NEGR1 Genetic Variants and Risk for Virological Failure With Genotypic Resistance in Botswana

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BACKGROUND

- Access to antiretroviral therapy (ART) in sub-Saharan Africa has expanded due to a large number of national and international initiatives.
- Published findings from the *Tshepo* clinical trial study previously demonstrated that NNRTI-assigned ART had no effect on the development of virological failure with genotypic resistance (VF/r) when evaluating persons randomized to receive nevirapine versus efavirenz-based ART.

OBJECTIVE

To explore for the first time the impact of host genetic factors on the development of VF/r among ART-treated adults in Botswana.

SUBJECTS AND METHODS

The *Tshepo* study. 3-year randomized clinical study following 650 ART-naïve adults (69.4% female) from Botswana who initiated first-line NNRTI-based ART. 48 out of 650 (7.4%) enrolled study participants developed VF/r.

Genotyping. The Illumina Human CardioMetabolo chip is a gene-centric array enriched in markers for metabolic and cardiovascular genes (198K), including mitochondrial, ADME, renal, and immune response genes.

Quality control (QC). Individuals with ≥5% of missing data, and SNPs with ≥2% missing data, minor allelic frequency≤1%, or deviation from the Hardy-Weinberg equilibrium (P<10^{-3} in controls) were excluded from analysis. Gender concordance and potential relatedness were also evaluated. Population stratification was assessed through a principal component analysis implemented in Eigensoft.

Statistical analysis. After QC, 133,822 SNPs were tested for association with VF/r in **46** cases *vs.* **522** participants who did not develop VF/r. Logistic regressions in an additive model were performed and adjusted for gender, age, CD4, HIV-RNA, and BMI at baseline, as well as for the first two eigenvectors from the population stratification analysis.

RESULTS

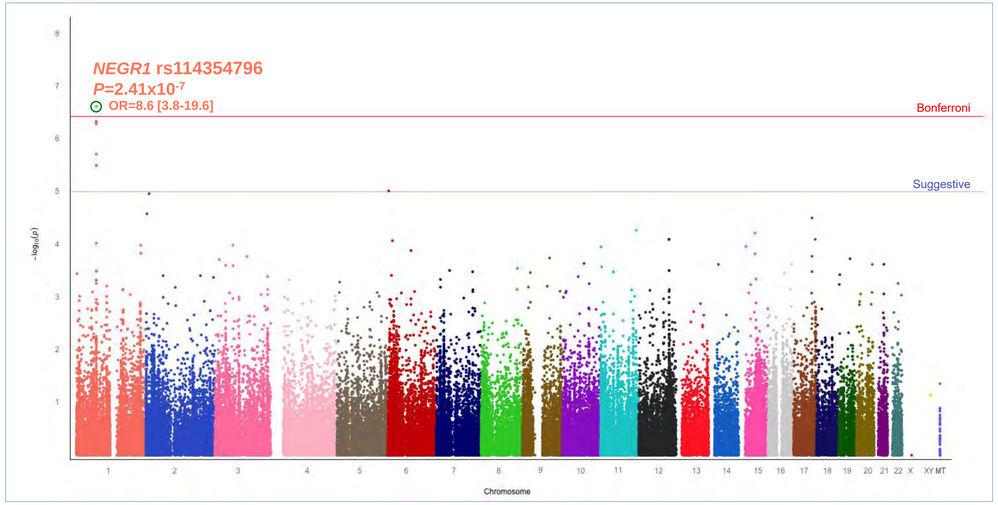

Figure 1: Manhattan plot. Distribution along the chromosomes of -$\log_{10}$(P-values) obtained for the virological failure association study. The red line marks the Bonferroni study-wide significance threshold (P=$3.7x10^{-7}$), and the blue line marks the suggestive significance threshold (P=10^{-5}).

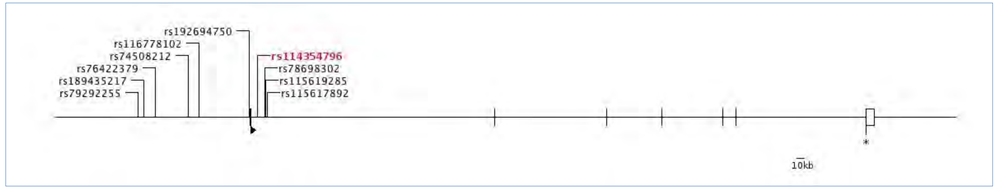

Figure 2: *NEGR1* genetic map. Boxes symbolize the exons. The ATG-START and STOP codons are represented by a black triangle and a star, respectively. The SNP meeting the genome-wide significance level is in **red** and the nine suggestive SNPs are in **black**. All these SNPs are in tight linkage disequilibrium ($0.55 \le r^2 \le 1$, $0.78 \le D' \le 1$) in the *Tshepo* study.

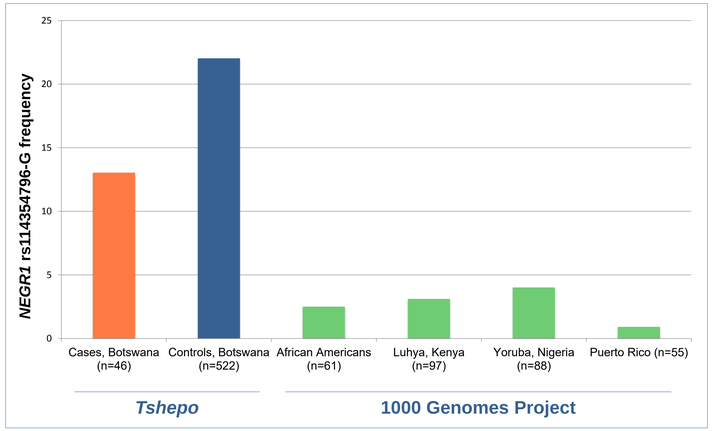

Figure 3: *NEGR1* rs114354796-G frequency. The allelic frequency is indicated for the *Tshepo* cases and controls, as well as for the 1000 Genomes Project populations in which the allele was represented.

CONCLUSIONS

- **We provide robust statistical evidence that *NEGR1* variants are associated with the development of VF/r among ART-treated adults in Botswana.**
- *NEGR1* is a neuronal development gene previously associated with several diseases and traits, including obesity, BMI, asthma, and SLE. The association of *NEGR1* variations with VF/r is **independent of BMI** (data not shown).
- The allelic frequency of the top *NEGR1* SNP in our Botswana group differs from 1000 Genomes reference populations. This emphasizes the genetic diversity of African populations and the **paramount importance on collecting genetic data in additional populations**.
- This first report of a non-*HLA* association with VF/r warrants **replication and functional investigation** to decipher the role played by *NEGR1* in development of HIV drug resistance.