Single Nucleotide Polymorphism in Gene Encoding Transcription Factor Prep1 Is Associated with HIV-1-Associated Dementia

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Abstract

**Background:** Infection with HIV-1 may result in severe cognitive and motor impairment, referred to as HIV-1-associated dementia (HAD). While its prevalence has dropped significantly in the era of combination antiretroviral therapy, milder neurocognitive disorders persist with a high prevalence. To identify additional therapeutic targets for treating HIV-associated neurocognitive disorders, several candidate gene polymorphisms have been evaluated, but few have been replicated across multiple studies.

**Methods:** We here tested 7 candidate gene polymorphisms for association with HAD in a case-control study consisting of 86 HAD cases and 246 non-HAD AIDS patients as controls. Since infected monocytes and macrophages are thought to play an important role in the infection of the brain, 5 recently identified single nucleotide polymorphisms (SNPs) affecting HIV-1 replication in macrophages in vitro were also tested.

**Results:** The CCR5 wt/Δ32 genotype was only associated with HAD in individuals who developed AIDS prior to 1991, in agreement with the observed fading effect of this genotype on viral load set point. A significant difference in genotype distribution among all cases and controls irrespective of year of AIDS diagnosis was found only for a SNP in candidate gene **PREP1** (p = 1.2 × 10⁻⁵). Prep1 has recently been identified as a transcription factor preferentially binding the −2,518 G allele in the promoter of the gene encoding MCP-1, a protein with a well established role in the etiology of HAD.

**Conclusion:** These results support previous findings suggesting an important role for MCP-1 in the onset of HIV-1-associated neurocognitive disorders.

Introduction

While the prevalence of HIV-1-associated dementia (HAD) has greatly decreased, first with the introduction of zidovudine [1,2] and later with combination antiretroviral therapy (cART) [3,4], neurocognitive impairment is still seen more frequently in HIV-1-infected patients than in seronegative individuals. In recent years a new terminology has been developed to classify this broadening clinical spectrum of neurocognitive impairment, including milder abnormalities. HAND (HIV-1-associated neurocognitive disorders) is the umbrella definition, comprising three entities: asymptomatic neurocognitive impairment, mild neurocognitive disorders (MND), and HAD. Clinical symptoms of HAND are cognitive impairment (memory, concentration), motor dysfunction and behavioral changes. Recent studies showed that MND occurred in 15–50% of the HIV-1-infected individuals [5–9], and HAD in 1–10% of the patients [4,5,7,8].

Although CD4+ T cells are the predominant cell type infected by HIV-1 and primarily associated with the disease course, circulating monocytes as well as macrophages can also become infected and contribute to the viral reservoir and disease progression [10]. Furthermore, monocytes and macrophages play a crucial role in certain HIV-1-related pathologies, including HAND [10]. Despite lack of strong evidence it is generally believed that HIV-1 migrates across the blood-brain barrier in monocytes that were infected in the blood [11,12]. Indeed, in the
brain, the monocyte-derived perivascular macrophages and microglia are the most commonly HIV-1-infected cells [13,14]. Complex mechanisms underlie the neurodegeneration, since neurons themselves are not infected by HIV-1. Local production of HIV-1 proteins [15–18] or other non-HIV compounds [19–24] by infected and activated macrophages and microglia cause neuronal damage. Furthermore, neuronal injury may occur as a consequence of the inflammatory process in the brain [25–27].

As is the case for many complex disorders, it remains unclear why some individuals are more at risk to develop HAND than others. The cause of the neurodegeneration is multi-factorial, and in addition to viral genetic factors [28–30], host genetic predisposition may also contribute to the susceptibility to these disorders. We previously reported a reduced prevalence of the 328 Van Rij [31], HAD; prospective cohort study (n = 121) Study population

In total we selected 86 AIDS patients with HAD (cases) from the Amsterdam Cohort Studies and ATHENA observational cohort from whom DNA was available for genotyping. Before the AIDS dementia complex was defined as a distinct clinical syndrome the diagnosis of dementia in these patients had been based on DSM-III (Diagnostic and Statistical Manual of Mental Disorders) criteria. Motor abnormalities were present in all these demented patients, which agreed retrospectively with the diagnostic criteria of the AIDS dementia complex [42]. When more precise

<table>
<thead>
<tr>
<th>Table 1. Overview of all common genetic variants tested for association with HIV-1-associated neurocognitive disorders.</th>
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<tbody>
<tr>
<td>Gene</td>
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<tr>
<td>APOE</td>
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<tr>
<td>CCL3</td>
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<tr>
<td>CCR2</td>
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<tr>
<td>CCR5</td>
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<td>MCP-1</td>
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<tr>
<td>TNFA</td>
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<tr>
<td>---</td>
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<tr>
<td>Prep1</td>
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</table>

HIV-1, HIV-1-associated neurocognitive disorders; HAD, HIV-1-associated dementia; HIVE, HIV-1 encephalitis.

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diagnostic criteria were introduced in 1991 and 2007 ([43,44]), these criteria were used to classify these patients. The absence of dementia was confirmed by neurological examination by a neurologist.

We compared the HAD patients with 246 AIDS patients without HAD (controls) (Table 2). A subset of these samples (49 cases and 186 controls) was included in a previous study that investigated CCR5 Δ32 and CCR2 V64I genotype frequencies between HAD cases and controls [31].

Cases and controls were matched for year of AIDS diagnosis (AIDS diagnosis was based on AIDS defining events according to the CDC AIDS definition 1987; Kaposi’s sarcoma was excluded as an AIDS defining event), time from AIDS diagnosis to death or to start cART, age at AIDS diagnosis and CD4+ T cell count at AIDS diagnosis. Cases receiving cART more than 6 months before their HAD diagnosis (n = 14), as well as controls who started cART more than 6 months before their AIDS diagnosis (n = 5) were excluded from the analysis. Median time from AIDS to developing HAD for the cases was significantly shorter than the time from AIDS to death or to start cART in the control population (p<0.0001; Mann Whitney test) (Table 2), indicating that time from AIDS to death or to start cART for the HIV-1-infected individuals in the control group was in principle long enough to develop HAD. Similar results were obtained when using Kaplan-Meier analysis, with no difference in time from AIDS to death (start cART used as censor) between cases and controls (p = 0.11, logrank test) and significant shorter time from AIDS to HAD for the cases, than time from AIDS to death (start cART as censor) for the controls (p = 0.00036, logrank test) (Figure 1A–B). Information on ancestry was only known for a limited number of patients and was based on reported ethnicity by the treating physician or reported country of birth.

### Candidate SNP selection and genotyping

Genotype distributions of polymorphisms previously associated with HAND (Table 1) as well as SNPs associated with in vitro HIV-1 replication in macrophages (cutoff p value = 5 × 10^-5) (Table S1) [41], were analyzed in this case-control study. PREP1

### Table 2. Characteristics of the studied population consisting of AIDS patients with or without HAD.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HAD patients (cases, n = 72)</th>
<th>Non-HAD patients (controls, n = 241)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time AIDS to death or start cART (months); median (range)</td>
<td>14 (0–114) n = 67</td>
<td>12 (0–81) n = 234</td>
<td>0.21</td>
</tr>
<tr>
<td>Time AIDS to HAD (months); median (range)</td>
<td>5 (0–114) n = 69</td>
<td>N.A.</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at diagnosis AIDS; average (range)</td>
<td>40 (22–63) n = 69</td>
<td>41 (23–71) n = 241</td>
<td>0.60</td>
</tr>
<tr>
<td>CD4+ T cell count (cells/μl) at AIDS, median (range)</td>
<td>120 (10–850) n = 39</td>
<td>105 (7–1,380) n = 166</td>
<td>0.48</td>
</tr>
<tr>
<td>Mode of HIV-1 transmission (IDU : other)</td>
<td>4 : 35</td>
<td>4 : 158</td>
<td>0.048</td>
</tr>
</tbody>
</table>

N.A., not applicable; HAD, HIV-1-associated dementia; IDU, injecting drug user.

1 Mann Whitney test.

2 Time to develop HAD after AIDS diagnosis among the cases was compared to the time from AIDS diagnosis to death or to start cART in the control group.

3 Unpaired t test.

4 CD4+ T cell counts within 6 months to the date of AIDS diagnosis.

5 Fisher’s exact test.

**Table 2.** Characteristics of the studied population consisting of AIDS patients with or without HAD.

**Figure 1.** Comparison of AIDS survival between HAD cases and non-HAD controls. (A) Kaplan Meier analysis for time from AIDS to death with start cART as censor (vertical lines), for both HAD cases (grey line) and non-HAD AIDS patients as controls (black line). (B) Kaplan Meier analysis for time from AIDS to death with start cART as censor (vertical lines) for the controls (black line) and for time from AIDS to HAD for the HAD cases (grey line). cART, combination antiretroviral therapy; HAD, HIV-1-associated dementia.

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was selected because of its preferred binding to the −2,518 G
allele in the promoter region of MCP-1. SNP rs2839619 was
selected from 17 SNPs in PREP1 after preliminary analysis
comparing 15 cases with 126 unmatched controls. This SNP seems
of particular interest since it was shown to be associated with
cholesterol metabolism [45], which is known to play a role in the
etiology of Alzheimer’s disease [46] and in addition, is in linkage
disequilibrium ($r^2 = 0.51$) with nearby intronic SNP rs234720 that
has been associated with cognitive test performance [47].

Peripheral blood mononuclear cells were used for isolation of
genomic DNA using QIAamp DNA blood mini kit (Qiagen,
Valencia, CA, USA) or NucleoSpin blood kit (Machery-Nagel,
Dueren, Germany). SNP genotypes for SNPs in Dyrk1A, Pde8A,
Ubr7 and PREP1 (Table S1) were available for 172 individuals
(18 cases, 154 controls) from a recent study on the effects of host
genetic variation on HIV-1 susceptibility and disease progression
[48]. For the remaining DNA samples and other SNPs not present
on the Illuma SNP beadchip, ABI TaqMan® SNP genotyping
assays (Applied Biosystems, Carlsbad, CA, USA) were used for
genotyping (Table S1). For all SNPs except the SNP in Tnfa,
genotyping assays were run on a LightCycler® 480 system (Roche,
Basel, Switzerland) using Probes Master (Roche) with the following
amplification cycles: 10 min 95°C; 50 cycles of 15 sec 95°C, 1 min 60°C.

The Tnfa SNP assay was run on an Applied Biosystems 7500
Fast Real-Time PCR System (Applied Biosystems) with Taqman
genotyping master mix (Applied Biosystems), and using the
following amplification cycles: 10 min 95°C; 40 cycles of 15 sec
95°C, 1 min 60°C. APOE allele types (E2, E3 or E4 [49]) were
determined by genotyping SNPs rs429358 (C or T) and rs7412 (C
or T). Ccr5 Δ32 and Ccr2 V64I genotyping was performed as
described previously [50,51].

Quantitative PCR

Buffy coat or full blood was obtained from 69 healthy blood
donors. Monocyte isolation and monocye-derived macrophage
(MDM) culture was performed as previously described [52]. Total
RNA was extracted from day 7 uninfected MDM using the High

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Cases (HAD)</th>
<th>Controls (no HAD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA AB BB</td>
<td>AA AB BB</td>
<td></td>
</tr>
<tr>
<td>APOE</td>
<td>E4 isoform²</td>
<td>52 16 1</td>
<td>158 52 5</td>
<td>0.95</td>
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<tr>
<td>CCL3</td>
<td>rs1130371</td>
<td>40 26 5</td>
<td>133 90 9</td>
<td>0.53</td>
</tr>
<tr>
<td>CCR2</td>
<td>rs1799864 (V64I)</td>
<td>64 8 0</td>
<td>206 34 1</td>
<td>0.66</td>
</tr>
<tr>
<td>CCR5</td>
<td>Δ32</td>
<td>66 6 0</td>
<td>203 38 0</td>
<td>0.13</td>
</tr>
<tr>
<td>Dyrk1A</td>
<td>rs12483205</td>
<td>38 25 8</td>
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<td>MCP-1</td>
<td>rs1024611 (∼−2518 A&gt;G)</td>
<td>3 27 41</td>
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¹Polymorphisms selected from earlier studies that tested for association between genotype and HAD.
²In the case of APOE AA, AB and BB refer to no APO E4, one APO E4 allele and two APO E4 alleles, respectively.
³SNPs selected from a previous study that found associations between these SNPs and HIV-1 replication in macrophages.
⁴Significant difference after correction for multiple testing (n = 12); Bonferroni threshold p = 4.2 × 10⁻³.

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Table 3. Genotype distribution among HAD (cases) and non-HAD (controls) HIV-1-infected patients for all polymorphisms tested.
cases with known non-Caucasian ethnicity, or for whom injecting drug use was reported as mode of HIV-1 transmission, a known risk factor for HAD [4], the difference in PREP1 SNP genotype distribution between cases (n = 64) and controls remained significant (n = 211) (*p = 4.3 × 10^-5"). Quantitative PCR experiments performed to investigate if the SNP in PREP1 was associated with either PREP1 or MCP-1 mRNA levels showed no difference in PREP1 or MCP-1 mRNA levels between the three PREP1 SNP genotypes (*p = 0.3 and 0.8, respectively, n = 69; one-way ANOVA; data not shown).

Remarkably, for none of the other polymorphisms tested, including those previously reported to be associated with HAD (CCR5 Δ32, promoter SNP in MCP-1, the −308 G>A SNP in TNEA, CCR2 V64I variant, Apo E4 isoform and a SNP in CCL5), a significant difference was found in genotype distribution between cases and controls. In a previous case-control study [31], we described a reduced prevalence of the CCR5 wt/Δ32 genotype among HAD patients. A subset of cases and controls from this published study overlaps with our current study population. Cases and controls that were additionally included for this study seroconverted on average later in time. We recently reported that in the HIV-1 epidemic in The Netherlands, the impact of certain host factor polymorphisms, including CCR5 Δ32, might be fading [53]. We therefore hypothesized that the protective impact of the CCR5 wt/Δ32 genotype on the onset of HAD also may have decreased over time. To test this hypothesis we divided cases and controls into two groups using the median year of AIDS diagnosis of the complete study population (1990). This approach was chosen over using seroconversion date since this information was unavailable for 29 of 42 cases with AIDS diagnosis ≤1990. Importantly, cases and controls with AIDS diagnosis >1990 matched all of the characteristics as described above (Table S4), and time from AIDS to death or to start cART for the HIV-1-infected individuals in the control group was in principle long enough to develop HAD (*p = 0.005 and *p = 0.001 for cases and controls with AIDS diagnosis ≤1990 and >1990, respectively, Mann Whitney test) (Table S4). Similar results were obtained when using Kaplan-Meier analysis and logrank test (data not shown). When the CCR5 wt/Δ32 genotype frequency was compared between cases and controls we observed a difference in the “AIDS diagnosis ≤1990” group (*p = 0.046), but not for the “AIDS diagnosis >1990” group (1.00) (Table S3), indeed suggestive of a fading protective effect. Assuming that other genetic effects may also have diminished over time in our cohort, we performed the same analysis for the remaining polymorphisms (Table S3). For 9 polymorphisms, no significant associations were observed in either group. The effect of the SNP in PREP1 was clearly independent of the year of AIDS diagnosis, since in both groups there were significantly fewer heterozygous individuals for SNP rs2839619 among cases than controls (*p = 0.001 and *p = 0.008 for the “AIDS diagnosis ≤1990” and the “AIDS diagnosis >1990 group”, respectively) (Table S3). Conversely, a significant (defined as *p < 0.05; however not significant after correction for multiple testing, n = 24) association with HAD was observed for the SNP in D1Rk1.4 only in the group with AIDS diagnosis after 1990.

Discussion

Here we describe the first combined evaluation of all previously identified genetic polymorphisms reported to be associated with the prevalence of HAND. In addition, we evaluated polymorphisms that we recently identified to be associated with HIV-1 replication in macrophages for their association with HAD. For one of the 12 polymorphisms tested, SNP rs2839619 in PREP1, we observed a significantly different genotype distribution when comparing AIDS patients with and without HAD. The prevalence of the heterozygous genotype was 55% among controls (and 53% in the HapMap CEU population, n = 226), as compared to only 24% among HAD cases, suggesting that the heterozygous genotype has a protective effect against the development of HAD (positive heterosis). Although multiple examples of heterosis exist ([54,55] and reviewed in [56]), the molecular basis for this heterozygous effect sometimes remains difficult to understand (reviewed in [56,57]).

Case-control studies are greatly influenced by variation in allele frequency across different subgroups [58] that may lead to identification of false positive associations. However, the association for the PREP1 SNP remained significant after excluding patients expected to be of non-European descent. Moreover, since the allele frequency for this SNP is similar for Caucasians, Asians and Africans (NCBI dbSNP) we do not expect that additional population stratification resulting from ethnicity would affect this association. Differences in population structure may be of importance when SNP rs2839619 is not the causal variant but rather tags another genetic variant, since for that particular SNP genotype distributions may vary between different populations. In addition, the outcome remained unaffected after correcting for injecting drug use as mode of HIV-1 transmission. Although it is known that Prep1 binds to the promoter region of CCR5, we were unable to demonstrate an association between SNP genotype and MCP-1 mRNA levels in MDM. However, MCP-1 is secreted by monocytes and macrophages, but expression is not limited to these cell-types. The cytokine is also expressed in for HAD possibly more relevant cells such as endothelial cells, astrocytes, microglia and neurons ([59] and references therein). Functional follow-up studies will need to delineate a mechanism that helps to explain the observed reduced frequency of heterozygous donors in the group of HAD patients.

None of the previously identified associations between genetic variants and HAND could be replicated in our present study, even when tested under a dominant or recessive model (data not shown). Many of the candidate gene polymorphisms suggested to play a role in the prevalence of HAND have not been reproduced widely in other cohorts (Table 1). Limitations in the availability of patient material, heterogeneity in HAND diagnoses, differences in case-control matching strategies and possible population substructure may have contributed to the absence of robust and replicable results. While we tried to carefully address many of these issues in this study, no robust replication of the reported associations was obtained, suggesting that meta-analyses of multiple HAND cohorts may be required to reliably evaluate the effect of host polymorphisms on HAND.

SNPs previously associated with HIV-1 Gag p24 levels in macrophage cultures were not found to be associated with HAD, although meta-analyses may be required to firmly establish this. This possibly suggests that the quantity of HIV-1 replication in macrophages is less important for the etiology of this phenotype as compared to the immune activation in the brain as a consequence of HIV-1 replication [12].

The protective effect observed for the CCR5 wt/Δ32 genotype was only observed in the group of individuals that had an AIDS diagnosis ≤1990 and no longer in the group that was diagnosed with AIDS >1990. Excluding cases and control from non-European descent, in whom the CCR5 wt/Δ32 genotype is less frequent, did not change the outcome of the analysis. In agreement with these findings, we observed a similar fading impact of the
**CCRF5 wt/Δ32** genotype on HIV-1 control over calendar time and at a population level [33].

We also observed a difference in D1941A SNP rs12483205 genotype distribution between cases and controls with AIDS diagnosis 1990. While the minor allele of this SNP was associated with reduced replication of HIV-1 in monocyte-derived macrophages in vitro [41] the observed difference here is the result of 33% more cases homozygous for the major allele, yet only 59% fewer cases with the heterozygous genotype as compared to the controls (data not shown). This difference could possibly be due to population stratification since in both Asian and sub-Saharan African populations the frequency of the homozygous major and heterozygous genotype is higher and lower respectively (dbSNP).

The association of a SNP in PREP1 with the onset of HAD further supports the biological importance of MCP-1 in the pathogenesis of this disease. Replication of this association in an independent cohort using matched HAD cases and controls will now be highly desirable. Functional studies will be required to delineate how the observed difference in allele frequencies can be explained in a biological context.

**Supporting Information**

Table S1  Overview of SNPs genotyped using the ABI TaqMan® SNP genotyping.

(DOC)

Table S2  Overview of primers used for qPCR experiments.

(DOC)

Table S3  Overview of genotype distribution comparisons between HAD cases and controls with AIDS diagnosis before or after 1991.

(DOC)

Table S4  Characteristics of HIV-1-positive patients with or without HAD, divided in a groups with AIDS diagnosis before or after 1991.

(DOC)

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Conceived and designed the experiments: ABW SMB. Performed the experiments: SMB TB DVM EMB MS BBN. Analyzed the data: SMB TB DVM AVS. Contributed reagents/materials/analysis tools: FDWP PH NS NAK ABW. Wrote the paper: SMB TB NAK ABW.

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