Published in final edited form as:

J Acquir Immune Defic Syndr. 2013 May 1; 63(1): 17–26. doi:10.1097/QAI.0b013e318285cd0c.

Polymorphisms of the Kappa Opioid Receptor and Prodynorphin Genes: HIV risk and HIV Natural History

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Abstract

Objective—Studies indicate cross-desensitization between opioid receptors (e.g., kappa opioid receptor, OPRK1), and chemokine receptors (e.g., CXCR4) involved in HIV infection. We tested whether gene variants of *OPRK1* and its ligand, prodynorphin (*PDYN*), influence the outcome of HIV therapy.

Methods—Three study points, admission to the Women's Interagency HIV Study (WIHS), initiation of highly active antiretroviral therapy (HAART) and the most recent visit were chosen for analysis as crucial events in the clinical history of the HIV patients. Regression analyses of 17 variants of *OPRK1*, and 11 variants of *PDYN* with change of viral load (VL) and CD4 count between admission and initiation of HAART, and initiation of HAART to the most recent visit to WIHS were performed in 598 HIV+ subjects including African Americans, Hispanics and Caucasians. Association with HIV status was done in 1009 subjects.

Results—Before HAART, greater VL decline (improvement) in carriers of *PDYN* IVS3+189C>T, and greater increase of CD4 count (improvement) in carriers of *OPRK1* –72C>T, were found in African Americans. Also, greater increase of CD4 count in carriers of *OPRK1* IVS2+7886A>G, and greater decline of CD4 count (deterioration) in carriers of *OPRK1* –1205G>A, were found in Caucasians. After HAART, greater decline of VL in carriers of *OPRK1* IVS2+2225G>A, and greater increase of VL in carriers of *OPRK1* IVS2+10658G>T and IVS2+10963A>G, were found in Caucasians. Also, a lesser increase of CD4 count was found in Hispanic carriers of *OPRK1* IVS2+2225G>A.

Conclusion—*OPRK1* and *PDYN* polymorphisms may alter severity of HIV infection and response to treatment.

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CONFLICT OF INTERESTS

The authors do not have an association that might pose a conflict of interest.

Keywords

polymorphism; *SNP*, viral load; CD4 count; WIHS; *OPRK1*; *PDYN*

INTRODUCTION

Opioids and opioid receptors have been implicated in aspects of the immune response to HIV infection, in part through modulation of expression of cytokines and chemokines and their receptors [e.g., 1–2]. Several polymorphisms in specific genes were found to be involved in mechanisms of HIV transmission and pathophysiology of HIV infection including variants of CC- chemokine receptors CCR5 and CCR2 that are directly involved in the mechanisms of HIV-1 penetration into the target cell [e.g., 3–4] and polymorphisms of the human leukocyte antigen (HLA) cluster associated with different rates of HIV progression [e.g., 5–8]. We hypothesize that the gene polymorphisms of the opioid receptors that have been shown to be involved in neurophysiological functions, and their ligands, may also influence the pathophysiology of HIV. HIV has been a focus of our studies since the early 1980s [9–12].

Opioid receptor genes have been implicated in the general regulation of specific aspects of immune response. Serum concentrations of the proinflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and interferon-gamma (IFN-gamma) were found to be significantly lower and the general health score significantly higher in subjects bearing the allele G of the functional mu opioid receptor (*OPRM1*) variant 118A>G, suggesting that carriers of the G variant may suppress proinflammatory cytokine secretion from peripheral immune cells [13].

Heterologous cross-deactivation of opioid and chemokine receptors has been shown in both *in vivo* and *in vitro* experiments [1, 14–18]. Activation of one of the G protein-coupled receptors (GPCR) results in phosphorylation of the C-terminus of another GPCR by protein kinases A or C, leading to decoupling of the latter receptor from G proteins, and loss of sensitivity to stimulation [19, 20]. Activation of the *OPRM1* and the delta opioid receptors (*OPRD1*) induce deactivation of CCR1, CCR2, CCR5, CXCR1 and CXCR2, but not CXCR4. In turn, ligands of CCR1, CCR2, CCR5, CCR7 and CX3CR1 deactivate *OPRM1* and *OPRD1* [16–18]. Beta-endorphin, an endogenous agonist for both *OPRM1* and *OPRD1* with higher affinity for *OPRM1*, significantly enhances interleukin-2 (IL-2) production in IL-1-stimulated EL-4 and LBRM33-1A5 lymphoid cell lines; naloxone completely abolished this enhancing effect [21]. In contrast, the *OPRK1*-selective synthetic full agonist U50 488H (trans-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate) inhibits the expression of the proinflammatory cytokines IL-1, IL-2, IL-6 and TNF-alpha (reviewed in 1). U50 488H inhibits the expression of CXCR4 on human CD4+ T cells, resulting in a reduction of viral entry [22, 23]. Treatment of microglial cell culture with U50 488H results in dose-dependent inhibition of expression of the monocytoprotic HIV-1 SF162 strain [24].

In this study, we looked for an association of 17 selected variants of *OPRK1* and 11 variants of prodynorphin (*PDYN*) with HIV status in subjects from the Women's Interagency HIV Study (WIHS) sites [e.g., 25–28]. Next, we tested for influence of specific genotypes of *OPRK1* or *PDYN* on the change of viral load and CD4 count across two periods: between admission to WIHS to the start of highly active antiretroviral therapy (HAART) and also, between the start of HAART to the most recent WIHS visit for which data was available. Our findings indicate potential involvement of the specific variants of *OPRK1* and *PDYN* in the pathophysiology of HIV infection.

METHODS

Study subjects: recruitment and diagnostic procedures

African American, Caucasian and Hispanic unrelated women were recruited by the WIHS. As of 2002, 3766 subjects were recruited, primarily in two cohorts- first, in 1994–2000 (the majority of cohort 1 was recruited in 1995–1996) and second, in 2001–2002 (available at: <https://statepiaps.jhsph.edu/wihs/invest-info/dossier.pdf>; accessed on June 15, 2012). In our study, only subjects who signed an extended updated informed consent to participate (from April 1, 2006 to September 30, 2007, 1506 subjects total) were included, thus excluding those who died before 2006. Subjects completed our Family Origin Questionnaire on three generations. Limited clinical information on the subjects was provided by WIHS, including HIV status, viral load and CD4 counts. During each clinic visit (every six months) subjects provided specimens and were physically examined and interviewed on history of illnesses, substance abuse, current medications and medication adherence.

We excluded 497 subjects for different reasons (Table 1): mixed ethnicity (421 subjects); seroconversion after admission to WIHS (6 subjects), subjects whose DNA concentration in specimen provided was insufficient (45 subjects), and subjects whose CD4 count was below 10 cells per ml (22 subjects), thus leaving 1009 subjects for analysis including 682 HIV+ subjects and 327 healthy controls. This cohort overlaps by 98% the cohort examined in our association study of *OPRM1* variants with response to HIV treatment [29]. The subset of these HIV+ subjects who had complete data across three study points R, S and T (R- entrance to WIHS study, S- initiation of HAART and T- most recent WIHS visit for which clinical data was available), were used for comparison of change of viral load and CD4 count at study intervals X (from entry to WIHS to start of HAART) and Y (start of treatment until most recent visit, Table 1B). A different subset of HIV+ subjects who had complete data across two of three study points (R to S or S to T) was used for analysis of the influence of genotype on change of viral load or CD4 count across intervals X or Y. Study point S was chosen to be in the range from 6 months before start of HAART up to one month after initiation of HAART. Lengths of the intervals X and Y varied widely among individuals and this was taken into account in the regression analysis (see Statistical Analysis section below).

OPRK1 and PDYN genotyping

The structures of *OPRK1* and *PDYN* are shown in Fig. 1A–H. Genetic markers were chosen based on the reported genotype frequency (>0.05 in African Americans or Caucasians, HapMap data), location in the gene (preference was given to variants from the coding region and promoter), and previous reports on association with various medical conditions. Some of the variants chosen for this study had been reported (HapMap) to be in linkage disequilibrium (LD) in two, but not all three ethnic groups of our study.

DNA from peripheral blood mononuclear cell pellets obtained from the WIHS repository was isolated using the PureGene® DNA purification kit (Gentra Systems, Minneapolis, MN) and treated with RNase A, yielding about 3.5 µg per subject. For genotyping, in each well of a 384 optical plate (ABI, Applied Biosystems, Foster City, CA), 1 µl of DNA (3 ng/µl) was mixed with 4 µl of the solution containing Universal PCR Mastermix (ABI), water and custom synthesized oligonucleotide primers and probes specific for each polymorphism (ABI, Supplemental Table 1). PCR cycling was performed according to an ABI protocol. Genotype analysis was done on the ABI Prism® 7900 sequence detection system using SDS 2.2 software (ABI). For each polymorphism, the results of TaqMan genotyping were confirmed by Sanger sequencing of 22 randomly- selected samples using the same primers.

Genotyping of the *PDYN* 68-bp repeat (rs35286251) was done by amplification of the area surrounding this variant in two replicates: first, using 5'-FAM-labeled forward and unlabeled reverse primers and then, using labeled reverse and unlabeled forward primers (sequences are in Supplemental Table 1). The amplification was performed using a step-down protocol; fragments were analyzed using an ABI 3730xl instrument and GeneMapper 4.0 software (ABI) as described earlier [30]. For calibration and confirmation, 20 homozygous samples were sequenced by the Sanger method.

Statistical analysis

As the CD4 counts and viral load were strongly skewed (long right-hand tail), they were natural log-transformed for analysis. For each variant, Hardy-Weinberg equilibrium (HWE) tests were determined in HIV- controls with likelihood ratio chi-square, separately for each ethnicity (84 tests). Differences in genotype frequencies among ethnicities were determined using chi-square tests in controls.

To assess the change in viral load and CD4 counts before and after initiation of HAART, analysis of variance with repeated measures was used for each ethnicity in the subset of HIV + subjects with values at each of three study points: R, S and T (Table 1B).

All but one (rs35286251) of the genetic markers were single nucleotide polymorphisms (SNPs). For these markers, genotypes AA, AB, and BB were coded 0, 1, and 2, respectively. This coding scheme is proportional to the number of B alleles in an individual and will reflect the allelic effect of a SNP, based on its genotype. Thus, working with these codes is not affected by deviations from HWE. For the 68-bp repeat for each individual, the two alleles (numbers of repeats) were added together to form this individual's genotype. In a second approach for the 68-bp repeat, allele lengths 1 and 2 repeats were considered S (short), allele lengths >2 repeats were considered L (long), and genotypes were assigned as follows: SS = 1, SL = 2, LL = 3 as described [31, 32]. All analyses were carried out separately for each of the three ethnicities and each marker.

Initially, a standard case-control association analysis was performed to see whether any one of the markers was associated with HIV status. For each marker, logistic regression was carried out with case/control as the dependent variable and marker genotype code as the independent (predictor) variable. Thus, results for each marker were associated with 1 degree of freedom.

For the second type of analysis, the aim was to see whether the rate of increase or decrease of viral load or CD4 count over intervals X and Y was associated with a marker genotype. This analysis (guided by J.O.) involved HIV+ subjects only. For each marker, each ethnicity, and each of the two intervals X and Y, a multiple regression analysis was carried out with natural log-transformed viral load as the dependent variable, and X (or Y) and marker genotype as independent (predictor) variables. Thus, in the analysis for a given SNP, two predictor variables, X (or Y) allowed for different lengths of these time intervals while genotype measured the potential effect of the given SNP on viral load (or CD4 count) for constant time interval length. In these analyses, the statistical software package (SYSTAT, <https://www.systat.com>) identified some individuals as being outliers or having large leverage (impact), that is, exerting an unusually large effect on the result. Whenever the resulting p-value for effect of genotype was 0.10 or less, analyses were repeated with these individuals excluded. If necessary, this procedure was repeated (when new "large leverage" individuals were identified) so that all results with $p < 0.10$ were statistically reliable in the sense that no individuals identified as outliers or having large leverage contributed to the final result.

Analysis of the linkage disequilibrium (LD) of the variants studied was performed separately for each ethnicity using Haploview 4.2 (<http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview>, accessed on March 15, 2011).

RESULTS

Analysis of overall change of viral load and CD4 counts at different study points (Data set 1; Table 1C)

When examining overall changes of the viral load and CD4 count, we used only those subjects who had clinical measurements at all three study points. We found a marked decline of the viral load from admission to WIHS until the start of HAART (interval X) in all three ethnic groups ($p < 0.0001$, Supplemental Figure 1), probably due to azidothymidine (AZT) therapy. Administration of AZT alone or in combination with other modified nucleotides has been shown to reduce viral load by 50 to 90% [33–35]. A significant decline in viral load from start of HAART until the most recent visit (interval Y) was found in African Americans and Caucasians ($p < 0.0001$), but not Hispanics. A significant increase of CD4 count after initiation of HAART was found in African Americans only ($p = 0.0003$; $p = 0.0001$). However, the number of subjects with $CD4 < 500$ at the last WIHS visit was considerably reduced in all ethnicities compared to this number at entry to WIHS or initiation of HAART.

Ethnic differences in genotype frequencies, HWE test, exclusion of low frequency variants

Allele and genotype frequencies of the polymorphisms studied are shown in Supplemental Table 2. In the control group, allele frequencies were significantly different among the three ethnicities for 25 out of 28 genetic markers studied (Supplemental Table 2), with the exception of *OPRK1* variants -298G>A (rs16918955) and coding 36G>T (rs1051660, Pro12Pro) and *PDYN* 1421T>C (rs10485703). Therefore, all tests were performed separately in each ethnicity. Polymorphisms with frequency < 0.05 in both case and control groups were excluded from analysis (Supplemental Table 2, highlighted in yellow). This includes variants of *OPRK1* -72C>T (rs9282806) in Hispanics, and 459C>T (Ser153Ser, rs7815824) and IVS3+3773C>G (rs16918884) in Caucasians. A significant deviation from HWE in controls was found for the variants of *OPRK1* IVS2+7886A>G (rs12548098) and IVS3+3773C>G (rs16918884) in African Americans ($p < 0.0001$). Raw data for these polymorphisms were reviewed and re-evaluated. No technical problems were found.

Multiple regression analysis of association of genotypes of OPRK1 and PDYN with viral load and CD4 count change before initiation of HAART (Data set 2; Table 1D)

A total of 324 multiple regression analyses were carried out ($3 \text{ ethnicities} \times 2 \text{ outcomes (viral load, CD4)} \times 2 \text{ intervals (X, Y)} \times 28 \text{ markers}$) after exclusion of 12 tests (see above). Of these 324 analyses, 225 identified individuals with large leverage, ranging between 1 (114 tests) and 8 (one test). Often it was the same individual who showed large leverage for different markers. The average number of individuals with large leverage among these 225 tests was 1.9.

Using multiple regression analysis in the group of HIV+ subjects only, before HAART, we found a significant effect of the number of T alleles of IVS3+189C>T (rs6035222) in *PDYN* on the viral load change (greater decline, $p = 0.0014$, Fig 2A, Table 2A) in African Americans. No variants of *OPRK1* showed an effect on viral load change before HAART.

We found a significant effect of the number of A alleles of the -1205G>A (rs3808627) in *OPRK1* on CD4 count change (greater decline, $p = 0.0224$, Fig. 3A, Table 2A) before HAART in Caucasians. Subjects bearing genotype AG of IVS2+7886A>G (rs12548098) of

OPRK1 showed a lesser decline of CD4 count before HAART also in Caucasians ($p=0.0095$, Fig. 3C). No subjects with the IVS2+7886GG genotype were observed in Caucasians. Subjects bearing genotype CT of -72C>T (rs9282806) of *OPRK1* showed a lesser decline of CD4 count before HAART in African Americans ($p=0.0404$, Fig. 3B). Four outliers were removed in analysis of -72C>T in African Americans, including the only individual bearing the -72TT genotype; two outliers were removed in analysis of the influence of -1205G>A and IVS2+7886A>G on CD4 count change in Caucasians. No variants of *PDYN* showed an effect on CD4 count change before HAART.

Multiple regression analysis of association of genotypes of *OPRK1* and *PDYN* with viral load and CD4 count change after initiation of HAART (Data set 2; Table 1D)

After initiation of HAART, a significant effect of the number of A alleles of the IVS2+2225G>A (rs6985606) of the *OPRK1* on viral load change (greater decline) was found in Caucasians ($p=0.0033$, Fig. 2B, Table 2B). Also in Caucasians, a lesser decline of viral load was found in individuals bearing genotypes TT and GT compared to GG genotype of IVS2+10658G>T (rs1365098, $p=0.0376$, Fig. 2C) and in individuals bearing genotypes GG and AG compared to AA genotype of IVS2+10963A>G (rs997917, $p=0.0376$, Fig. 2D). Genotypes of the polymorphism IVS2+10658G>T completely predict genotypes of IVS2+10963A>G.

In Hispanics, individuals with genotype AA and GA of IVS2+2225G>A showed a lesser increase of CD4 count after initiation of HAART ($p=0.0401$, Fig. 3D, Table 2B). Two individuals were removed from this analysis as outliers. No variants of *PDYN* showed an effect on viral load or CD4 count change after initiation of HAART.

Logistic regression analysis of *OPRK1* and *PDYN* variants and HIV status (Included data is shown on Table 1A)

Using logistic regression, in all controls and cases we found that in African Americans, frequency of the minor allele A of the *OPRK1* promoter variant -1205G>A (rs3808627) and allele A of the possible *PDYN* promoter variant -11128G>A (rs1997794) are lower in the HIV+ group than in controls, indicating possible protection from infection (Table 2C).

In this initial study all findings in multiple regression analyses or logistic regression analyses were only point-wise significant and did not withstand multiple test correction. Further studies are needed to validate these genetics findings and the functionality of the variants.

Analysis of linkage disequilibrium (LD)

LD analysis was performed in the group of HIV- controls only, separately for each ethnic group (Supplemental Figure 2). In the *OPRK1* gene, very high LD ($r^2 > 0.95$) was found for the variant pair rs997917/rs1365098 in all three ethnicities; for the pairs rs3802281/rs3802282 and rs963549/rs702764 in Caucasians and African Americans; pair rs16918875/rs16918955 in Caucasians; and pair rs3802281/rs963549 in African Americans and Hispanics. Several pairs of *OPRK1* variants including rs3802282/rs702764, rs3802281/rs702764 and rs3802282/rs963549, were found to be in high LD ($r^2 > 0.75$) in all three ethnicities.

In the *PDYN* gene, in all three ethnicities, very high LD ($r^2 > 0.95$) was observed among variants rs2235749, rs910079, and rs910080, and also between rs10854244 and rs6045935.

DISCUSSION

Before the initiation of HAART, we found a significant effect of the number of T alleles of intronic *PDYN*IVS3+189C>T on the slope of viral load decline in African Americans. This polymorphism has been found to be in association with alcohol dependence [36]. No other variants of *PDYN* were found to have a significant effect on either viral load or CD4 count change before or after HAART. However, a promoter allele *PDYN*-11128A (rs1997794) was found to be in association with HIV status (protection) also in African Americans. This variant might be involved in regulation of promoter activity and might influence gene expression through formation of a non-canonical AP-1 binding site [37]. This variant has been found to be in association with opioid dependence in Chinese females [38] and with alcohol dependence in Caucasians [36]. In tests of cognitive function, carriers of the minor allele of this variant, and also variant 1508T>C (rs910080), had higher episodic memory scores than homozygote carriers of the major allele [39]. Decline of cognitive function is one of the challenges of HIV+ patients. We did not find this polymorphism 1508T>C to be in association with either viral load or CD4 count change in our study.

The 68-bp *PDYN* promoter repeat (rs35286251) is involved in regulation of gene expression [40–42]. Three or more copies of this variant (or “long” allele vs. one or two copies or “short” allele) have been found to be associated with opioid dependence in Chinese [43] and with cocaine dependence as well as with cocaine/alcohol codependence in African Americans [31, 32]. Epidemiologic studies have shown that drug abuse may exacerbate HIV disease progression [e.g., 44]. However, we did not find an effect of this polymorphism on change of viral load or CD4 count either before or after HAART, using the long-short allele paradigm or using the sum of repeats from two alleles in each individual.

Some of the *OPRK1* variants that were found to interact with viral load or CD4 count change in this study have been found to be in association with alcohol dependence in Caucasians [36], including IVS2+2225G>A (rs6985606), IVS2+7886A>G (rs12548098) and IVS2+10963A>G (rs997917). Other studies indicate an association of haplotype GGCTTCT of the *OPRK1*, consisting of -3731C>T (rs12675595), 36G>T, IVS2+2225G>A, IVS2+10963A>G, 843A>G, 1176G>A and 4139A>G (rs7820807), with alcohol dependence also in Caucasians [45].

We found an association of the *OPRK1* -1205G>A of the possible promoter region with HIV status in African Americans. Studies have shown that the *OPRK1* is potentially involved in modulation of stress responsivity and depression (e.g., 46–47). Stress, depressed mood, and dysthymic disorders have been shown to be associated with unsafe sex practices (e.g. 48) and, therefore, HIV infection. As the result of self-medication for depression, abuse of drugs, in turn, may lead to infection with HIV through sharing of needles or by using unsafe sex practices under the influence of drugs (e.g., 9–12).

No association of the synonymous *OPRK1* 36G>T variant with HIV status, or change of viral load or CD4 count was found in our study. This variant has been previously found to be in association with vulnerability to develop opiate addiction [49–50].

Previous studies indicate possible involvement of *OPRK1* in activation or inhibition of proinflammatory cytokines IL-1, IL-2, IL-6 and TNF-alpha that are involved in inflammatory response to HIV infection [1] and also, in possible regulation of expression of CXCR4 responsible for HIV entry into cells [22, 23]. *OPRK1* polymorphisms analyzed in this study may potentially alter either expression of the *OPRK1* gene or its mRNA splicing thus altering cytokine activation or *CXCR4* expression, which may result in observed alteration of viral load or CD4 change.

Polymorphisms of the *OPRK1* gene have also been studied for association with other medical conditions including nausea and vomiting in cancer patients receiving opioids and pressure pain sensitivity in females, but no significant findings were made. Contradictory results were found in association of *PDYN* gene variants with temporal lobe epilepsy and schizophrenia; no association of *PDYN* polymorphisms with atopic dermatitis was found.

The large number of African Americans in this study allows interpreting associations found in this ethnicity with greater confidence. A limitation of this study is a relatively small number of Hispanic and Caucasian subjects.

Our findings may be considered evidence of the involvement of *OPRK1* and *PDYN* variants and also, the OPRK1 and PDYN systems in response to HIV treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Susan Russo for editorial assistance. We acknowledge the NIH-NIAID, which supported The Women's Interagency HIV Study and principal investigators of these studies (see the detailed acknowledgements provided below). We also acknowledge Susan Holman for facilitation and Judith Cook for help in our studies. We thank the women participating in the WIHS for their time, cooperation and support. This work was supported by the National Institutes of Health P60 DA05130 (MJK), MH79880 (MJK) and MH076537 (HC).

"Data in this manuscript were collected by the Women's Interagency HIV Study (WIHS) Collaborative Study Group with centers (Principal Investigators) at New York City/Bronx Consortium (Kathryn Anastos); Brooklyn, NY (Howard Minkoff); Washington DC, Metropolitan Consortium (Mary Young); The Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt); Los Angeles County/Southern California Consortium (Alexandra Levine); Chicago Consortium (Mardge Cohen); Data Coordinating Center (Stephen Gange). The WIHS is funded by the National Institute of Allergy and Infectious Diseases (U01-AI-35004, U01-AI-31834, U01-AI-34994, U01-AI-34989, U01-AI-34993 and U01-AI-42590) and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (U01-HD-32632). The study is co-funded by the National Cancer Institute, the National Institute on Drug Abuse and the National Institute on Deafness and Other Communication Disorders. Funding is also provided by the National Center for Research Resources (UCSF-CTSI Grant Number UL1 RR024131)".

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Figure 1. Structure of *OPRK1* and *PDYN* genes

A. Location of the polymorphisms of the *OPRK1* gene selected for these studies in chromosome 8; B. mRNA of the *OPRK1* gene; C. Protein structure of the *OPRK1* gene; D. The list of the polymorphisms in the *OPRK1* gene used in this study; E. Location of the polymorphisms of the *PDYN* gene selected for these studies in chromosome 20; F. mRNA of the *PDYN* gene; G. Protein structure of the *PDYN* gene; H. The list of the polymorphisms in the *PDYN* gene used in this study; TM1–7, transmembrane domains; 5'-UTR and 3'-UTR, untranslated regions; VEON-Vertebrate endogenous opioids neuropeptide (21–68 amino acids); BN- Beta neoendorphin (175–183 amino acids); DYN,

Dynorphin (207–223 amino acids); LEUM, Leumorphin (226–254 amino acids); RIM, Rimorphin (226–238 amino acids); LEU-ENK, Leu-enkephalin (226–230 amino acids). Nucleotide positions in the gene are assigned using a start point (+1) translation initiation site ATG.

Figure 2.

Influence of the genotypes of *OPRK1* and *PDYN* variants on change of ln of viral load from admission to WIHS to initiation of HAART (A) and from initiation of HAART to the most recent visit (B–D).

The left Y-axis shows ln values of viral load (used in statistical analysis); the right Y-axis shows the actual (untransformed) values of viral load. Clinical improvements were found in carriers of the minor allele of the *PDYN* IVS3+189C>T in African Americans and *OPRK1* IVS2+2225G>A in Caucasians. Clinical deterioration was found in carriers of the minor allele of *OPRK1* IVS2+10658G>T and IVS2+10963A>G in Caucasians.

Figure 3.

Influence of the genotypes of the *OPRK1* variants on change of CD4 count from admission to WIHS to initiation of HAART (A–C) and from initiation of HAART to the most recent visit (D).

The left Y-axis shows $\ln$ CD4 count (used in statistical analysis); the right Y-axis shows the actual (untransformed) values of CD4 count. Clinical improvements were found in carriers of the minor allele of the *OPRK1* –72C>T and IVS2+7886A>G in Caucasians. Clinical deterioration was found in carriers of the minor allele of *OPRK1* –1205G>A in Caucasians and *OPRK1* IVS2+2225G>A in Hispanics.

Table 1.

A. Demography of the subjects involved in this study and reasons for exclusions

Group of subjects	HIV–	HIV+	Total
Total number of samples received			1506
Included			
African Americans	213	463	676
Caucasians	46	112	158
Hispanics	68	107	175
Total included (HIV+ and HIV-)	327	682	1009
Subjects for whom clinical data for 2 of the 3 study points was available		598	
Excluded			
Mixed ethnicity	134	287	421
Low DNA concentration			45
Subjects whose CD4 count was found below 10		22	22
Seroconverted after admission to WIHS study			6
Started HAART before admission to WIHS		1	1
Started HAART after the most recent visit		1	1
Missing longitudinal data		1	1
Total Excluded			497

B. Definition of study points (study design)

	Admission to WIHS	Initiation of HAART	Most recent visit
Study points:	R	S	T
Time intervals:	X (R to S)		Y (S to T)

C. Demography of HIV+ subjects used for clinical data analysis of viral load (VL) or CD4 count change when data on all three study points (R, S and T) were available

Number of subjects in each group		
Ethnicity	VL	CD4
African Americans	264	277
Caucasians	80	88
Hispanics	74	76
Total	418	441

D. Demography of the HIV+ subjects used for statistical genetics (logistic regression) analysis of association of the *OPRK1* and *PDYN* polymorphisms with change of viral load (VL) or CD4 count when data on two of three (R and S or S and T) study points were available

Number of subjects in study group				
Ethnicity	VL- interval X	VL- interval Y	CD4- interval X	CD4- interval Y
African Americans	375	378	397	397

D. Demography of the HIV+ subjects used for statistical genetics (logistic regression) analysis of association of the *OPRK1* and *PDYN* polymorphisms with change of viral load (VL) or CD4 count when data on two of three (R and S or S and T) study points were available

Ethnicity	Number of subjects in study group			
	VL- interval X	VL- interval Y	CD4- interval X	CD4- interval Y
Caucasians	94	95	102	102
Hispanics	91	92	99	99
Total	560	565	598	598

Significant results of an association of the *OPRK1* and the *PDYN* variants with viral load (VL) and CD4 count change and with HIV status

Table 2

A. Regression analysis of the genotypes of *OPRK1* and *PDYN* in HIV+ subjects only with viral load and CD4 count change over X^a (Data set 2, Table 1D)

Variants	p-values of VL change over X			p-values of CD4 count change over X		
	Amer. (n=375)	Cauc. (n=94)	Hisp. (n=91)	A. Amer. (n=397)	Cauc. (n=102)	Hisp. (n=99)
-1205G>A (rs3808627, <i>OPRK1</i>)	N/S	N/S	N/S	N/S	0.0224 (D) ^b	N/S
-298G>A (rs16918955, <i>OPRK1</i>)	N/S	N/S	N/S	0.0690	N/S	N/S
-72C>T (rs9282806, <i>OPRK1</i>)	N/S	N/S	N/S	0.0404 (I) ^b	N/S	N/S
IVS2+7886A>G (rs12548098, <i>OPRK1</i>)	N/S	N/S	N/S	N/S	0.0095 (I) ^b	N/S
IVS2+13236T>A (rs7016778, <i>OPRK1</i>)	N/S	N/S	N/S	N/S	0.0713	N/S
846C>T (Val282Val, rs16918875, <i>OPRK1</i>)	N/S	N/S	0.0727	N/S	N/S	N/S
-13315A>T (rs10854244, <i>PDYN</i>)	0.0880	N/S	N/S	N/S	N/S	N/S
IVS3+189C>T (rs6035222, <i>PDYN</i>)	0.0014 (I) ^b	N/S	N/S	N/S	N/S	0.0533
600T>C (His200His, rs6045819, <i>PDYN</i>)	0.0541	N/S	N/S	N/S	N/S	N/S

B. Regression analysis of the genotypes of *OPRK1* and *PDYN* in HIV+ subjects only with viral load (VL) and CD4 count change over Y^a (Data set 2, Table 1D)

Variants	p-values of VL change over Y			p-values of CD4 count change over Y		
	Amer. (n=378)	Cauc. (n=95)	Hisp. (n=92)	A. Amer. (n=397)	Cauc. (n=102)	Hisp. (n=99)
36G>T (Pro12Pro, rs1051660, <i>OPRK1</i>)	N/S	N/S	N/S	N/S	0.0842	N/S
IVS2+2225G>A (rs6985606, <i>OPRK1</i>)	N/S	0.0033 (I) ^b	N/S	N/S	N/S	0.0401 (D) ^b
IVS2+10658G>T (rs1365098, <i>OPRK1</i>) ^c	N/S	0.0376 (D) ^b	N/S	N/S	N/S	N/S
IVS2+10963G>A (rs997917, <i>OPRK1</i>) ^c	N/S	0.0376 (D) ^b	N/S	N/S	N/S	N/S
IVS3+4504C>A (rs7817710, <i>OPRK1</i>)	N/S	0.0590	N/S	N/S	N/S	N/S
846C>T (Val282Val, rs16918875, <i>OPRK1</i>)	N/S	N/S	0.0756	N/S	N/S	N/S
IVS3+189C>T (rs6035222, <i>PDYN</i>)	0.0561	N/S	N/S	N/S	N/S	N/S
1421T>C (rs10485703, <i>PDYN</i>)	0.0575	N/S	N/S	N/S	N/S	N/S

C. Logistic regression analysis of the polymorphisms of the *OPRK1* and *PDYN* and HIV status (Data set from Table 1A)

Variant	p-values of analysis in each ethnic group		
	A. Americans (n=213 HIV ⁻ , n=463 HIV ⁺)	Caucasians (n=46 HIV ⁻ , n=112 HIV ⁺)	Hispanics (n=68 HIV ⁻ , n=107 HIV ⁺)
-1205G>A (rs3808627, <i>OPRK1</i>)	0.0297 ^d	0.6232	0.9682
-11128G>A (rs1997794, <i>PDYN</i>)	0.0200 ^d	0.5297	0.2668

N/S, association is not significant; A.Amer., African Americans; Cauc., Caucasians; Hisp., Hispanics

^a values of p<0.10 are shown, point-wise significant values (p<0.05) are indicated in bold italic;

^b effect of genotypes consisting of minor allele on viral load change or CD4 count change was evaluated; greater decline of viral load or increase of CD4 count was interpreted as improvement of clinical conditions (D); greater increase of viral load or decrease of CD4 count was interpreted as deterioration of clinical conditions (D);

^c genotypes of polymorphism IVS2+10658G>T were completely predicted by genotypes of the polymorphism IVS2+10963A>G in Caucasians;

^d frequency of minor allele A is lower in HIV⁺ group