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Race-dependent association of single nucleotide polymorphisms in TrkB receptor in people living with HIV and depression

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Abstract

Human immunodeficiency virus (HIV)-associated cognitive disorders (HAND) is characterized by impaired motor and intellectual functions, as well as mood disorders. Brain-derived neurotrophic factor and its receptor TrkB (or NTRK2) mediate the efficacy of antidepressant drugs. Genomic studies of BDNF/TrkB have implicated common single nucleotide polymorphisms in the pathology of depression. In the current study, we investigated whether single nucleotide polymorphisms (SNPs) (rs1212171, rs1439050, rs1187352, rs1778933, rs1443445, rs3780645, rs2378672, and rs11140800) in the *NTRK2* has a functional impact on depression in HIV-positive subjects. We have utilized the Central Nervous System (CNS) HIV Antiretroviral Therapy Effects Research (CHARTER) cohort. Our methods explored the univariate associations of these SNPs with clinical (current and lifetime) diagnosis of depression via chi square. The distribution of alleles was significantly different for African-Americans and Caucasians (non-Hispanic) for several SNPs, so our regression analyses included both “statistical controls” for race group and models for each group separately. Finally, we applied a method of simultaneous analysis of associations, estimating the mutually shared information across a system of variables, separately by race group. Our results indicate that there is no significant association between clinical diagnosis of major depression and these SNPs for either race group in any analysis. However, we identified that the SNP associations varied by race group and sex.

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Author contributions

VA designed experiments, contributed to data analysis, and co-wrote the manuscript; FY contributed to data analysis and manuscript writing; IM contributed to experimental design and manuscript writing; SLL contributed to experimental design and materials; RET designed experiments, contributed to data analysis, and co-wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest

Author Futoshi Yumoto was employed by company Resonate, Inc. All other authors declare no competing interests.

Keywords

BDNF; HIV; HAND; rs1212171; race differences; major depression

Introduction

In the United States, rates of current depression are significantly higher at 36% in people living with human-immunodeficiency virus (PLH) (Asch et al. 2003; Rabkin 2008) compared to 6.8% in the general population (CDC 2011). In other HIV populations, prevalence of major depressive disorder or moderate to major depression has ranged from 26% among 212 people in Denmark (Slot et al. 2015) to 28% among 4422 people in the Swiss HIV Cohort Study (Anagnostopoulos et al. 2015). Depressive symptoms affected 15.7% of 2863 HIV-positive people in Western Europe and Canada (Robertson et al. 2014) and 48.8% of 690 people in Italy (Marando et al. 2016). Among 180 people with newly-diagnosed HIV infection in Houston, 67% had depressive symptoms (Bhatia et al. 2011). Individuals with psychiatric comorbidities have lesser quality of life and lower adherence to HIV medications when compared to patients without psychiatric disorders (Cohen et al. 2007; Weaver et al. 2005). Thus, the importance of establishing whether there is a genetic component for depression in HIV positive individuals is paramount.

Brain derived neurotrophic factor (BDNF) modulates synapse formation and plasticity by activating the tyrosine receptor kinase, TrkB (Park and Poo 2013; Tanaka et al. 2008). Moreover, both experimental (Duman and Monteggia 2006; Pollak et al. 2008) and clinical (Bocchio-Chiavetto et al. 2010; Post 2007; Shimizu et al. 2003) studies have designated BDNF/TrkB signaling as an important event that modulates the pathophysiology of depression; therefore, it has been suggested that mood disorders may arise from impaired BDNF-TrkB signaling (Castren et al. 2007; Pla et al. 2014). Likewise, loss of BDNF/TrkB has negative consequences for neuronal network and cognitive behavior (Zuccato and Cattaneo 2009) and major depressive disorders (Chen et al. 2001; Mitre et al. 2017). Impaired BDNF/TrkB levels is one of key feature found in PLH who develop HIV-associated neurological disorders (HAND) (Bachis et al. 2012), an array of clinical signs that includes cognitive and motor impairments, and mood disorders (McArthur et al. 2010). Moreover, the decrease in BDNF (Bachis et al. 2012) and TrkB (Speidell et al. 2020) expression has been shown promote loss of neurons in an animal model of HAND. Because the ability of antidepressants to regulate neuronal plasticity requires an intact central nervous system, changes in the expression of TrkB receptor in PLH could perturb neural function that leads to depression.

Single nucleotide polymorphisms (SNPs), which have been used to examine genetic variables and their putative contributions to pathology, can alter gene expression. The TrkB gene contains several SNP that appears to be associated with psychosis and depression, including rs2289656 (Deflesselle et al. 2018; Dong et al. 2009), and rs1212171 (Avdoshina et al. 2013). However, little is known about whether this association is sex- and race-dependent. Moreover, there are several SNPs yet to be explored for PLH. In addition, a few specific statistical methods have commonly been used in the analysis of SNPs.

These are univariate analyses, whether t-test (or nonparametric equivalent) or chi-square (or nonparametric equivalent); multivariate analyses (linear or logistic regressions) where race or sex were controlled for (i.e., included as a covariate); or multivariate models run separately for sex or race groups. While analysis programs have been published to estimate associations between potentially-interacting genetic variables and disease outcomes (Jiang et al. 2011; Li et al. 2005; Wang et al. 2009), such approaches have not been widely used for diagnostic entities like depression. These methods are not always appropriate when the outcomes are self-reported because self-reported outcomes are so variable, both within and across persons, that establishing evidence of an association is extremely difficult and can be highly sample-dependent.

In this study, using the methodologies described above, we explored the association between TrkB polymorphisms rs1212171, rs1439050, rs1187352, rs1778933, rs1443445, rs3780645, rs2378672, and rs11140800 and depression in PLH of different race and sex. These SNP were selected because they are not more frequently studied in PLH despite the fact that they might play a role in the antidepressant action (Colle et al. 2015).

Materials and Methods

Study population.

CHARTER is a prospective, observational study of neurobehavioral outcomes of HIV disease, which enrolled ambulatory, HIV-infected adults from 2003 to 2007 at six U.S. medical centers: San Diego, CA; Baltimore, MD; Galveston, TX; New York, NY; Seattle, WA; and St Louis, MO. All data utilized for these analyses were de-identified. Study participants underwent detailed, structured interviews and laboratory assessments to obtain information on HIV disease and treatment-related factors, including nadir CD4+ T-cell counts, combined antiretroviral therapy (cART) history, nucleoside reverse-transcriptase inhibitor (NRTI) exposure, history of a major depressive disorder (MDD), substance use/dependency, comorbidity, and demographics. Data were also collected on current CD4+ T-cell count, HIV RNA levels in plasma, and hepatitis C virus serology. Details regarding CHARTER study eligibility criteria, enrollment and follow-up procedures have been published previously (Heaton et al. 2010; Heaton et al. 2011; Heaton et al. 2015). CHARTER is approved by the Institutional Review Boards of all participating sites and abides by the Declaration of Helsinki; all study participants provided written informed consent. For this study, we utilized data from 1055 CHARTER participants (77% male) with (14.4%) and without (85.6%) a current clinical diagnosis of MDD. Of these 1055 participants, 933 of them self-identified as African American or Caucasian, and as these were the largest race groups in the cohort, these are the participants we included in these analyses. Demographic information is presented in Table 1. The clinical diagnosis of MDD was determined using DSM-IV at the screening visit. Lifetime diagnosis of major depressive disorder (LTMDD) designation (yes/no) is defined as having ever received this diagnosis, whether or not it is current. CHARTER participants also complete a self-report of depressive symptoms at the baseline visit. The Beck self-reported depressive symptom scale (Beck et al. 1996) was discretized to have four levels, 0=0–13 (“minimal symptomatology”), 1=14–19 (“mild”), 2=20–28 (“moderate”), and 3= 29–63 (“severe”).

Single-nucleotide polymorphisms genotyping.

Genomic DNA was extracted from peripheral blood mononuclear cells collected at the baseline CHARTER visit using PUREGENE (Gentra Systems, Inc, Minneapolis, MN). Eight TrkB single-nucleotide polymorphisms (rs1212171, rs1439050, rs1187352, rs1778933, rs1443445, rs3780645, rs2378672, and rs11140800) were analyzed using the Affymetrix Genome-Wide Human SNP Array 6.0™. Genotyping was conducted by the Vanderbilt Technologies for Advanced Genomics (VANTAGE) at Vanderbilt University.

Statistical analysis.

Statistical analyses were carried out using SPSS v 25 (IBM, Chicago, IL) for descriptive and traditional statistics (chi square, logistic regression with and without “statistical” control of covariates, described further below). BayesiaLab v.7 (Bayesia S.A.S., Laval, France) was used for Bayesian network (simultaneous) modeling. The dependent variable of interest when a dependent variable was included was LTMDD. Three regression methods were used. In the first regression method, we controlled for sex and ethnicity statistically, including these variables in the single model of the relationships between SNPs and LTMDD; in the second, we controlled for the influence of these variables by modeling the relationships between SNPs and LTMDD separately for the different race groups. Finally, the simultaneous analysis approach, described below, considered the SNPs as a system of related predictors of LTMDD. These regression models test whether multivariable modeling of SNPs, assuming SNPs to be independent of each other (Wickens 1995), lead to the same inferences as modeling of SNPs as a system of associated variables. Additionally, in each of these four analytic paradigms, we included additional variables to reinforce any interpretations about associations with the dependent variable. Specifically, using the same analytic method, we sought to estimate both whether the target associations were found and also whether or not expected associations among other variables (not the dependent variable of interest) were found (e.g., associations between having a diagnosis of AIDS with having cART; associations between clinical diagnosis of depression and self-reported depressive symptoms).

Nominally, was set at 0.05 for the family of analyses in each paradigm (although there is no such control required in the Bayesian network modeling, described below). No formal classification was planned in these analyses of the association between any SNPs and clinical depression.

Univariate analysis: Chi Square Method.

Collapsing across HIV status, sex was not balanced across race ($x2=65.8$, $p<0.001$). Just 11% of the Caucasian (non-Hispanic) sample was female while 33% of the African-American group was female. Appropriate multiple comparisons corrections for the univariate analyses would include adjusting the overall α by 8 SNPs × 2 (MDD, LTMDD) or 16 ($\alpha/16$, Bonferroni) analyses in total; or, application of the Holm correction (Holm 1979) to the family of 16 analyses. There was one family of 16 analyses for males, and one for females, in each of the two ethnicity groups. However, if none of the univariate analyses yielded a p-value small enough to ‘survive’ either of these corrections (i.e., any $p<0.00031$), then no corrections would (need to) be applied.

Multivariate analyses: Logistic Regression Methods.

First, we compared a null model (with just an intercept, predicting LTMDD while statistically controlling for sex (Male (M)/Female (F)) and race (African-American and Caucasian (non-Hispanic)) to a model with all SNPs entered simultaneously to predict LTMDD after controlling for ethnicity and sex in the first step. Stepwise regression entering one SNP per step would not be plausible since there is no scientific reason to enter any of them ahead of any other. Secondly, we repeated this logistic modeling separately for the two race groups, i.e., not "controlling statistically" for ethnicity. For the two multivariate cases ("controlling for" ethnicity, and modeling separately for each race group), we obtained the Nagelkerke R^2 values that estimate the amount of variance shared between depression and any given SNP as an effect size approximation. R^2 values were obtained for change in explanatory power beyond sex (M, F), ethnicity, and the intercept (first case), and for explanatory power beyond just the intercept and sex. Explanatory power (Nagelkerke R^2 values) less than 5% were characterized as "not meaningful", even if they arose from statistically significant ($p<0.05$) models. Strength of association was characterized as zero if it was derived from any model that was not statistically significant. Although the analyses were not specifically intended to generate estimates of the strengths of associations (the beta coefficients of the regressions), non-significant models suggest the strengths of associations were not different from zero, while significant models generated Nagelkerke R^2 values we could readily interpret across models.

Simultaneous analyses: Bayesian Network inference methods.

In the simultaneous modeling, we leveraged the mutual information in order to uncover robust associations with a target event; our main interest was in the binary (Yes/No) outcome of LTMDD, and we repeated the modeling using another target event as a control: the binary (Yes/No) outcome of whether the HIV positive status had changed to AIDS. In each of these inferred models, the program used supervised structural learning (searching for the overall data structure) using an augmented Markov blanket learning algorithm to constrain the structure so that only one "parent" per variable is identified, but no relationships with the target variable (LTMDD or AIDS, depending on the model) were imposed. This was intended to result in the "discovery" of relationships that are expected, i.e., LTMDD should share mutual information with both the binary (Yes/No) outcome of current diagnosis of major depressive disorder and with the current four-level discretization of the self-reported depressive symptom scale (Beck Depressive Inventory); similarly, AIDS should share mutual information with whether or not an individual was on cART. Thus, the AIDS modeling served as a validation that the structural inference method did not "miss" some relationships that were expected. In addition, to increase the likelihood of an interpretable model, we also specified a threshold above which association strengths (structural coefficients) needed to be in order to be estimated to be 0.40 (following (Comrey and Lee 1992). This general approach is similar to what would be employed in an exploratory factor analysis – searching for underlying structure and not seeking to quantify the associations that the structure may suggest (Bollen 1989). Finally, we cross validated model results using 500 resamples adding 0.01 of a standard deviation's worth of perturbation (random noise) to the data, and refitting. The final models were accepted only if no new relationships with mutual information greater than 0.01 were identified after

the cross-validation. Mutual information (Kullback-Leibler), and not probability, was used for association estimation because linearity in relationships cannot be assumed for these variables, and we are determining whether or not there is any evidence of associations, not trying to estimate or quantify the associations we might find using this method. The resulting tree was visualized in automatic layout for interpretability (Conrady and Jouffe 2015).

We carried out post hoc modeling of the SNPs simultaneously without a target, to test whether it could ever be supportable to treat SNPs or ethnicity as if they are controllable, exchangeable, or imputable without separate modeling to accommodate any differences across groups. Similar to the first modeling approach, we leveraged the mutual information shared among these SNPs in order to uncover robust associations among them, without any target event to predict. Unsupervised learning (Taboo) with a pre-specified maximum number of parent nodes = 2 was selected (this setting was cross-validated with the maximum set = 3 to determine if results were sensitive to this setting). In Bayesia Lab, the Taboo learning algorithm is a hybrid where a greedy search (which may be stopped by a local minimum, i.e., inappropriate stopping) is followed by Taboo, which remembers previous links and finds the final network structure that optimizes the structure across all of these (Conrady and Jouffe 2015). As with the supervised learning, to increase the likelihood of an interpretable model in the unsupervised modeling, we also specified a threshold above which association strengths (structural coefficients) needed to be in order to be estimated to be 0.4 (following (Comrey and Lee 1992). Finally, we cross validated the model results using 500 resamples adding 0.01 of a standard deviation's worth of perturbation (random noise) to the data, and refitting. The final models were accepted as "validated" only if no new relationships with mutual information greater than 0.01 were identified after the cross-validation. Mutual information (Kullback-Leibler) was used for association estimation. All simultaneous modeling was done for the Caucasians (non-Hispanic) (n=442) and the African-Americans (n=491) separately. We thus modeled the SNPs simultaneously in two different ways. First, we used a supervised machine learning algorithm, the Augmented Markov Blanket, to infer the associative structure between SNPs and each target outcome. The second approach was to apply unsupervised structural learning (Taboo) learning to infer the associative structure between the SNPs themselves, without any given target. Model results provide estimated shared information for just those associations that had structural coefficients above the threshold. The program runs dynamically, imputing missing values based on the inferred network structure. There was no missing demographic data and no missing outcome data for non-Hispanic Caucasians; no missing values for whether or not the individual was on ART and 1% missing values for estimated HIV duration; current and LT MDD; current and LT diagnosis of any substance abuse in African-Americans. There was 0–3% missingness for non-Hispanic Caucasians and 0–2% missingness for African-Americans on the SNPs.

Results

Associations between LTMDD and SNPs, sex and race by univariate analysis.

We used chi squared for univariate analyses, the most explicit treatment of SNPs as independent. We included sex and ethnicity as additional dependent variables because in practice, multivariate analyses might be preceded with univariate ones like these to identify covariates that should be included. Associations between sex and either LTMDD or current MDD were not significant when both racial groups were analyzed together ($p>0.35$ for both MDD outcomes). When the associations were analyzed for the racial groups separately, we found no relationship for Caucasian (non-Hispanic) (both $p>0.46$). However, we found a significant relationship between sex and LTMDD for African-Americans ($p<0.01$). Specifically, 45% of African-American had at least one lifetime MDD event and of these, 40% were female, while only 27.8% of those in this race group who had never had a MDD event were female; in contrast, in Caucasian (non-Hispanic), only roughly 10% of LTMDD were female. These examinations of sex, race group, and depression suggest that the explorations of associations between LTMDD and SNPs should include sex and race as a covariate.

Chi square analyses were done on each SNP for their relationships with LTMDD and current MDD. Sex and race groups were analyzed separately (i.e., in four groupings). In these univariate analyses, all unadjusted results were not significant ($p>0.05$) except for the allele distributions of: rs1443445G across current MDD (Yes/No) for African-American females ($p=0.048$), rs1212171T and current MDD for Caucasian (non-Hispanic) females ($p=0.021$), and, for African-American males only, rs1439050T and LTMDD ($p=0.044$) and rs11140800A and current MDD ($p=0.042$). In summary, the univariate analyses did not find a relationship between any of the eight SNPs and either current or lifetime MDD when analyzed independently, even when taking sex and race group into account.

TrkB polymorphisms and prediction for MDD.

Two common traditional multivariate approaches (see Materials and methods) were taken to explore associations between the TrkB SNPs and the depression outcomes, both featuring logistic regression. Logistic regressions included sex in the first, followed by race group in the second, and all SNPs in the third, steps. This allowed for statistical control of both sex and ethnicity in the predictions by SNPs (independent variables) of LTMDD and current MDD (dependent variables in separate models). The explanatory power for every model ranged from 1.1% to 3.9%. While many of these achieved statistical significance, none could be characterized as clinically meaningful because of this uniformly low strength of association. When sex was added to the model predicting LTMDD, Nagelkerke R^2 was zero, and it was 0.001 when predicting current MDD (Table 2). When race group was added to predict LTMDD after controlling for sex, Nagelkerke R^2 was 0.025 (2.5% explanatory power was gained; $p=0.036$) and 0.003 (0.3% explanatory power gained) for current MDD (Table 2). When all SNPs were added, Nagelkerke R^2 for LTMDD was 0.039, and it was 0.012 for current MDD; i.e., all SNPs together added 3.9% explanatory power for LTMDD and 1.2% for current MDD (Table 2). The modeling to predict LTMDD identified a contribution of rs11140800A to LTMDD ($p=0.032$, uncorrected). No SNP was individually

significantly predictive for current MDD (all p>0.10) after controlling for sex and race group. Because none of the results was statistically significant, the conclusion from a variety of analytic perspectives is that the strength of association between the SNPs and either LTMDD or current MDD is not meaningfully different from zero when race was controlled statistically.

Logistic regressions were next done separately for Caucasians (non-Hispanic) and African-Americans (i.e., *not* controlling statistically for race). Models included sex in the first, followed by all SNPs in the second steps. For Caucasian (non-Hispanic) participants, when sex was added to predict LTMDD, Nagelkerke R^2 was 0.02 and it was zero when predicting current MDD. When all SNPs were added to the model to predict LTMDD after controlling for sex, Nagelkerke R^2 was 0.033 and 0.011 for current MDD. All SNPs together added 3.3% explanatory power for LTMDD and 1.1% for current MDD among Caucasians (non-Hispanic), and no individual SNP was significantly predictive in either model (all uncorrected p>0.10). For African-American participants, when sex was added to the null model to predict LTMDD, Nagelkerke R^2 was 0.017 and it was 0.004 when predicting current MDD. When all SNPs were added to this base model, Nagelkerke R^2 for predicting LTMDD was 0.033 and it was 0.038 for current MDD. Thus, all SNPs together added 3.9% explanatory power for LTMDD and 3.8% for current MDD among African-Americans, and no SNP was individually significantly predictive (all p>.064) of either outcome.

Simultaneous Modeling.

As noted, the eight SNPs were analyzed simultaneously, together with a four-level discretized version of the Beck Inventory (BDII Total), the binary (Yes/No) versions of LTMDD and current MDD (separate networks for each of these outcomes). We also included the binary (Yes/No) outcomes of whether there was a current, or a lifetime diagnosis of any substance abuse, whether the HIV positive status had turned to AIDS, and whether or not the individual was on cART. We expected the three “depression” outcomes to have associations strong enough for BayesiaLab to discover them. We expected AIDS and cART to have similar results. Figures 1A (African-Americans) and 1B (Caucasians, non-Hispanic) show the two models for LTMDD (as the target).

The expected associations between the three depression variables were uncovered for both cohorts. The associative networks differ for African-Americans and Caucasians (non-Hispanic) participants, although all the variables are the same. SNPs are associated with each other in both race group models, and these associations are relatively stronger (among SNPs) than the associations between any SNP and any of the depression variables. The strengths of associations between SNPs differ depending on race group. Figures 1C (African-Americans) and 1D (Caucasians, non-Hispanic) show the two models for AIDS status. The expected associations between AIDS and cART variables were uncovered for both cohorts. SNPs are associated with each other in both race group models and these associations are stronger (among SNPs) than the associations between any SNP and any of the depression variables. The associative networks differ for African-American and Caucasian (non-Hispanic) participants, although all the variables are the same. Without

utilizing or even quantifying the strengths of associations between SNPs, it can be seen that these networks differ depending on racial group.

The AIDS/cART modeling was included to ensure that whatever relationships were uncovered in the target (MDD) model were not spurious. Figures 2A and 2B show that, as expected from the supervised modeling (with the targets shown in Figure 1), these SNPs associate together (i.e., are not independent). The strengths of associations differ depending on the SNPs and the associations differ qualitatively by race group. AIDS status association with SNPs were not expected, but an association between cART therapy and AIDS status was expected.

Discussion

SNPs and other variations in the human genome have been used as potential markers to predict various pathological conditions (Wang et al. 1998), response to drugs, or sensitivity to environmental factors (Katara 2014). SNPs in the BDNF and TrkB/NTRK2 genes have been reported to modulate the efficacy of antidepressant drugs (Hennings et al. 2019) and be associated with acute suicide (Deflesselle et al. 2018); however, less is known about other polymorphisms in the NTRK2. *NTRK2* SNPs rs10868223, rs11140778, rs1565445, rs1659412, were reported to be associated with antidepressant efficacy (Hennings et al. 2013) but these results were not replicated (Colle et al. 2015). A particular SNP located in the *NTRK2* is believed to contribute to differences in susceptibilities to comorbidities such as MDD in individuals with different ethnic backgrounds (Gupta et al. 2013; Lin et al. 2014; Roetker et al. 2013; Zhou et al. 2015). Decreased expression of TrkB has been shown to participate in the loss of synapses in a rodent model of HAND (Speidell et al. 2020). These animals also exhibit depression and anxiety (Bachis et al. 2016). Thus, changes in TrkB expression could have a significant role in the neuropathology of HAND. We have previously suggested an association between depression and a SNP in the *NTRK2* gene in HIV positive women (Avdoshina et al. 2013). In this study, we have used an independent data set that includes males as well as females, and utilized a clinical (rather than self-reported) dependent variable of major depression to confirm/refute this association. The sample size was large and well characterized and homogenous in terms of diagnosis. Using this wider cohort, we found no evidence of associations between NTRK2 SNPs and MDD or LTMDD. However, we found that there is a difference in polymorphism distributions/connections between races and sex.

The results presented here document stronger associations among the SNPs than between any of them and the target, LTMDD. Expected associations were seen between AIDS status and on/off cART; between long-term and current diagnosis of substance abuse (data not shown); and between LTMDD, current MDD, and self-reported depressive symptomatology in both race groups. Finding these expected associations suggests that our failure to discover meaningful associations between SNPs and indicators of depression is not likely to be due to an atypical data set or other analytic difficulties (e.g., too big or too small of a sample). The results suggest that the lack of associations is not an artifact of the methods we used, whether depression is indicated with a clinical diagnosis, or as self-reported symptoms.

Our results suggest that these SNPs should not be analyzed as if they are independent-biologically, this is known to be implausible, but typical analytic methods may treat genetic variables as independent. The logistic and simultaneous analysis models demonstrate that race should not be controlled statistically, also a common approach, because the modeling results differ in qualitative ways. We used Bayesian network analyses to leverage the mutual information shared among these SNPs, without a target for the SNPs to predict, in order to uncover robust associations among these SNPs. This post hoc modeling of the SNPs simultaneously accomplished two sensitivity tests. First, it effectively tested whether the associations between SNPs uncovered in the previous modeling might be due to the inclusion of the target variables; finding associations among the SNPs in the post hoc models shows that more simplistic analyses are not appropriate, because the SNPs are not independent. Second, these models also tested whether it could ever be supportable to treat SNPs or race group as if they are controllable statistically, exchangeable, or imputable without separate modeling to accommodate the differences. The results suggest that none of these assumptions is supported.

We have also found that, although too small to be actionable, the strength of association between MDD and all SNPs for African-American cohort was more than three times stronger than for Caucasian (non-Hispanic) cohort and three times greater than the overall estimated strength (1.2%) when the groups were analyzed together. Although the SNP distributions were clearly different for our race groups, the statistical control of race group may not be as effective for precise estimation of effects in any analysis involving these SNPs. In fact, African American population in US is characterized by immense genetic diversity, originating from African (harboring the most genetic lineages) (Beltrame et al. 2016) and European ancestries (Gautam et al. 2019). Thus, these contributions may have significant impact on how we find an association between these SNPs and MDD. It will be interesting to expand our study to a population in which self-reported ancestry is more accurate.

We have also analyzed sex as an important variable because sex differences can exist in the patterns of gene expression. In addition, endogenous and exogenous environmental factors can differentially affect sex-specific genetic risk factors (Khramtsova et al. 2019). We found that sex is an important stratifying variable, although our sample sizes did not support the stratification by both race and sex in the Bayesian network modeling. Our data suggest that analysis plans for SNPs should at least include sensitivity analyses to determine if collapsing across meaningful groups (sex, ethnicity) affects estimates like it did in these cases.

There are important methodological problems in analyzing SNPs and other biological network members as if they are orthogonal to each other (Jiang et al. 2011; Li et al. 2005; Wang et al. 2009). Importantly, investigators working in the domain do not believe that the SNPs act independently; the set of analyses we utilized shows that this knowledge of interdependence among SNPs is not captured or reflected in typical analysis methods. While interaction terms are worth considering in statistical modeling, logistic regression becomes less powerful the more variables are included in the models. We did not explore this option because the full complement of interaction terms would be limited, in terms of assumptions of linearity in the interactions themselves, and also in terms of power.

For these reasons, as well as the flexibility that the Bayesian network modeling options afforded, we opted to model them as a system. Our results show that these SNPs are not independent of one another. Thus, when SNPs are modeled, or included in models of other diseases, it is important to first study the network of associations rather than treating SNPs as orthogonal to one another. Although software/programs exist to estimate associations in the presence of multi-collinear SNPs, these methods do not provide evidence about the network of SNPs. Using Bayesian network modeling yielded both information about SNP networks and also empirical evidence that race should not be controlled statistically; instead, different models may be needed for each group, depending on the genetic variables under study. The differences we reported between relationships that are due to race groups, and the associations among SNPs themselves, should be kept in mind for imputation as well as for hypothesis and inference testing in future.

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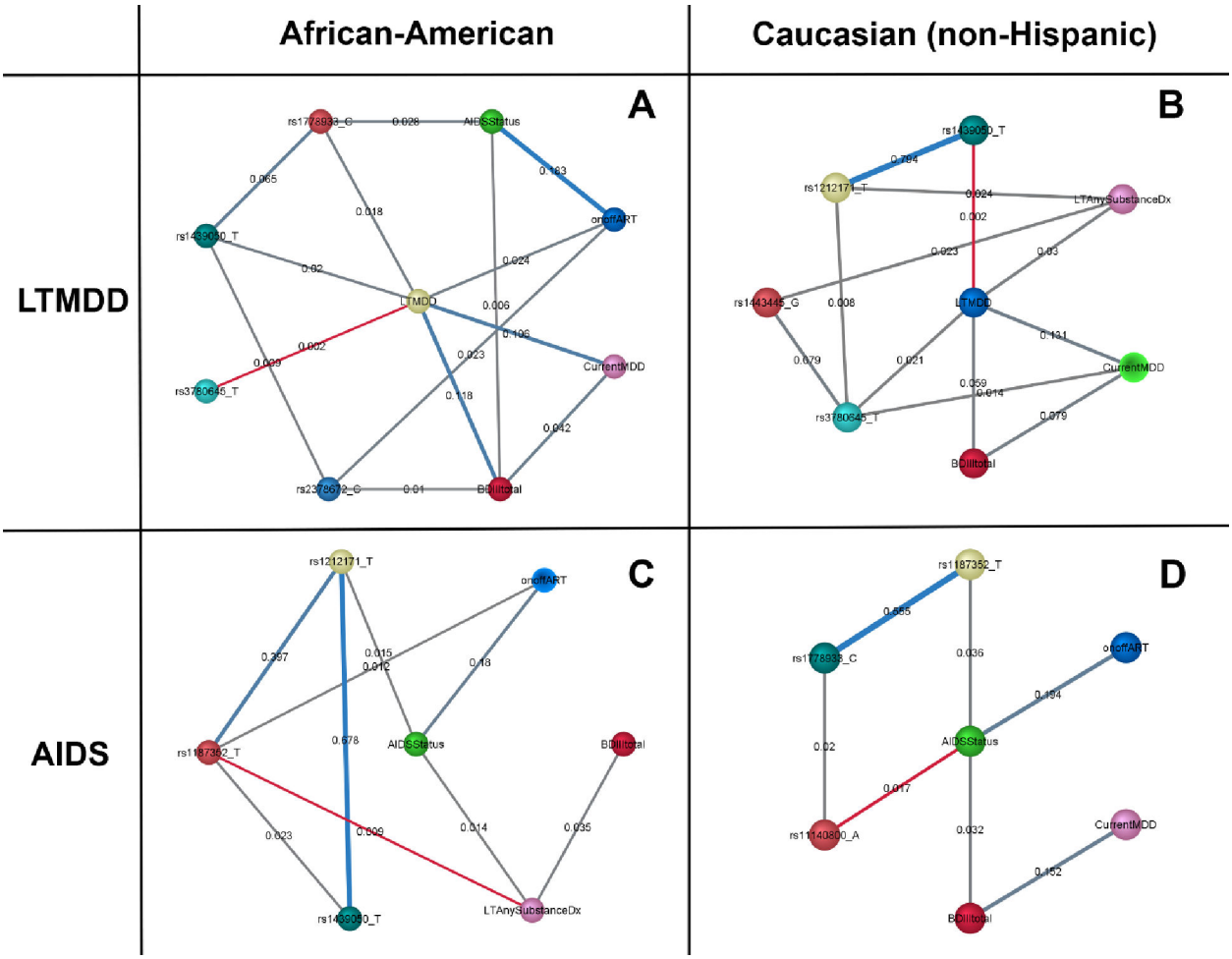

Figure 1. Inferred Bayesian Network of LTMDD and AIDS status.

(A). Inferred Bayesian Network: SNPs predicting LTMDD =Yes for 491 African-American HIV positive men and women. **(B)**. Inferred Bayesian Network: SNPs predicting LTMDD =Yes for 442 Caucasian (non-Hispanic) HIV positive men and women. **(C)**. Inferred Bayesian Network: SNPs predicting AIDS Status =Yes for 491 African-American HIV positive men and women. **(1)**. Inferred Bayesian Network: SNPs predicting AIDS Status=Yes for 442 Caucasian (non-Hispanic) HIV positive men and women. Simultaneous modeling was accomplished using the Augmented Markov Blanket with the indicated target outcome (LTMDD or AIDS). Color is included solely for distinguishing nodes. Paths between nodes represents relative strengths of association; thicker = stronger association. Values summarize the shared (mutual) information between those two (connected) nodes. Nodes represent either SNPs or clinical outcomes. Each figure shows the strength of association as values and also in the relative sizes of the connections between nodes for all identified structural coefficients. However, mutual information is dimensionless and has no fixed range; thus, the values provided in the figures are only included as a numeric analog to the visual information provided by the relative widths of paths between variables, and not to provide specific quantification of the strengths of these associations.

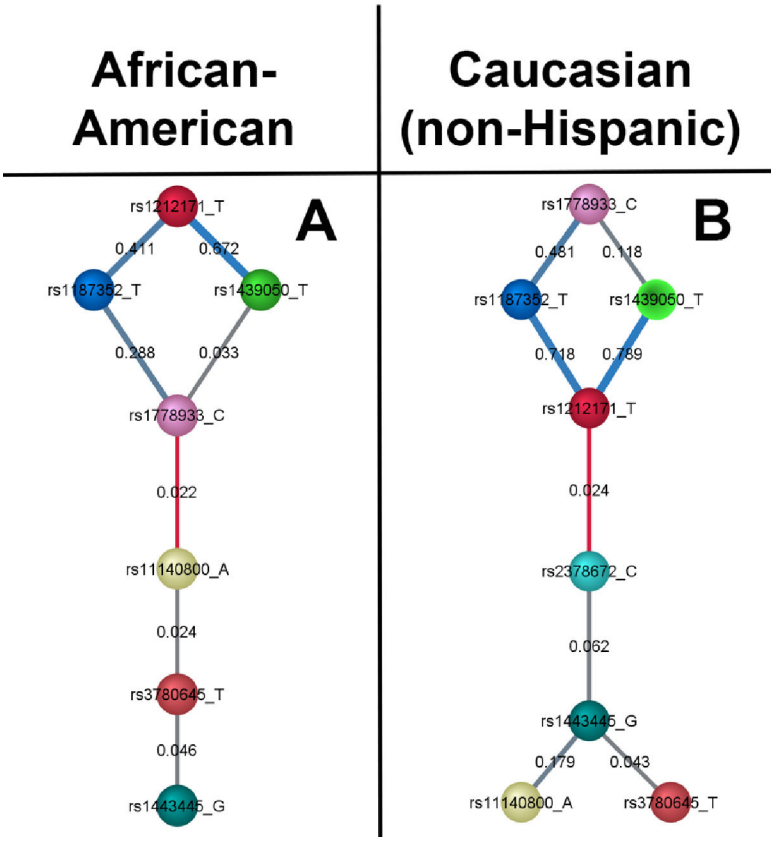

Figure 2. Inferred Bayesian Network of TrkB gene SNPs (Taboo).

(A). Inferred Bayesian Network of SNPs: 491 African-American HIV positive men and women. (**B)**. Inferred Bayesian Network of SNPs: 442 Caucasian (non-Hispanic) HIV positive men and women. Simultaneous modeling was accomplished using Taboo (unsupervised learning). Color is included solely for distinguishing nodes. Paths between nodes represents relative strength of association; thicker = stronger association. Values summarize the shared (mutual) information between those two (connected) nodes. Nodes represent SNPs, no clinical outcomes are included.

Table 1.

Demographic information

		Caucasian (non-Hispanic)	African-American
N		442	491
Age (years)	**min**	19	18
	max	68	69
	mean (SD)	43.53 (9.19)	43.45 (7.96)
Education (years)	**min**	7	7
	max	20	20
	mean (SD)	13.5 (2.58)	12.01 (2.14)
Estimated duration of HIV (months)		119.9 (82.2)	125.7(71.3)
% Female		11	33
% with AIDS		2.6	63
% on cART		56	71
% with current MDD		15	13
% with LT MDD		58	45
BDI-II severity level	**minimal (%)**	177 (40)	229 (46.6)
	mild (%)	124 (28.1)	130(26.5)
	moderate (%)	96 (21.7)	90 (18.3)
	severe (%)	44(10)	40 (8.1)

MDD = current clinical diagnosis of major clinical depression;

LTMDD = lifetime clinical diagnosis of major clinical depression

BDI-II= Beck Depressive Inventory

Table 2.

Nagelkerke R Square results

SNP	Nagelkerke R Square change for SEX	Nagelkerke R Square change for ETHNICITY	Nagelkerke R Square change for LT MDD
rsl212171_T	0.014	0.140	0.002
rs 1439050_T	0.012	0.100	0.001
rsl 187352_T	0.007	0.036	0.000
rsl778933_C	0.003	0.051	0.003
rsl 1140800_A	0.035	0.257	0.017
rsl443445_G	0.000	0.012	0.001
rs3780645_T	0.002	0.024	0.000
rs2378672_C	0.004	0.104	0.000