Leveraging Multi-Ancestry Polygenic Risk Scores for Body Mass Index to Predict Antiretroviral Therapy-Induced Weight Gain*

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Widespread availability of antiretroviral therapies (ART) for HIV-1 have generated considerable interest in understanding the pharmacogenomics of ART. In some individuals, ART has been associated with excessive weight gain, which disproportionately affects women of African ancestry. The underlying biology of ART-associated weight gain is poorly understood, but some genetic markers which modify weight gain risk have been suggested, with more genetic factors likely remaining undiscovered. To overcome limitations in available sample sizes for genome-wide association studies (GWAS) in people with HIV, we explored whether a multi-ancestry polygenic risk score (PRS) derived from large, publicly available non-HIV GWAS for body mass index (BMI) can achieve high cross-ancestry performance for predicting baseline BMI in diverse, prospective ART clinical trials datasets, and whether that PRSbmi is also associated with change in BMI over 48 weeks on ART. We show that PRSbmi explained ~5-7% of variability in baseline (pre-ART) BMI with high performance in both European and African genetic ancestry groups, but that PRSbmi was not associated with change in BMI on ART. This study argues against a shared genetic predisposition for baseline (pre-ART) BMI and ART-associated weight gain.

Keywords: HIV; AIDS; Polygenic Risk Scores; BMI; Pharmacogenomics.

1. Introduction

1.1. Many antiretroviral therapies for HIV are associated with weight gain

There are ~1.2 million individuals in the United States and ~38 million worldwide living with HIV-1.1 With >30 FDA-approved antiviral agents for treating HIV-1, many available in combination co-formulated tablets, and with long-acting injectable agents now available, HIV is now a chronic treatable infection in most patients with access to contemporary antiretroviral therapy (ART). However, there remains considerable interindividual variability in HIV treatment responses including drug toxicity, immune recovery, and drug-drug interactions. Variable responses may be influenced by polymorphisms in drug absorption, distribution, metabolism, and

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elimination (ADME) genes and/or off-target genes. Beyond the need to develop novel therapies and optimize current therapies are newer priorities which include achieving functional or sterilizing cure of HIV and reducing HIV-associated inflammation and immune activation so as to prevent end-organ complications.

Weight gain following ART initiation is common with most modern ART regimens. The greatest weight gain has been observed in individuals of African ancestry, especially among women of African ancestry. While environmental and social factors likely play a role, there is also the potential for an underlying genetic predisposition. As a few examples among many, it has been shown that, among patients who switched from efavirenz- to integrase strand transfer inhibitor (INSTI)-based ART, CYP2B6 genotype was associated with weight gain, possibly reflecting withdrawal of inhibitory effects of higher efavirenz levels. Analyses using Phase 1 clinical trials data showed that CYP2B6 slow metabolizers who switch from efavirenz to dolutegravir will have more prolonged subtherapeutic dolutegravir levels. In ART-naive AIDS Clinical Trial Group (ACTG) studies, CYP2B6 slow metabolizers had less weight gain at week 48 in participants receiving efavirenz with tenofovir disoproxil fumarate (TDF) but not those receiving efavirenz with abacavir. We previously discovered and replicated an association between CYP2B6 15582C→T (rs4803419) and efavirenz Cmin in self-identified Black, Hispanic, and white individuals, showed that this single nucleotide polymorphism (SNP) improved prediction of efavirenz plasma exposure in individuals living with HIV in South Africa, and showed that this polymorphism is associated with decreased plasma nevirapine clearance in Asians. While we and others have identified potential genetic associations with weight gain, a large proportion of variation remains unexplained. Given this discrepancy, it is plausible that susceptibility to ART-associated excessive weight gain will be affected by each individual’s overall genetic predisposition at many genetic loci.

1.2. Polygenic risk scores allow for prediction of complex traits such as body mass index

Polygenic risk scores (PRS) are the cumulative, mathematical aggregation of risk derived from the contributions of many DNA variants across the genome. PRS are a powerful technology in the field of disease risk prediction and have been shown to be correlated with disease incidence in coronary artery disease, type 2 diabetes, atrial fibrillation, breast cancer, schizophrenia, and many other traits. In recent years there have been advances in PRS methodology that incorporate diverse ancestry groups, quantitative and qualitative phenotypes, and consider different linkage disequilibrium (LD) reference panels. In addition, PRS and SNP-based heritability estimation have been applied to body mass index (BMI) in large biobank populations and genome-wide significant SNPs have been shown to explain ~6% of trait interindividual variation in BMI (while considering all common SNPs, the estimate is greater than 20%). When considering the underlying genetic predisposition to weight gain in response to ART, is it possible that the underlying genetic background for BMI in populations without HIV will also be predictive of weight gain in response to ART? In this paper, we explore whether susceptibility to ART-associated weight gain is influenced by each individual’s overall genetic predisposition to higher BMI as reflected by PRS for BMI (PRSBM) derived from large datasets from populations without
Figure 1 shows an overview of our study design, which is described in more details in Methods.

2. Methods

2.1. Data and Study Participants

2.1.1. GWAS Summary Statistics

We used publicly available summary statistics from existing genome-wide association studies (GWAS) for BMI in European and African ancestry populations. The European ancestry summary statistics come from the GIANT consortium’s meta-analysis of ~700,000 individuals of European ancestry which contained 2,336,269 SNPs.\(^{21}\) The African ancestry summary statistics come from the African American Anthropometry Genetics Consortium’s GWAS of 42,752 individuals which included ~18,000,000 variants.\(^{22}\) Both sets of summary statistics were subset to the ~1.1 million HapMap3 SNPs included in the PRS-CSx LD reference for the PRS-CSx analysis.\(^{18}\)

2.1.2. AIDS Clinical Trials Group Data

These study data are from a retrospective analysis of a clinical trials cohort from efavirenz-containing arms of prospective, randomized ACTG protocols. Data were from ART-naive individuals who initiated efavirenz-containing regimens in ACTG studies A5095 (NCT00013520), A5142 (NCT00050895), and A5202 (NCT00118898) in the United States and consented to genetic testing.\(^{23}\) All participants provided written informed consent for genetic research and
provided DNA for analysis. Drug class components of regimens were randomly assigned (efavirenz-based versus comparator) except for nucleoside reverse transcriptase inhibitor (NRTI) choice in A5142. Eligible individuals met the following criteria: initial efavirenz-containing regimens included TDF or abacavir; available weight data at entry and week 48 (± 4 weeks); >100 CD4 T-cells/mm3 at baseline and week 48; HIV-1 RNA <400 copies/mL at week 48; and available CYP2B6 genotypes. This cohort did not receive INSTIs. The participants’ sex was 78.4% male (n = 413) and 21.6% female (n = 114). Data on participants’ gender was not available.

2.2. Quality Control

2.2.1. Genotypic Data

DNA was extracted from whole blood collected from consenting participants, and DNA extracted. Samples were labelled with coded identifiers. Stored DNA was genotyped in seven different phases using different genotyping arrays. Phases 1, 2, and 3 were genotyped at the Broad Institute with HumanHap650Yu3_A for phases 1 and 2, and Human1M-Duov3_B for phase 3. For phases 4-7, genotyping was performed at the Vanderbilt Technologies for Advanced Genomics (VANTAGE) facility using the Human Core Exome chip for phase 4, HumanOmmni2.5Exome-8-v1.1_A1 chip for phase 5, the HumanOmni25-8v1-2_A1 chip for phase 6, and the Illumina Infinium Multi-Ethnic Global BeadChip (MEGAEX) for phase 7.

Post-genotype quality control was performed by Vanderbilt Technologies for Advanced Genomics Analysis and Research Design (VANGARD). All quality control steps were performed using PLINK version 1.9.28 Genotyping efficiency per participant was > 99% for all samples, and discordant samples between genotype sex and reported sex were removed from the datasets prior to imputation. After quality control steps, each genotyping phase was imputed separately using the TOPMed reference panel after transforming to genome build 38 using liftOver and stratification by chromosome to parallelize the imputation process.29 The seven imputed datasets were merged using PLINK, and we excluded imputed polymorphisms with imputation R² scores < 0.3, genotyping call rates < 95%, or minor allele frequency (MAF) < 0.05.29 Genotype data were transformed back to genome build 37 using liftOver to allow compatibility with the PRS-CSs LD reference panels. Genetic ancestry was inferred using principal component analysis with 1000 Genomes as the reference, to assign each participant to a superpopulation of African (AFR), Admixed American (AMR), East Asian (EAS), European (EUR), South Asian (SAS), or Other.

2.3. Polygenic Risk Score Construction

2.3.1. Pruning and thresholding

A PRS for baseline BMI (PRSBMI) was created using PRSice 2.3.5 (2021-09-20) for LD clumping and p-value thresholding with default optimization parameters.37 A multi-ancestry LD reference was generated using data from the 1000 Genomes Project.38 Optimal p-value thresholds were estimated in a subset of the target data comprising 20% of the total target set (n=1055527) for both the European and African ancestry summary statistics. This threshold was then used to calculate an EUR-derived PRSBMI and AFR-derived PRSBMI for the remaining 80% of individuals. This approach was also used to separately optimize p-value thresholds for predicting BMI change on ART.
2.3.2. PRS-CSx

PRS-CSx (version July 29, 2021) was used to construct a multi-ancestry PRS<sub>BMI</sub>, where both the European and African ancestry summary statistics were jointly adjusted by the model using default optimization parameters to learn the shrinkage factor. The output was then converted to risk scores using the PLINK ‘--score’ function as described in the PRS-CSx documentation. The resulting PRSs were analyzed independently for their performance in each ancestry group and were also linearly combined to create a multi-ancestry PRS<sub>BMI</sub>. A mixing parameter for the combined PRS<sub>BMI</sub> was optimized in a subset of the target data comprising 20% of the total target set (n=105/527) and was optimized to minimize the difference in mean PRS<sub>BMI</sub> between the AFR and EUR ancestry groups. The resulting PRS<sub>COMB</sub> took the form of PRS<sub>COMB</sub> = PRS<sub>EUR</sub> + α*PRS<sub>AFR</sub> where α is the mixing parameter.

2.4. Computational and statistical analysis

All data analyses were performed using python3, scipy, and pandas in a jupyter notebook. The distribution of PRS<sub>BMI</sub> scores was compared between ancestry groups to evaluate systematic ancestry-dependent trends and biases. Performance of each PRS<sub>BMI</sub> was evaluated as the R<sup>2</sup> value of the PRS<sub>BMI</sub> in the test set against the phenotype of interest (baseline BMI or change in BMI). Linear regression was used to calculate a p-value for each PRS<sub>BMI</sub>. For the pre-ART BMI phenotype, we also adjusted for the first 10 principal components, age, sex, and baseline weight in our regression and calculated the incremental performance of our PRS<sub>BMI</sub> by comparing the PRS<sub>BMI</sub> + covariates R<sup>2</sup> to the covariates-only model and recorded the p-value for the PRS<sub>BMI</sub> parameter in the PRS<sub>BMI</sub> + covariates model. For BMI change, we also adjusted for the first 10 principal components, age and sex, as well as baseline BMI.
3. Results

3.1. PRS-CSx produces a high-performing multi-ancestry PRS for baseline BMI

Fig. 2. Distribution of PRS from PRS-CSx in each ancestry group. (A) European-derived PRS vs baseline BMI. (B) African-derived PRS vs baseline BMI. (C) Combined PRS + PRS vs baseline BMI. (D) Combined PRS vs BMI change from baseline to week 48 on ART.

3.1.1. PRS generated from European summary statistics systematically overestimate BMI in African ancestry individuals

Consistent with other work applying PRS across ancestry groups, the EUR-derived PRS (PRS EUR) from PRSice and PRS-CSx both perform best in the EUR ancestry subset of our data and have significant performance decreases in other ancestry groups. Before covariate adjustment, PRS EUR from PRSice performs better at predicting baseline BMI in EUR than the PRS EUR from PRS-CSx, with an $R^2$ of 0.080 versus 0.070. However, the PRSice PRS EUR performs very poorly in AFR compared to the PRS-CSx PRS EUR, with $R^2$ in AFR of 0.0032 and 0.055 respectively. Scatterplots of the PRS vs BMI show that the discrepancy in performance is accompanied by a systematic overestimation of AFR BMI in the PRSice PRS EUR (Supplementary Figure 1). This trend is also present in the PRS-CSx results (Figure 1A). Full PRS performance results are provided in Supplementary Table 1. Interestingly, the performance of the PRSice PRS EUR in AMR was high, with an $R^2$ of 0.110.

3.1.2 PRS generated from African summary statistics produces a bimodal distribution

Similar to the trend in PRS EUR, the AFR-derived BMI PRS (PRS AFR) performs better in the AFR ancestry subset of our data, with $R^2$ in AFR of 0.052 and 0.062 for PRSice and PRS-CSx
respectively. However, the PRS_{AFR} from PRSice performs much worse in EUR than the PRS-CSx one does, with $R^2$ of 0.0063 and 0.034 respectively. In both the PRSice and PRS-CSx results, the distribution of PRS varies by ancestry, but the difference is particularly pronounced between AFR and EUR, where scores in the AFR population and EUR population from both PRSice and PRS-CSx are entirely disjoint, with the highest AFR score being lower than the lowest EUR score (Figure 1B, Supplementary Figure 1).

### 3.1.2. Linear combination of the European and African PRSs

- **Table 1. Multi-ancestry PRS-CSx PRS_{COMB} performance for BMI prediction in each ancestry group**

<table>
<thead>
<tr>
<th>Target Ancestry</th>
<th>$R^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUR (n=206)</td>
<td>0.0725</td>
<td>9.1e-5</td>
</tr>
<tr>
<td>AFR (n=128)</td>
<td>0.0795</td>
<td>1.3e-3</td>
</tr>
<tr>
<td>AMR (n=43)</td>
<td>0.0674</td>
<td>0.060</td>
</tr>
<tr>
<td>Multi-ancestry (n=422)</td>
<td>0.0663</td>
<td>8.1e-8</td>
</tr>
</tbody>
</table>

Given that PRS_{EUR} overestimates BMI in AFR compared to EUR and that PRS_{AFR} underestimates BMI in EUR compared to AFR, we combined the two PRS additively, tuning a mixing parameter such that we minimized the difference in mean combined PRS (PRS_{COMB}) between the AFR and EUR test sets (Table 1). Beyond outperforming both PRS_{AFR} and PRS_{EUR} in AFR test set, the PRS_{COMB} also improves performance in the EUR set. The PRS_{COMB} also improves performance for admixed individuals (AMR) over the PRS-CSx PRS_{EUR} which achieved an $R^2$ of 0.056. For comparison purposes, we explored a similar linear combination of the PRSice scores, but to avoid further reducing the sample size, we opted to optimize the combination in the entire test set by also minimizing the difference in mean PRS. Despite the possibility of overfitting to the test data, we found that this approach resulted in drastically diminished performance in the AFR test set, with an $R^2$ of 0.0016. This seems to indicate that linear combination of PRS_{EUR} from pruning and thresholding is not as effective for creating an unbiased multi-ancestry PRS_{BMI}. Full PRS_{BMI} performance results for predicting BMI in each ancestry group are provided in Supplementary Table 1. Additionally, when we adjust our PRS_{BMI} for the first 10 principal components, age, sex, and height, the incremental performance of PRS-CSx PRS_{COMB} on the entire population is greater than the incremental performance of the PRSice PRS_{COMB} with $R^2$ increases of 0.053 and 0.038 respectively over the covariates alone. Furthermore, we see that the incremental performance of the PRS-CSx PRS_{COMB} is greater than the incremental performance of the single-ancestry PRS-CSx PRSs (Supplementary Table 2).
3.2. **PRS_{BMI} is not correlated with weight change on antiretroviral therapy**

<table>
<thead>
<tr>
<th>Target Ancestry</th>
<th>$R^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUR (n=206)</td>
<td>0.0085</td>
<td>0.186</td>
</tr>
<tr>
<td>AFR (n=128)</td>
<td>8.97e-07</td>
<td>0.992</td>
</tr>
<tr>
<td>AMR (n=43)</td>
<td>0.020</td>
<td>0.305</td>
</tr>
<tr>
<td>Multi-ancestry</td>
<td>0.0073</td>
<td>0.080</td>
</tr>
</tbody>
</table>

With our high-performing multi-ancestry PRS_{BMI} from PRS-CSx, we then measured its performance in predicting BMI change from baseline to week 48 following initiation of ART. Across all ancestry groups, the PRS_{BMI} was not a significant predictor of weight change and had small $R^2$ values in all analyses (Table 2). The performance of the other PRSs for BMI change prediction can be found in Supplementary Table 3 with concurrent results. When we subsequently adjust for the first 10 principal components, age, sex, and baseline BMI, we see negligible change in prediction performance or statistical significance (Supplementary Table 4). This evidence further supports the conclusion that weight gain following ART shares little to no underlying genetic predisposition with baseline BMI.

4. Discussion

Our work carries interesting implications for the underlying biology of ART-associated weight gain and for the application of PRS derived from large population GWAS for predicting potentially related traits. First, we were able to successfully construct PRS for BMI (PRS_{BMI}) using large, publicly-available GWAS summary statistics for BMI in different ancestry groups. We showed that while pruning and thresholding produced higher performance in EUR using the EUR summary statistics, PRS-CSx produced a better multi-ancestry PRS, with the exception of the AMR population subset, where pruning and thresholding-based combined PRS performed higher than any other ancestry or PRS. A larger validation set of AMR individuals will be needed to see whether this performance holds, but this could be a consequence of the use of a multi-ancestry subset of the dataset to tune the p-value threshold. Notably, we also demonstrated that our PRS_{BMI} derived from summary statistics from a population without HIV is highly predictive of BMI pre-treatment in individuals with HIV. Through the use of PRS-CSx, we were subsequently able to create a multi-ancestry PRS_{BMI} that performed very well in both EUR and AFR populations. This followed from the peculiar observation that the PRS_{AFR} from both PRSice and PRS-CSx showed a disjoint bimodal distribution where PRS_{AFR} is drastically lower in the AFR subset of the population. Since PRS_{EUR} tends to overestimate BMI in the AFR subset, the PRS_{AFR} can be seen as a "correction factor" for the PRS_{EUR}, increasing scores for EUR and decreasing scores for AFR to mitigate the bias. Despite this trend appearing from both PRSice and PRS-CSx, PRSice did not produce a very effective multi-ancestry PRS.

Despite the strong correlation between our PRS_{BMI} and baseline BMI, the PRS_{BMI} was not well correlated with BMI change in response to ART, and we did not find statistically significant evidence that PRS_{BMI} is associated with BMI change in response to efavirenz-based therapy, even when adjusting for covariates including baseline BMI. Our results provide compelling evidence...
that an individual’s genetic predisposition based on a common variant PRS for higher BMI may not contribute to greater ART-associated weight gain. It is still possible that other genetic models and/or low frequency variants not captured by PRS may play a role in ART-associated weight gain. Future research on the causes of ART-associated weight gain should explore distinct mechanisms beyond our canonical understanding of the genetics of obesity and BMI.

There are limitations to this work which may have influenced our results. First, our PRS testing sample size was limited to approximately 500 individuals, and when subdivided by ancestry the sample sizes become smaller, limiting our power to find associations between our PRS and target traits. As such, it remains a possibility that PRS could be associated with ART-associated weight gain, but at a smaller effect size than we could detect given our statistical power. Additionally, due to particularly small sample sizes of East Asian and South Asian individuals, we mostly focused on cross-ancestry performance in EUR, AFR, and AMR populations, as well as in the entire population. Finally, it is also worth noting that integrase inhibitor-associated weight gain is greater than efavirenz-associated weight gain and that integrase inhibitors are currently the preferred initial therapy for most people. The ACTG cohorts included in this study did not receive INSTIs; thus the effect sizes may be larger if this investigation was repeated in a cohort of individuals who experienced weight gain after receiving INSTIs.

Subsequent work in this area could investigate how other covariates may influence BMI change. In further exploration of the use of large sample-size GWAS to construct PRS for drug response traits, one could study other phenotypes, such as how GWAS for liver function tests (such as alanine transaminase (ALT) and aspartate transaminase (AST)) may be predictive of adverse liver events, or whether a PRS derived from GWAS for major depressive disorder is predictive of neurological effects of ART. These approaches have the potential to leverage large, publicly available datasets to generate new discoveries in smaller pharmacogenetic cohorts. As more associations or lack thereof are found, we continue to narrow down the likely biological causes of adverse drug reactions such as excessive weight gain, bringing us closer to the true etiology.

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Supplementary Figures/Tables

Supplementary Figure 1. PRSice PRS for BMI plotted against baseline BMI
Supplementary Table 1. Performance of each PRS for predicting baseline BMI
Supplementary Table 2. Incremental performance of each PRS for predicting baseline BMI
Supplementary Table 3. Performance of each PRS for predicting BMI change
Supplementary Table 4. Incremental performance of each PRS for predicting BMI change

All supplemental data can be found at the links above or at: https://ritchielab.org/publications-supplementary-data/psb-2023/actg-bmi-prs.

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