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# Dopamine receptor D3 genetic polymorphism (rs6280TC) is associated with rates of cognitive impairment in methamphetamine-dependent men with HIV: preliminary findings

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**Abstract** Macrophages are one of HIV-1's principal targets and chiefly responsible for translocating HIV into the central nervous system (CNS). Previous research suggested an increase in macrophages being infected by HIV in the presence of methamphetamine (METH) or increased extracellular dopamine (DA). Experimental studies indicate that this is mediated by DA receptors, including DA receptor D3 (DRD3), which is expressed in macrophages. A single nucleotide polymorphism (SNP) of the DRD3 gene (rs6280TC) modulates its dopamine binding affinity, resulting in the possibility that inheriting a variant of this SNP increases macrophage susceptibility to HIV infection in the presence of METH and DA, particularly in the CNS where METH is sequestered, leading to cognitive impairment (CI). Thus, we conducted a retrospective clinical investigation to evaluate whether rs6280TC is associated with CI among HIV-positive METH users. We stratified 310 males by HIV serostatus (HIV-positive, -negative) and METH dependence (METH-positive, -negative) and then by rs6280TC genotype (CC, CT, and TT). Genotypic groups within each of four HIV/METH groups were compared for rates of CI. We hypothesized that only HIV-positive/METH-positive carriers of the C allele, which increases the DRD3's binding to DA, would be more likely to develop CI. Cochran–Armitage test for trends in proportions yielded significant ($p<0.05$) association between three genotypes and impairment rates in the hypothesized order, but only among HIV-positive/METH-positive subjects. The results also confirmed that C allele carriers (CC and CT, 53.3%) in this group had higher impairment rates ($p=0.05$) than TT carriers (33.3%). These findings support the theory that rs6280TC influences the frequency of CI in HIV-positive/METH-positive males.

**Keywords** HIV · Methamphetamine · Cognitive impairment · SNP · Dopamine D3 receptor

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## Introduction

Methamphetamine (METH) abuse is commonly comorbid in HIV-1-infected individuals because of a mutual association with risky behaviors such as injection drug use (Strathdee and Stockman 2010) and risky sexual behavior (Cheng et al. 2010; Marquez et al. 2009). Clinical evidence suggests that METH users have increased rates of HIV-1 transmission (Mathers et al. 2008), reduced viral suppression within the central nervous system (CNS; Ellis et al. 2003), and greater rates of cognitive impairment (Scott 2007) than non-METH users. The combined direct and indirect neurotoxic effects on the CNS from HIV viral proteins as well as METH-induced oxidative stress and disruption of neurotransmission are thought to exacerbate neurodegeneration (Reiner et al. 2009), with an increased risk for HIV-associated neurocognitive disorders (Nath

2010; Nath et al. 2001). However, confirmation of METH's role in increasing HIV viral load in the CNS and the subsequent consequence of this in increasing risk for cognitive impairment remain unclear. Recently, experimental evidence has suggested that METH may have a direct effect on HIV viral replication within the CNS.

This effect has been demonstrated in simian models of neuroAIDS (Marcondes et al. 2010) where METH administration led to significantly increased viral loads (VL) in the frontal lobe, caudate, and hippocampus, but not in plasma or cerebrospinal fluid (CSF). Since macrophages are one of the targets of HIV and are known to traffic into the brain from the systemic circulation (Eugenin et al. 2006), the number of activated brain macrophages ($CD14^{+}CD16^{+}$ inflammatory macrophages) were investigated. These were found to be significantly increased in monkeys that were given regular doses of METH as compared with controls. They also found significantly increased numbers of "highly CCR5-expressing" $CD14^{+}CD16^{low}$ macrophages in METH-treated monkeys. However, the authors left open the question of the underlying mechanisms of this effect.

One potential mechanism involves dopamine receptor D3 (DRD3) and monocyte-derived macrophages. DRD3 is one of the five currently identified dopamine receptors and is classified as a dopamine 2-like receptor due to its structural and functional similarities to the dopamine D2 receptor (Missale et al. 1998). In vitro models using human tissue have confirmed the enhanced infection and replication of HIV virus in the presence of METH and a role for the dopamine (DA) receptors. For instance, Liang et al. (2008) infected human monocyte-derived macrophages (MDMs) with HIV in the presence and absence of METH. They reported that treatment with METH significantly enhanced HIV infection (HIV RT activity) and up-regulated CCR5 expression of MDMs in a dose-dependent fashion. They also found that DA receptor antagonists targeting DRD1 neutralized the METH effect of HIV reverse transcription activity, but could not override the METH effect of increased CCR5 receptor expression in macrophages. However, because they do not report experimentation with other DA receptor antagonists, it is possible that a D2-like antagonist may have abrogated this chemokine receptor effect.

Given that stimulant drugs of abuse, and particularly METH, bring about extremely high levels of extracellular DA in the CNS, the investigation of Gaskill et al. (2009) of the role of DA in HIV infection within primary human MDMs deserves review. They infected cultured MDMs with HIV in the presence or absence of DA. They found that MDM cultures inoculated with HIV in the presence of DA significantly increased viral infection, as measured by concentrations of HIV capsid protein p24Gag. They determined that the mechanism for this effect was through infection of greater proportions of HIV-infected macrophages in DA-exposed cultures rather than through enhanced viral replication. Using DA receptor agonists, they determined that the D2-like family of DA receptors mediated this effect, not the D1-like family. Finally, they were able to confirm the work of others (McKenna et al. 2002) that primary human MDMs express functional DA receptors. In sum, there appears to be gathering evidence for the role of METH, DA, and DA receptors in modulating HIV viral load in the brain. This experimental evidence is in consonance with a large extant literature documenting the role of catecholamines (including DA) functioning as immunomodulators (Flierl et al. 2008). Therefore, it seems important to focus the study of the effects of a potent dysregulator of the catecholamine system, such as METH, on immune functioning in neuroAIDS.

Our primary interest in this study was to examine the influence of a functional single nucleotide polymorphism (SNP) in the DRD3 gene (rs6280) involving a serine (T) to glycine (C) amino acid substitution at codon 9 on rates of cognitive impairment in an HIV-infected METH-dependent cohort. Based on the current literature showing that: (1) a T to C substitution increases DRD3's DA binding affinity (Lundstrom and Turpin 1996), (2) HIV infection of MDMs are increased in the presence of METH and high levels of extracellular DA and mediated through DA receptors (Liang et al. 2008; Gaskill et al. 2009), and (3) increases in HIV viral infection of the brain can result in cognitive impairments; we hypothesized that carriers of the C allele may

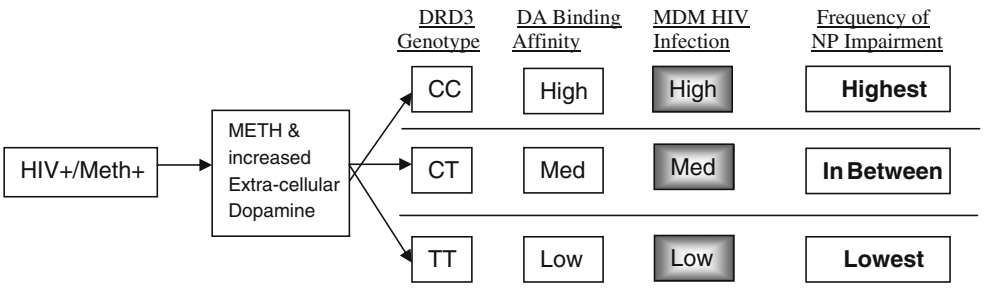


**Fig. 1** Susceptibility to HIV infection

Note: the shaded part of the model is subject to inference because these are living subjects and we cannot biopsy brain tissue

produce macrophages that are more susceptible to HIV infection in the context of METH use and therefore be more likely to develop cognitive impairments than T allele carriers (see Fig. 1). Furthermore, we hypothesized that the implicit specificity of this model would dictate that the same genotypic effect would not be observed in the other three primary groups: uninfected people who do not use METH (HIV-negative/METH-negative), uninfected METH users (HIV-negative/METH-positive), and HIV-infected people who do not use METH (HIV-positive/METH-negative).

## Methods

### Participants

Participants were volunteers evaluated at the HIV Neurobehavioral Research Center (HNRC) at the University of California in San Diego as part of a cohort study focused on central nervous system effects of HIV and METH. The current study comprised 310 ethnically diverse men classified into one of the following four groups: HIV-seronegative/METH nonusers (HIV-negative/METH-negative, $n=56$); HIV-seronegative/METH-dependent (HIV-negative/METH-positive, $n=77$); HIV-seropositive/METH nonusers (HIV-positive/METH-negative, $n=84$); HIV-seropositive/METH-dependent (HIV-positive/METH-positive, $n=93$). At the time and region in which the data were gathered, there was a paucity of women diagnosed with HIV infection and even fewer with HIV reporting METH use. Therefore, the few women from whom we obtained data were excluded from these specific analyses in order to avoid the potentially confounding effect of sex on genetic findings.

All participants underwent a comprehensive characterization procedure that included collection of demographic, neuromedical, psychiatric, as well as neuropsychiatric information. HIV serological status was determined by enzyme-linked immunosorbent assays plus a confirmatory test. Lifetime METH dependence was determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID-IV). However, participants were not actively using other substances, with the exception of cannabis and alcohol. Potential participants were excluded if they met lifetime dependence criteria for other drugs, unless the dependence was judged to be remote (>5 years ago) and episodic in nature by a doctoral-level clinician. Alcohol dependence within the last year was also an exclusion criterion.

Additional information for each participant was collected as it relates to current depressed mood as well as lifetime diagnosis of major depression disorder (MDD) and/or bipolar disorder I or II. Current depressed mood was assessed utilizing the Beck Depression Inventory-I (BDI-I; Beck et al. 1961), and MDD and bipolar disorder were ascertained using the SCID-IV. Information was also collected to determine lifetime dependence on sedatives, cannabis, opioids, cocaine, hallucinogens, and alcohol using the SCID-IV. For all HIV-positive participants, HIV RNA plasma and CSF copies were ascertained as part of a larger neuromedical evaluation. It should be noted that only a subset of participants submitted to a lumbar puncture, so CSF VL data are available for only 61 of 84 HIV-positive/METH-negative and 71 of 93 HIV-positive/METH-positive participants. All participants gave written consent prior to enrollment, and all procedures were approved by the Human Research Protection Program of the University of California, San Diego.

### NP battery

The neuropsychological (NP) assessment and interpretive methods have been described elsewhere (Cherner et al. 2002). Participants completed a comprehensive neuropsychological evaluation assessing seven ability domains (i.e., learning, memory, attention/working memory, processing speed, abstraction/executive functioning, verbal fluency, and motor speed/dexterity). Raw test scores were converted to *T* scores (standard scores with a mean of 50 and standard deviation of 10) using demographically corrected norms to adjust for age, education, gender, and ethnicity, as available for each measure. The demographically corrected *T* scores for each test were then converted into deficit scores using the method developed by Heaton et al. (1995). The deficit scores assign performances within the normal range (*T* scores≥1 standard deviation below the mean), a value of zero, thus reflecting only degree of impairment in 1/2 SD increments (i.e., *T* scores between 39 and 35=1; 34–30=2, 29–25=3, 24–20=4, and <20=5). The individual test deficit scores were then averaged within each domain to derive the domain deficit score that reflects the severity of deficit within each ability area. Previous work has demonstrated that deficit scores achieve good diagnostic agreement with classifications based on blind ratings by clinicians, with a cut point for impairment set at ≥0.50 (Carey et al. 2004; Heaton et al. 1995). In addition, this method has the advantage of data reduction to minimize multiple comparisons and has shown robust relationships with documented brain injury (Heaton et al. 1995; Masliah et al. 1997).

### DNA extraction and genotyping

DNA was extracted from peripheral blood mononuclear cells stored (3–5 years) at −70°C using the QIAamp DNA Mini kit (Qiagen, Valencia, CA; catalog #51185). The *DRD3* Ser9Gly polymorphism (rs6280) was assayed along

with 17 other SNPs as part of a concurrent genetic association project at the HNRC. A multiplex PCR technique designed using Sequenom SpectroDESIGNER software (version 3.0.0.3) was employed by inputting a sequence containing 100 bp of flanking sequence on either side of the *DRD3* rs6280 polymorphism. The SNP was then grouped into multiplexes so that the extended product would not overlap in mass with any other oligonucleotide present in the reaction mix and where no primer–primer, primer–product, or nonspecific interactions would occur. The PCR was carried out in 384-well reaction plates in a volume of 5 μL using 10 ng genomic or whole genome-amplified DNA. All subsequent steps, up until the reaction, were spotted onto the SpectroCHIP and carried out in the same reaction plate. After PCR, any unincorporated dNTPs from the PCR were removed from the reaction by digestion with Shrimp alkaline phosphatase. dNTPs were removed so that they could not play any role in the extension of the oligonucleotide at the SNP site. The extension reaction was then carried out in the presence of the extension oligonucleotide and a termination mix containing mass-modified dideoxynucleotides, which extended the oligonucleotide over the SNP site with one base. Before spotting onto the SpectroCHIP, the reaction was cleaned by incubation with a cation exchange resin, which removed any salts present. The extension product was then spotted onto a 384-well spectroCHIP before being flown in the matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. Data were collected, in real time, using SpectroTYPER Analyzer 3.3.0.15, SpectraAQUIRE 3.3.1.1, and SpectroCALLER 3.3.0.14 (Sequenom) algorithms. All genotyping was performed by an accredited commercial laboratory (Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Cambridge, MA CLIA no. 22D1005307).

### Statistical analyses

Each of the four primary groups (i.e., HIV-negative/METH-negative; HIV-negative/METH-positive; HIV-positive/METH-negative; HIV-positive/METH-positive) were first compared on their background data and comorbidities using chi-square and analysis of variance (ANOVA) for dichotomous and continuous variables, respectively. Additionally, the three genotypic groups (CC, CT, and TT) within the primary groups above were compared on background and comorbidities using the Cochran–Armitage test for trends in ordered proportions. Then, rates of NP impairment across the four primary groups were compared using chi-square. Finally, mean log VLs and rates of cognitive impairment were compared across the three DRD3 genotype groups within each of the four HIV/METH primary groups using ANOVA and chi-square, respectively.

## Results

### HIV, METH, and NP impairment

Although not central to our main hypotheses, comparisons of NP impairment rates across the four primary groups showed that the HIV-positive/METH-positive group had the highest rate of global NP impairment (43%) compared with the HIV-negative/METH-negative (18%), HIV-negative/METH-positive (36%), and HIV-positive/METH-negative (30%) groups ($\chi^2$=10.77, $p$=0.01). The log CSF VL of the two HIV-positive groups did not differ significantly [$F$(1,131)=0.01, $p$=0.91]. The HIV-positive/METH-positive group's log CSF VL was $M$=2.27 (SD=0.81), while the HIV-positive/METH-negative group's was $M$=2.28 (SD=0.92). These groups were not different from one another with respect to most demographics, excepting the number of years of education completed (data not shown). The two METH-positive groups had approximately 1 year less education on average than the two METH-negative groups; however, assessment of NP impairment was based on NP norms corrected for age, education, and ethnicity. The two HIV-positive groups did not differ in HIV disease characteristics such as HIV RNA in plasma or CSF, current and nadir CD4 counts, and treatment status. The two METH-positive groups did not differ in rates of lifetime substance dependence or psychiatric comorbidities. The rank order of impairment rate by the above primary groups was also observed by (Rippeth et al. 2004). Therefore, we anticipated this result because a subset of our subjects was also part of the study by Rippeth and colleagues.

### DRD3 genotype and NP impairment in the context of HIV and METH

In order to address our primary hypotheses regarding the effect of genotype on global NP impairment, within the context of HIV and METH dependence, we decomposed the HIV-positive/METH-positive group into DRD3 genotypic groups: homozygotes for the major allele (TT) which has the lowest affinity for DA, homozygotes for the minor allele (CC) with the highest affinity, and heterozygotes (CT) in the intermediate. As indicated in Table 1, among HIV-positive/METH-positive participants, the three DRD3 genotypic groups were well matched, differing only on proportion of Caucasian and non-Caucasian subjects (Table 1). A clear "gene dose"-dependent trend was observed between the number of C alleles and rates of global NP impairment in the HIV-positive/METH-positive group, but no clear association is observed among the HIV-negative/METH-negative, HIV-negative/METH-positive, or HIV-positive/METH-negative groups, as displayed in

**Table 1** Characteristics of HIV-positive/METH-positive sample by DRD3 genotypes

| | DRD3 genotypic group | | | |
|---|---|---|---|---|
| | CC ($n$=11) | CT ($n$=34) | TT ($n$=48) | $p$ |
| Age, mean (SD) | 38.8 (4.9) | 36.1 (7.5) | 37.4 (7.0) | ns |
| Education mean, (SD) | 12.8 (4.1) | 12.1 (3.1) | 12.2 (2.6) | ns |
| Ethnicity | | | | |
| Caucasian (%) | 36.4 | 64.7 | 77.1 | |
| Non-Caucasian (%) | 63.6 | 34.3 | 22.9 | .04 |
| DSM-IV psychiatric disorder (% lifetime) | | | | |
| Major depression | 45.5 | 32.4 | 54.4 | ns |
| Bipolar disorder | 9.1 | 5.9 | 10.9 | ns |
| BDI-I total score, mean (SD) | 10.5 (9.1) | 13.9 (9.2) | 14.1 (9.3) | ns |
| DSM-IV substance dependence (% lifetime) | | | | |
| Sedative | 0.0 | 0.0 | 0.0 | – |
| Cannabis | 27.3 | 20.6 | 6.5 | ns |
| Opioid | 0.0 | 2.9 | 0.0 | ns |
| Cocaine | 9.1 | 14.7 | 17.4 | ns |
| Hallucinogens | 0.0 | 5.9 | 0.0 | ns |
| Alcohol | 27.3 | 23.5 | 34.8 | ns |
| % Current meth dependence | 9.1 | 26.5 | 21.7 | ns |
| % HCV co-infected | 27.3 | 41.2 | 35.4 | ns |
| HIV disease parameters | | | | |
| HIV RNA, plasma | | | | |
| % Detectable | 45.5 | 62.5 | 54.4 | ns |
| (log copies/mL), mean (SD) | 3.6 (1.4) | 3.6 (1.0) | 3.5 (1.1) | ns |
| HIV RNA, CSF[a] | | | | |
| % Detectable | 25.0 | 42.3 | 37.8 | ns |
| (log copies/mL), mean (SD) | 2.2 (1.19) | 2.4 (0.86) | 2.2 (0.68) | ns |
| Current $CD4^+$ count | 527 (481) | 457 (243) | 432 (337) | ns |
| Nadir $CD4^+$ | 358 (280) | 316 (225) | 224 (176) | ns |
| HIV treatment status | | | | |
| % Currently on ARV medication | 54.5 | 44.1 | 39.1 | ns |
| % Treatment-naive | 27.3 | 35.3 | 41.3 | ns |

[a] Log CSF viral load data available for $n$=8 CC, $n$=26 CT, and $n$=37 TT

Fig. 2. A Cochran–Armitage test for monotonic trends in proportions was applied to evaluate the association of genotype and rates of cognitive impairment in the four primary groups (Armitage 1955; Sasieni 1997). Consistent with the hypotheses, it was found to be significant only in the HIV-positive/METH-positive group [$\chi^2_{(1,N=93)}$=4.40, $p$<0.05]. Examination of impairment rates for each of the cognitive ability domains by genotype within the HIV-positive/METH-positive group (Table 2) suggest a near-significant ($p$=0.06) differences across the three genotype groups in the executive functions domain. However, when the C allele carriers were collapsed into one group and compared against the homozygous T carriers (CC/CT vs. TT), rates of impairment in the executive functions domain were significantly different ($p$=0.03), while rates in the speed of information processing domain approached significance ($p$=0.06).

To further explore the potential effect of the C allele on global NP impairment rates within the HIV-positive/METH-positive group, odds ratios were calculated as an estimate of the magnitude of the effect observed. We compared the CC vs. TT and also collapsed CC and CT groups and compared against the TT homozygous group to evaluate the effect of being a minor allele (C) carrier. The comparison of the two homozygous groups (CC vs. TT) yielded a near-significant chi-square [$\chi^2_{(1,N=59)}$=3.45, $p$=0.06] and an odds ratio of 3.5 (95% CI=0.89–13.7). The comparison of the collapsed group (CC and CT) vs. the homozygous TT group yielded a result at the threshold of significance [$X^2_{(1,N=93)}$=3.79, $p$=0.05] and an odds ratio of

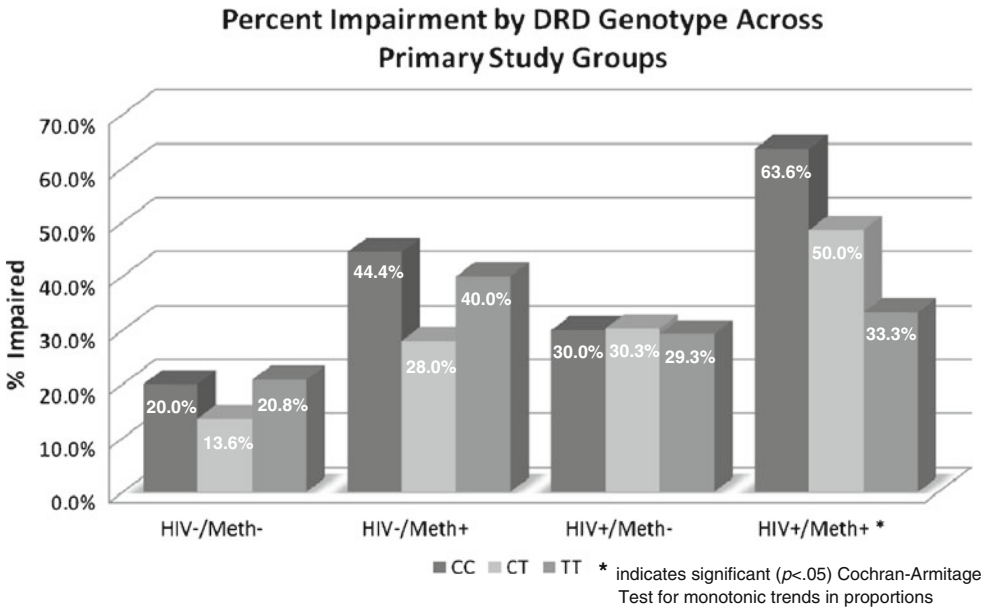


**Fig. 2** Percent impairment by the DRD genotype across primary study groups

2.28 (95% CI=1.00–5.29). Analyses similar to those described above (CC vs. TT and CC/CT vs. TT) among the other three primary groups (HIV-negative/METH-negative, HIV-negative/METH-positive, and HIV-positive/METH-negative) were not significant (all $p>0.49$).

DRD3 genotype and VL in the context of HIV and METH

As hypothesized, there was no association between DRD3 genotype and VL or $CD4^+$ cell count in peripheral blood. Also, as expected, CSF VL did not differ by DRD3 genotype within the HIV-positive/METH-positive group [$F_{(2,70)}=0.75$, $p=0.48$]. No differences in CSF VL by DRD3 genotype were observed in the HIV-positive/METH-negative group either ($p=0.85$).

## Discussion

The principal findings of this study support our hypotheses that there is an association between the DRD3 gene SNP (rs6280TC) and rates of cognitive impairment, but only in the context of HIV-positive subjects who are currently or recently dependent on METH (HIV-positive/METH-positive). Moreover, this association was allele dose-dependent such that homozygous C allele carriers were observed with the highest frequency of cognitive impairment (CC, 63.6%), followed by heterozygous C allele carriers (CT, 50%) and the group with no C alleles (TT, 33.3%). This lends preliminary support to the theory that the C allele variant in the DRD3 gene presents a liability to immune cells, such as macrophages, increasing their vulnerability to infection with HIV-1 in the presence of METH and the attendant higher levels of DA in the brain. This may lead to the growing viral burden of MDMs, particularly in the brain and in perivascular regions, which leads to increased CNS inflammation, oxidative stress, and, subsequently, cognitive impairment.

A clear stair-step pattern of NP impairment rates was observed across DRD3 genotypic groups only in the HIV-positive/METH-positive group and was further shown to yield a significant Cochran–Armitage test for trends in proportions (Nath et al. 2001; Reiner et al. 2009). Effect size estimates based on odds ratios also indicated a possible noteworthy allele effect. For instance, when the groups representing genotypic extremes were compared (CC vs. TT) on NP impairment rates, a near-significant, relatively robust odds ratio of 3.5 was observed for the CC genotypic

**Table 2** % Impaired by domain across DRD3 genotype groups within the HIV-positive/METH-positive primary group

| NP domain | CC | CT | TT | $p^1$ | CC/CT | $p^2$ |
|---|---|---|---|---|---|---|
| Verbal | 27.3 | 20.6 | 27.1 | 0.78 | 22.2 | 0.58 |
| Executive functions | 63.6 | 47.1 | 29.2 | 0.06 | 51.1 | 0.03 |
| SIP | 36.4 | 32.4 | 16.7 | 0.17 | 33.3 | 0.06 |
| Learning | 36.4 | 47.1 | 33.3 | 0.45 | 44.4 | 0.27 |
| Memory | 36.4 | 38.2 | 27.1 | 0.54 | 37.8 | 0.27 |
| Working memory | 36.4 | 29.4 | 18.8 | 0.35 | 31.1 | 0.17 |
| Motor | 36.4 | 29.4 | 22.9 | 0.60 | 31.1 | 0.37 |

$p^1$ = $p$ value of three-way chi-square comparing impairment rates across three genotypes (CC, CT, and TT); $p^2$ = $p$ value of two-way chi-square comparing impairments rates of C allele carriers and TT homozygotes (CC/CT vs. TT)

*SIP* speed of information processing

groups. When genotypes containing the C allele variant coding for greater receptor affinity for DA were collapsed (CC/CT vs. TT), we observed a more pronounced contrast ($p=0.05$) and an odds ratio of 2.28 for C allele carriers. It is notable that none of the genotypic comparisons within the other primary groups was significant.

Although the CC group within the HIV-positive/METH-positive group was more ethnically diverse than the CT or TT groups, ethnic background was not associated with impairment status across all of these groups. Moreover, three of seven (42.9%) cognitively impaired subjects within the CC group were Caucasian. Therefore, it seems clear that ethnic background is not a better explanation for the cognitive outcomes.

The results of this retrospective examination of NP and genotypic data offer preliminary, albeit indirect clinical corroboration for the experimental literature on the mechanistic roles of METH and DRD3 in potentially increasing viral load in the brain via infected MDMs. Specifically, the results support our model of the effect of DRD3 C allele in HIV-positive/METH-positive groups, which has greater binding affinity for DA, and may ultimately lead to greater rates of HIV-associated cognitive impairment in a gene dose-dependent fashion. Interestingly, HIV-positive/METH-positive subjects who had two copies of the T allele (lower DRD3 DA binding affinity) were observed to have rates of cognitive impairment (33.3%) comparable to HIV-positive/METH-negative subjects (29.8%).

Although the two HIV-positive primary groups and the disaggregated genotypic groups within each had quite comparable levels of HIV RNA within the CSF, with similar percentages below the lowest detectable limit (50 copies/mL), the observed differences in cognitive impairment rates may reflect a legacy of increases in brain tissue VL that may or may not have since been suppressed by treatment. For example, prior research has consistently demonstrated that nadir CD4 lymphocyte count is more predictive of cognitive impairment than current CD4 (Rippeth et al. 2004; Sasieni 1997; Scott et al. 2007). This suggests that a history of immune compromise is more critical to the likelihood of impairment than present status. In any case, CSF VL is only a surrogate for brain tissue VL, and there is evidence that it may serve as an imperfect indicator of the latter (Marcondes et al. 2010; Langford et al. 2006), particularly after treatment has been initiated.

The results point to a variety of factors to consider in a future prospectively designed clinical study. Based on effect sizes observed in this study, and the DRD3 SNP allelic distribution within the population and observed in our sample, future efforts have a precedent to anticipate sample sizes needed to adequately power such an investigation. Based on these clinical findings, future in vitro work applying the methods of Gaskill et al. (2009) and Liang et al. (2008) should investigate the effect of METH and DA on viral activity in MDMs that differ only in their DRD3 genotypes. The finding that the genotype of MDMs modulates viral activity would close the gap between their work and what we observed within the present study. Future in vitro and clinical investigations should also investigate genetic polymorphisms of all DA receptors and variants of genes coding for other components in the DA pathway, from synthesis to metabolism, which may also play key functional roles. In fact, our group has previously shown an association between the Val158Met polymorphism in the catechol-*o*-methyltransferase gene and executive functioning impairment in the context of HIV and METH (Bousman et al. 2010) and is currently conducting in vitro work to further elucidate this finding. Elucidation of all the associations between DA genes and HIV-associated cognitive impairment may lead to multivariable models that account for more of the variation in impairment rates than a single SNP alone and may potentially highlight pathways involved in mediating such impairment. Future studies should also more narrowly define METH using groups. The signal may carry more fidelity if all METH-positive subjects were currently dependent or dependent within the more recent past (e.g., ≤3 months) and with greater proportions of subjects being antiretroviral (ARV)-naive. Similarly, histopathological analysis of postmortem brain tissue of HIV-positive subjects who were dependent on METH during their lifetimes could also show an association with the DRD3 genotype.

As expected, we did not find differences in plasma or CSF viral loads between the two HIV groups and between the DRD3 genotype groups with the HIV-positive/METH-positive primary group. Valcour et al. (2010) have suggested, based on a review of several studies, that HIV proviral DNA within peripheral blood mononuclear cells (PBMCs) may be a more sensitive biomarker of HIV-associated cognitive impairment than HIV RNA. Thus, given the evidence for the effect of METH and the attendant levels of dopamine on the increased infectivity of monocyte-derived macrophages (Liang et al. 2008; Gaskill et al. 2009), future research efforts should endeavor to quantify HIV DNA in PBMCs.

Notwithstanding the limitations of the retrospective design, there certainly appears to be a notable genetic effect of the DRD3 rs6280TC SNP on rates of cognitive impairment in METH-dependent HIV-positive subjects that is worth investigating further. Subsequent research corroborating this finding could suggest the development of prophylactic interventions for intractable METH dependence in HIV-positive patients. For example, treatment with DA receptor antagonists targeted toward specific DA receptors may attenuate genotypic differences in DA binding affinity (Lundstrom and Turpin 1996) and therefore

attenuate rates of cognitive impairment. A more practical clinical implication may be that HIV-positive patients need to be genotyped before being prescribed dopaminergic drugs to treat movement disorders or stimulants to treat a variety of conditions such as attention deficit and adjunctive treatments for depression.

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**Conflict of interest** Dr. Heaton receives royalties from the publisher of the Wisconsin Card Sorting Test. All authors declare they have no other conflicts of interest.

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