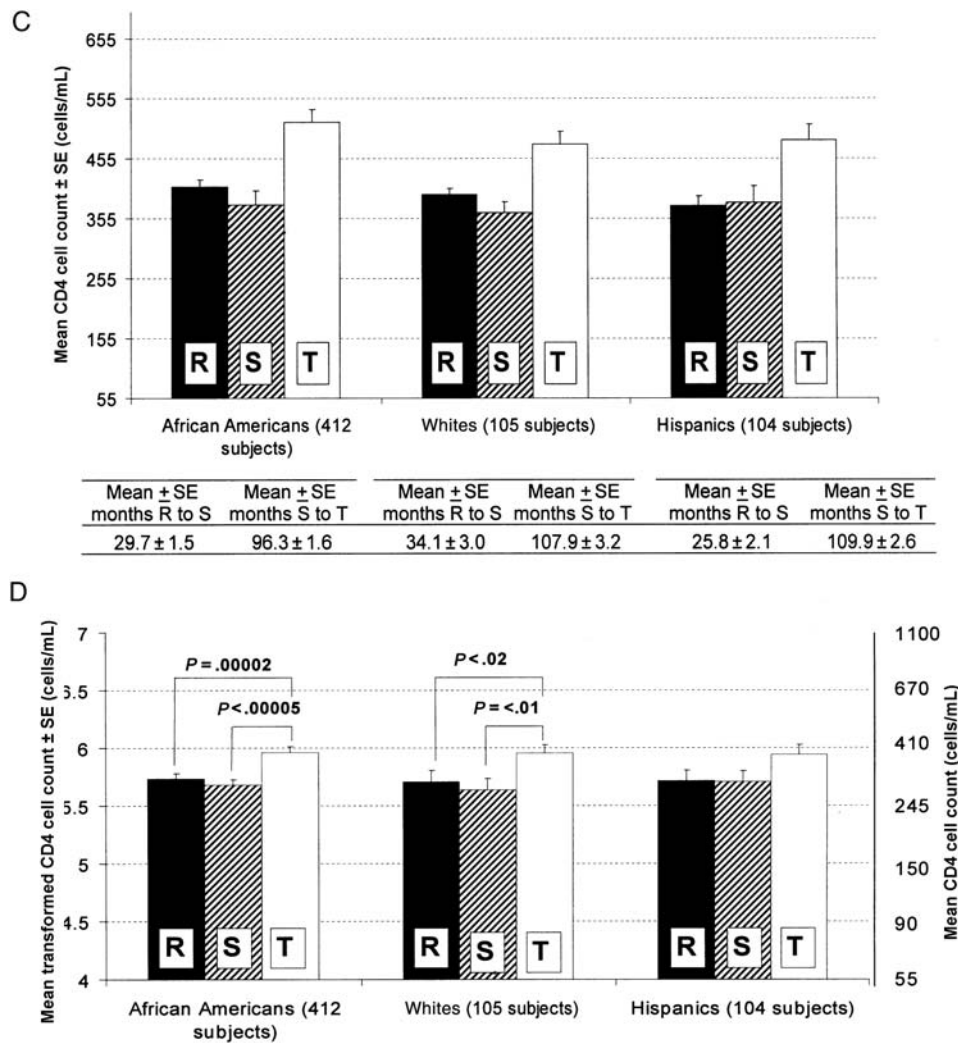


Figure 2. continued.

using the recessive model ( $P = .0214$  and  $P = .0395$ , respectively, Table 4). This effect was not found using approach 1.

#### Logistic Regression Analysis of OPRM1 Polymorphisms and HIV Status

By use of logistic regression in controls and cases (approach 1; Table 5), we found that, in whites, the minor alleles 2044A, IVS1 + 1050A, IVS1 + 14123A, IVS1 + 32711C, and IVS2 + 31A had lower frequencies in the HIV-positive group as compared to controls. In African Americans, the minor allele IVS2 + 691C was found in greater frequency in the HIV-positive group, and the minor allele 11189G was found in lower frequency in the HIV-positive group.

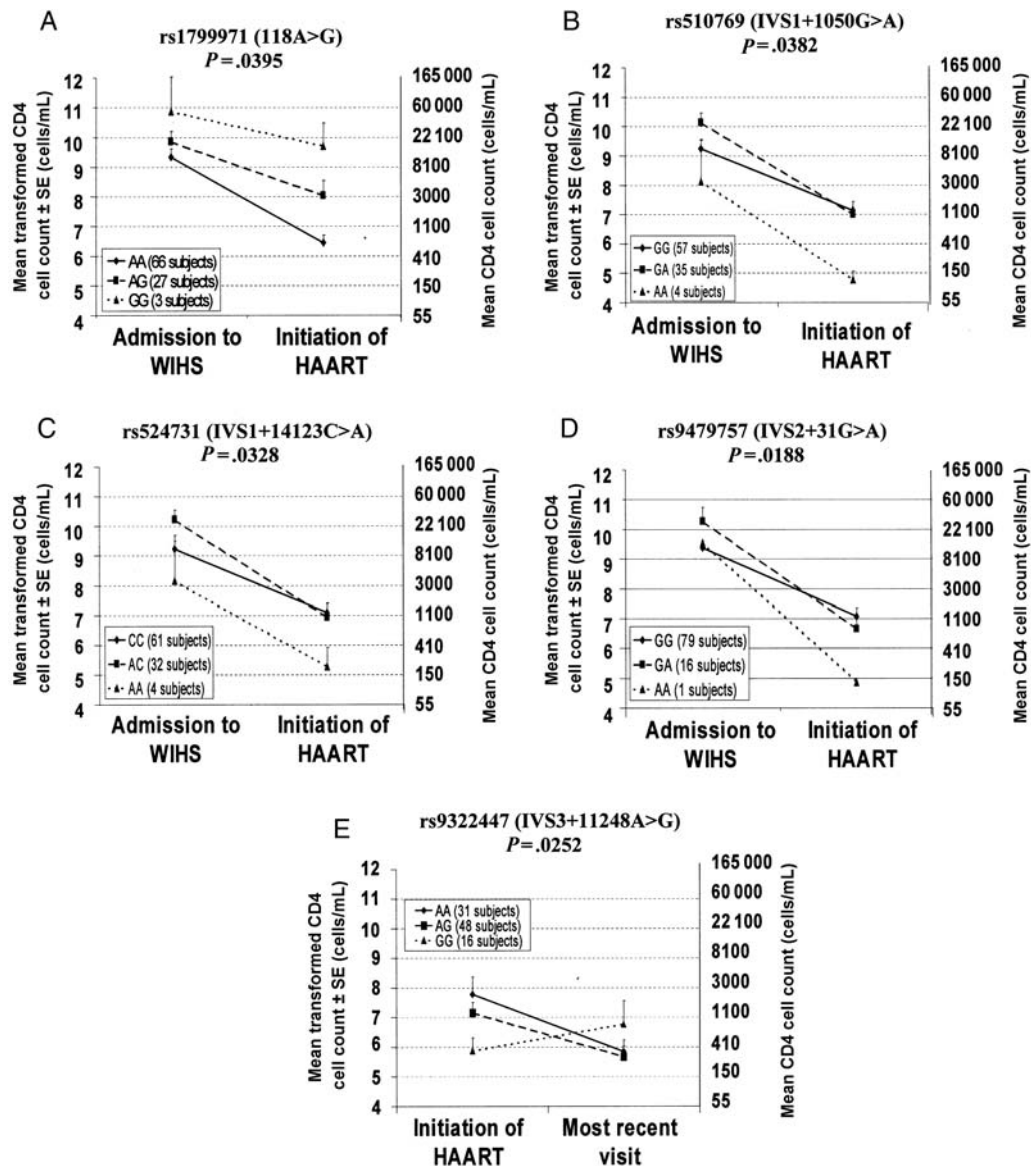
By use of approach 2 (Table 5), we found a significantly lower frequency of genotypes with the minor alleles -2044 (CA + AA), IVS1 + 14123 (CA + AA), IVS1 + 32711 (TC + CC), and IVS2 + 31 (GA + AA) in the HIV-positive group of whites,

using the recessive model. Also in whites, a lower frequency of genotype IVS1 + 32711CC was found in the HIV-positive group, using the dominant model. A lower frequency of genotypes 11189 (AG + GG) was found in the HIV-positive group of African Americans, using the recessive model.

All findings in multiple regression analyses or logistic regression analyses were only point-wise significant.

#### Analysis of LD

LD analysis was performed for cases and controls combined, separately for each ethnic group. Complete LD ( $r^2 = 1$ ) was not detected for any combination of variants. However, several variants were in high LD ( $r^2 > 0.7$ ). Variants IVS1 + 1050G > A (rs510769) and IVS1 + 14123C > A were in high LD ( $r^2 > 0.7$ ) in whites and Hispanics. Variants IVS1 + 14123C > A and IVS1 + 32711T > C (rs3778151) were in high LD ( $r^2 > 0.7$ ) in whites only. Variants IVS2 + 691G > C (rs2075572) and



**Figure 3.** Association between genotypes of *OPRM1* variants and changes in natural log-transformed viral loads between admission to Women's Interagency HIV Study (WIHS) and the start of highly active antiretroviral therapy (HAART), among whites (A–D) and Hispanics (E). In all panels, genotypes of the homozygous major alleles are denoted by the solid line. Abbreviations: rs, Single Nucleotide Polymorphism Database (dbSNP) reference sequence; SE, standard error.

IVS3 + 11248A > G were in high LD in all ethnicities ( $r^2 > 0.66$ ). In African Americans, high LD was observed between variants -1320G > A and -111C > T ( $r^2 = 0.90$ ).

## DISCUSSION

We selected 3 study points that identify crucial and well-defined points in the clinical history of HIV-infected patients. Date of infection was not verified for most patients. We found high variability in viral loads in the original data (Figure 2A), especially at study point T, which results in different patterns

of viral load change in African Americans as compared to that in whites; when data were natural log transformed, we observed the same patterns of viral load change in all 3 ethnicities (Figure 2B). The analyses of viral load and CD4 cell count changes during intervals X and Y indicate strong decline of viral load over both intervals in all 3 ethnic groups (Figure 2B). However, no improvement in CD4 cell count was found in any ethnic group before the start of HAART (Figure 2D). Before the start of HAART, some subjects received monotherapy, including AZT, or 2-drug combination therapy. Administration of AZT alone may result in reduction of viral load by

**Table 4. Significant Findings From Multiple Regression Analysis of the Association Between *OPRM1* Genotypes and Changes in Viral Load and CD4 Cell Count During Interval X Only and/or During Interval Y Only Among Subjects With Human Immunodeficiency Virus (HIV) Infection**

Variant, by HIV marker and interval	P of Change (Approach 1) <sup>a</sup>			P of Change (Approach 2)
	African Americans	Whites	Hispanics	
Viral load, interval X	n = 390	n = 96	n = 94	
118A>G	LF <sup>b</sup>	<b>.0395<sup>c</sup></b>	.8788	White rec, <b>.0432</b>
IVS1+1050G>A	.9597	<b>.0382<sup>d</sup></b>	.8767	White rec, <b>.0304</b>
IVS1+14123C>A	.1743	<b>.0328<sup>d</sup></b>	.8610	White rec, <b>.0213</b>
IVS2+31G>A	.6165	<b>.0188<sup>d</sup></b>	.6597	White rec, <b>.0206</b>
Viral load, interval Y	n = 393	n = 98	n = 95	
IVS3+27669G>A	.5724	.2761	<b>.0252<sup>e</sup></b>	Hispanic dom, <b>.0143</b>
CD4 cell count, interval Y	n = 412	n = 105	n = 104	
IVS1+1050G>A	.9545	.1124	<b>.0183<sup>f</sup></b>	Hispanic dom, <b>.0292</b>
IVS1+14123C>A	.5666	.0556	<b>.0150<sup>f</sup></b>	White rec, <b>.0214</b> ; Hispanic dom, <b>.0170</b>
IVS1+32711T>C	.2506	.0575	.1095	White rec. <b>.0395</b>
IVS2+31G>A	.6394	.0693	<b>.0493<sup>f</sup></b>	Hispanic rec. <b>.0493</b>

Values in bold italics indicate point-wise significant associations ( $P < .05$ ). Interval X was defined as the period between admission to WIHS and initiation of HAART, and interval Y was defined as the period between initiation of HAART and the most recent visit to WIHS for which clinical data were made available.

Abbreviations: dom, dominant model; HAART, highly active antiretroviral therapy; rec, recessive model; WIHS, Women's Interagency HIV Study.

<sup>a</sup> The number of subjects in each analysis differed because of missing data (Table 1).

<sup>b</sup> LF, Low Frequency, Ethnic groups in which the genotype frequency of specific single-nucleotide polymorphisms was  $<0.05$  were eliminated.

<sup>c</sup> Subjects with minor allele G showed a smaller decrease.

<sup>d</sup> Subjects with minor allele A showed a larger decrease.

<sup>e</sup> Subjects with minor allele G showed a smaller decrease.

<sup>f</sup> Subjects with minor allele A showed a larger increase.

50% in antiretroviral-naïve patients; administration of AZT with other modified nucleotides, including 2',3'-dideoxyinosine, 2',3'-dideoxycytidine, and 2',3'-dideoxy-3'-thiacytidine, reduces the viral load by 80%–90% [33]. AZT treatment was also shown to be effective in reducing maternal-infant HIV transmission [35].

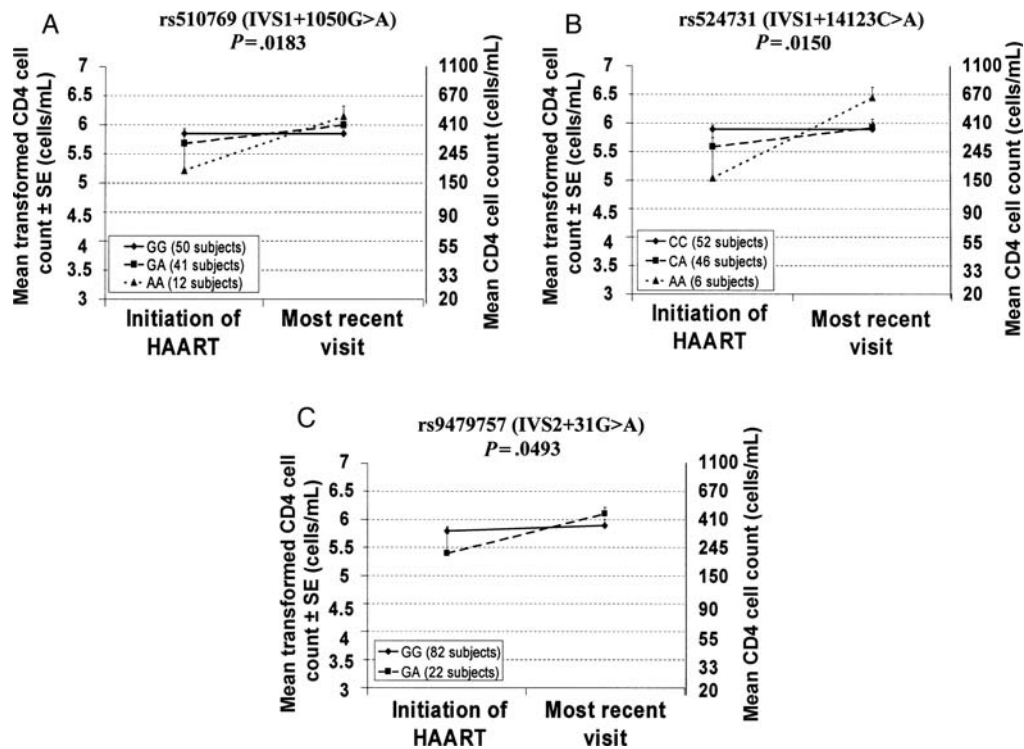
Whites with minor allele G of the *OPRM1* variant 118A > G (Asn40Asp), a functional stress responsivity variant, had a smaller decrease in viral load over interval X. In vitro studies by our group showed that the variant Asp40 of *OPRM1* binds beta-endorphin, an endogenous ligand of *OPRM1*, with 3 times the affinity of wild-type *OPRM1* [1], suggesting that subjects bearing this polymorphism might have different response to opioid medications. Cell studies by our group showed that the Asp40 variant of *OPRM1* had lower forskolin-induced cAMP accumulation and lower receptor-binding-site availability [36]. Studies of postmortem human brain specimens showed that allele 118G is expressed at a lower level than 118A [37]. This polymorphism was shown to be associated with a number of neurobiological conditions, including heroin addiction [38], alcoholism [39], and pharmacological response to naltrexone treatment for alcoholism [9].

The 118G allele has been associated with proinflammatory cytokine levels in a study involving healthy Japanese subjects,

among whom the allele frequency of the 118G was 59.3% [40]. Cross-desensitization interactions between *OPRM1* and chemokine receptors that are directly involved in HIV proliferation have been shown [13, 14]. Therefore, alteration of *OPRM1* activity in subjects bearing the 118G variant may result in altered activity of chemokine receptors (coreceptors for HIV replication), producing a worse clinical outcome in HIV-positive patients. Indeed, a smaller decrease in viral load over interval X in HIV-positive whites bearing the 118G allele was found in this study. Alteration in viral load change in subjects bearing the 118G allele might also be directly related to altered stress responsivity previously found in healthy people bearing this allele [1].

We did not find association between any polymorphism and change in CD4 cell count during interval X in any ethnic group. However, we found an association of the intronic IVS1 + 1050G > A, IVS1 + 14123C > A, and IVS2 + 31G > A variants with the change in CD4 cell count over interval Y in Hispanics. The same polymorphisms were found to be associated with a change in viral load over interval X in whites. In both cases, these polymorphisms showed similar effect. In whites, subjects with minor alleles IVS1 + 1050A, IVS1 + 14123A, and IVS2 + 31A had a larger decrease (ie, improvement) in viral load before the start of HAART. In





**Figure 4.** Association between genotypes of *OPRM1* variants and changes in natural log-transformed CD4 cell counts (+1) between the start of highly active antiretroviral therapy (HAART) and the most recent visit to the Women’s Interagency HIV Study for which clinical data were made available, among Hispanics. In all panels, genotypes of the homozygous major alleles are denoted by the solid line. Abbreviations: rs, Single Nucleotide Polymorphism Database (dbSNP) reference sequence; SE, standard error.

**Table 5. Significant Findings From Logistic Regression Analysis of *OPRM1* Polymorphisms and Human Immunodeficiency Virus (HIV) Infection Status**

Variant	P (Approach 1)			P (Approach 2)
	African American (n = 213 HIV– and 478 HIV+)	White (n = 46 HIV– and 115 HIV+)	Hispanic (n = 68 HIV– and 111 HIV+)	
-2044C>A	LF <sup>a</sup>	<b>.0078<sup>b</sup></b>	LF <sup>a</sup>	White rec, <b>.0078<sup>d</sup></b>
IVS1+1050G>A	.1322	<b>.0278<sup>b</sup></b>	.7352	NS
IVS1+14123C>A	.4837	<b>.0083<sup>b</sup></b>	.7866	White rec, <b>.0149<sup>d</sup></b>
IVS1+32711T>C	.1894	<b>.0038<sup>b</sup></b>	.1983	White rec, <b>.0145<sup>e</sup></b> ; white dom, <b>.0352<sup>f</sup></b>
IVS2+31G>A	.3837	<b>.0171<sup>b</sup></b>	.6479	White rec, <b>.0092<sup>g</sup></b>
IVS2+691G>C	<b>.0491<sup>c</sup></b>	.3268	.1357	NS
IVS3+27669G>A	<b>.0262<sup>b</sup></b>	.6006	.2545	African American rec, <b>.0304<sup>h</sup></b>

Values in bold italics indicate point-wise significant associations ( $P < .05$ ).

Abbreviations: dom, dominant model; HIV+, HIV positive; HIV–, HIV negative; NS, not significant; rec, recessive model.

<sup>a</sup> LF, Low Frequency, Ethnic groups in which the genotype frequency of specific single-nucleotide polymorphisms was  $<0.05$  were eliminated.

<sup>b</sup> Minor allele frequency was decreased in the HIV+ group.

<sup>c</sup> Minor allele frequency was increased in the HIV+ group.

<sup>d</sup> Decreased frequency of genotypes (CA+AA) was found in the HIV+ group.

<sup>e</sup> Decreased frequency of genotypes (TC+CC) was found in the HIV+ group.

<sup>f</sup> Decreased frequency of genotype CC was found in the HIV+ group.

<sup>g</sup> Decreased frequency of genotypes (GA+AA) was found in the HIV+ group.

<sup>h</sup> Decreased frequency of genotypes (AG+GG) was found in the HIV+ group.

Hispanics, they had a greater increase (ie, improvement) in CD4 cell count since the start of HAART.

In whites, the smallest group in our study, we found a number of polymorphisms, including a possible promoter variant, -2044C > A, and a number of intronic variants, IVS1 + 1050G > A, IVS1 + 14123C > A, IVS1 + 32711T > C, and IVS2 + 31G > A, to be associated with HIV status. The frequency of the minor allele of these variants was lower in the HIV-positive group, indicating a possible protective effect from infection. Both IVS1 + 1050G > A and IVS1 + 32711T > C were found by our group to be associated with vulnerability to opioid addiction in whites [41]. Allele T of IVS1 + 32711T > C was found to be associated with positive subjective response to the first episode of heroin use in Chinese subjects [42]. Haplotype block (A-A), consisting of 118A > G and IVS1 + 1050G > A, was found to be associated with feelings of amphetamine-induced euphoria, energy, and stimulation in whites [43]. Several polymorphisms that we found to be associated with HIV status in whites (IVS1 + 1050G > A, IVS1 + 14123C > A and IVS2 + 31G > A; Table 5) were also found to be associated with a decrease in viral load over interval X in whites and with an increase in CD4 cell count in Hispanics over interval Y (Table 4). Further study in a white group with a larger number of subjects is needed.

We found in African Americans, the largest ethnic group in our study, associations between HIV status and both the intronic IVS2 + 691G > C and the polymorphism from the 3' untranslated region IVS3 + 11248A > G. Previously, a minor haplotype GCG, consisting of variants IVS2 + 31G > A, IVS2 + 691G > C, and IVS3 + 183A > G (rs10485057), was found to be associated with smoking initiation and nicotine dependence [44]. Two of these variants, IVS2 + 31G > A and IVS2 + 691G > C, in our study were found to be associated with HIV status in whites and African Americans, respectively.

Studies by our group and others have shown that OPRM1 is involved in modulation of the HPA axis, one of the main systems involved in stress regulation [1, 7]. Stress, depressed mood, and dysthymic disorders have been reported to be associated with unsafe sex practices [45]. Numerous studies indicate that impulsivity is a statistically significant predictor of sexual risk behavior [46] and, therefore, HIV infection. Abuse of drugs as the result of self-medication for depression, in turn, may lead to infection with HIV and/or other infectious diseases, through sharing of needles or by engaging in unsafe sex while under the influence of drugs [19–21]. Many studies have reported an association of OPRM1 variants with vulnerability to heroin addiction [31, 38, 41]. Also, OPRM1 variants have been found to be associated with polysubstance abuse, including cocaine use [47] and alcoholism [39]. Cocaine use or injection drug use, reported by 27% of HIV-positive subjects and 35% of HIV-negative subjects, was associated with inconsistent condom use [48]. Many earlier

studies, including studies by our group, linked drug abuse, specifically, use of psychostimulants, including cocaine, with HIV-related risk behavior [49].

We found clinical improvement in subjects of different ethnicities bearing alleles IVS1 + 1959A, IVS1 + 14123A, and IVS2 + 31A (in whites, decline of viral load over interval X; in Hispanics, increase of CD4 cell count over interval Y) and association of the same variants with HIV status. This provides evidence of the involvement of OPRM1 variants and the OPRM1 system in the response to HIV treatment. A number of studies showed that intronic variants located in miRNA binding sites may influence gene expression [50]. Testing the influence of these polymorphisms on expression of OPRM1 in cell cultures could be performed in future studies. We are now testing polymorphisms that we found to have an effect on CD4 cell count or viral load change in the current study for their association with psychiatric conditions, using the same WIHS cohort.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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