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# *RYR3* gene variants in subclinical atherosclerosis among HIV-infected women in the Women's Interagency HIV Study (WIHS)

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## Abstract

**Background—**Single nucleotide polymorphisms (SNPs) in the Ryanodine receptor 3 (*RYR3)* gene are associated with common carotid intima media thickness (CCA cIMT) in HIV-infected men. We evaluated SNPs in the *RYR3* gene among HIV-infected women participating in Women's Interagency HIV Study (WIHS).

**Methods—**CCA cIMT was measured using B-mode ultrasound and the 838 SNPs in the RYR3 gene region were genotyped using the Illumina HumanOmni2.5-quad beadchip. The CCA cIMT



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genetic association was assessed using linear regression analyses among 1213 women and also separately among White (n=139), Black (n=720) and Hispanic (n=354) women after adjusting for confounders. A summary measure of pooled association was estimated using a meta-analytic approach by combining the effect estimates from the three races. Haploblocks were inferred using Gabriel's method and haplotype association analyses were conducted among the three races separately.

**Results—**SNP *rs62012610* was associated with CCA cIMT among the Hispanics ($p=4.41\times 10^{-5}$), *rs11856930* among Whites ($p=5.62\times 10^{-4}$), and *rs2572204* among Blacks ($p=2.45\times 10^{-3}$). Meta-analysis revealed several associations of SNPs in the same direction and of similar magnitude, particularly among Blacks and Hispanics. Additionally, several haplotypes within three haploblocks containing SNPs previously related with CCA cIMT were also associated in Whites and Hispanics.

**Discussion—**Consistent with previous research among HIV-infected men, SNPs within the *RYR3* region were associated with subclinical atherosclerosis among HIV-infected women. Allelic heterogeneity observed across the three races suggests that the contribution of the *RYR3* gene to CCA cIMT is complex, and warrants future studies to better understand regional SNP function.

### Keywords

RYR3; single nucleotide polymorphisms; HIV infection; CCA; cIMT; subclinical atherosclerosis

## Introduction

Acquired immunodeficiency syndrome (AIDS) related events and deaths have declined precipitously, but morbidity and mortality in the context of HIV infection has increased [1–4]. Specifically, cardiovascular disease has emerged as one of the leading public health concerns with higher incidence rates among HIV-infected individuals [1–3, 5]. In the Women's Interagency HIV Study (WIHS), cardiovascular disease was one of the most common causes of death among HIV-infected women [2]. Studies have reported that HIV-infected women have higher rates of coronary artery disease (CAD) and higher mortality compared to HIV-infected men [2, 6, 7]. Likewise, HIV-infected women have greater rates of myocardial infarction than HIV-infected men [3, 8].

Carotid intima media thickness (cIMT) is often measured at the common carotid artery (CCA) and internal carotid artery and several studies found that CCA cIMT is a reliable and valid subclinical measure of early atherosclerosis and a strong predictor of incident cardiovascular events in HIV-uninfected individuals [9–11]. A systematic review and meta-analysis confirmed this strong relationship between CCA cIMT and subsequent vascular events [12]. Progression of atherosclerosis, assessed by CCA cIMT, is more rapid among HIV-infected individuals compared to HIV-uninfected controls [13, 14]. Although, HIV infection and its management with antiretroviral therapy, especially protease inhibitors is postulated to contribute to subclinical atherosclerosis [1, 15, 16], reports are inconsistent [17–20]. A meta-analysis indicated that protease inhibitors are not strong independent risk factors for CCA cIMT [21]. All factors contributing to the process of atherosclerosis are not fully known, particularly within the context of HIV infection.

Heritable factors account for 38%-66 % of the variance in the CCA cIMT [22, 23]. The limited genetic studies on CCA cIMT, including genome wide association studies (GWAS) were performed primarily in HIV-uninfected individuals and most studies show that CAD associated variations are not associated with CCA cIMT [24–29]. However, the contributing factors of HIV, treatment and immunosuppression could involve different sets of genes and biological pathways in HIV-infected patients. More recently, we reported two SNPs (i.e.,

rs2229116 and rs7177922) in tight linkage disequilibrium (LD) in the *RYR3* gene on chromosome 15 associated with CCA cIMT in White HIV-infected men from the Fat Redistribution and Metabolic Change in HIV Infection Study in a GWAS [30]. These findings were further replicated in the Multicenter AIDS Cohort Study (MACS) among White HIV-infected men [31]. *RYR3* encodes an intra-cellular receptor that acts as a calcium ion-channel and is expressed on the endoplasmic reticulum of cardiac cells [32]. In this hypothesis-based study, we examined the association between genetic variations within the *RYR3* gene and subclinical atherosclerosis using CCA cIMT amongst an ethnically diverse group of HIV-infected women in the WIHS.

## Methods

### Study population

The WIHS is the largest US multi-center prospective cohort study of HIV-infected and risk-matched HIV-uninfected women enrolled across six sites (i.e., Bronx/Manhattan, NY; Brooklyn, NY; Washington DC; San Francisco Bay Area, CA; Los Angeles/Southern California/Hawaii; Chicago, IL) [33]. A total of 3,766 women were enrolled during the first two enrollment waves, 2,054 HIV-infected and 569 HIV-uninfected women during the initiation phase in 1994/95 and another 737 HIV-infected and 406 HIV-uninfected women during the expansion phase in 2001/02 [33, 34]. The study examines the effects of antiretroviral treatment, especially highly active anti-retroviral therapy (HAART) among diverse HIV-infected population group without AIDS or associated clinical conditions [34]. HIV-uninfected women at high risk of acquiring the infection and having similar characteristics were also recruited to form a comparison group but are not included in the current study. In a vascular disease sub-study in April 2004, CCA cIMT was measured in 1,331 HIV-infected women from WIHS [19], and 1,230 of them in three races (140 Whites, 729 Blacks, and 361 Hispanics) with genotypes available in *RYR3* gene region were considered in our analyses. Other race/ethnicity groups could not be analyzed since they were not sufficiently large for analysis.

### Carotid Intima Media Thickness (cIMT)

Measurements for CCA cIMT were obtained following a standard protocol of the vascular disease sub-study across all study sites, as previously described [19]. Briefly, the measurements were taken by sonographers who received uniform training at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center. The complete ultrasound included: 1) standardized measurements of the far right wall of the distal CCA for IMT, stiffness and lesions and 2) scanning the proximal internal carotid, external carotid and right carotid bulb at the bifurcation for lesions [19]. The ultrasound utilizes computerized and automated edge-tracking multiframe image processing to measure IMT in multiple sequential frames over several cardiac cycles, most frequently 80 frames spanning 2 cardiac cycles [35]. The coefficient of variation was 1.8% (Intraclass correlation=0.98; n=113) for repeated IMT measurements with the initial images guiding the repeat scans.

### DNA Extraction

Blood samples were collected at each semiannual visit for laboratory testing. Genomic DNA was isolated from peripheral blood leucocytes using the Pure-gene DNA isolation kit (Gentra Systems, Minneapolis, MN). The PICO Green dsDNA quantification kit (Molecular Probes, Eugene, OR) and the Perkin Elmer HTS7000 BioAssay Reader were used for DNA quantification and reading the microplate, respectively. All samples were stored at −20C [36].

## Genotyping and Quality Control

A total of 838 SNPs within the *RYR3* gene were genotyped within the genomic regions spanning 33603163 to 34158303 base pairs and ~20 kb flanking the gene (NCBI build 37, hg19) in chromosome 15 using the Illumina HumanOmni2.5-quad beadchip (Illumina, San Diego). SNPs were excluded from the analysis based on missing genotype [i.e. 3 SNPs in all race groups with > 10% missing data], Hardy-Weinberg equilibrium [i.e. 3 SNPs with $p < 0.001$ in Hispanics and Blacks respectively], and minor allele frequency (MAF) < 0.01 [i.e., 197 in Whites, 130 in Hispanics and 88 SNPs in Blacks]. Of the 1230 individuals, seventeen did not have non-genetic variables (i.e., age, CD4 cell count, HAART) or ancestry informative markers derived principal components (PCs) that were adjusted for in the models. After quality control, the association results for CCA cIMT in the race-specific adjusted linear regression analyses were based on 638 SNPs in 139 Whites, 744 SNPs in 720 Blacks, and 702 SNPs in 354 Hispanics. The combined (adjusted) analysis included a total of 657 SNPs, after exclusion of 3 SNPs with > 10% missing data, 107 SNPs with Hardy-Weinberg equilibrium < 0.001 and 71 SNPs with MAF < 0.01 in all races, in 1213 individuals. The results of the three race/ethnic groups were further pooled to obtain a summary estimate of association by employing a meta-analytic approach and included 786 unique SNPs.

## Statistical Analysis

All analyses were performed using the PLINK (v1.07) software with default settings [37]. Quantitative trait analysis for the base10 log-transformed CCA cIMT was performed using an additive linear regression model among all individuals and also separately among Whites, Blacks and Hispanics. All analyses were adjusted for age, CD4 cell count, HAART and PCs of the genetic covariance. Genetic ancestry components were estimated using 157 ancestry informative marker SNPs selected from Illumina HumanOmni2.5-quad beadchip using Helix Tree (Golden Helix, Bozeman, MT) to obtain principal components. Briefly, the number of PCs which distinguished the major racial/ethnic groups in the sample was sought by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernible clustering of patients by their self-reported race/ethnicity was possible (data not shown). The first 9 PCs and the first 3 PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) in the overall adjusted analysis and race/ethnicity specific analysis, respectively.

The method proposed by Li and Ji was employed to correct for multiple testing for the SNPs potentially in high LD and clustered in the genomic region [38]. Briefly, this method calculates what is known as the "effective number" ($M_{eff}$) of independent tests from correlated tests and improves upon the method proposed by Cheverud [39] by accommodating for moderately correlated tests and providing a less conservative estimate. Thus, the p-value thresholds for the multiple testing corrections in Whites, Blacks, and Hispanics were estimated to be $1.91\times 10^{-4}$, $1.47\times 10^{-4}$ and $1.75\times 10^{-4}$, respectively. Further, pooled analysis of the effect estimates from the three individual race/ethnicity groups was performed to obtain a more precise summary estimate of the SNP associations by employing a meta-analytic approach in PLINK [37]. Meta-analysis in PLINK evaluates SNPs that are informative in at least two studies/groups and provides both the fixed- and random-effects estimates. Herein, we report the fixed-effects estimates and p-values.

Gabriel's LD method based haploblocks were determined in PLINK using all SNPs with an MAF > 0.05, spanning the *RYR3* gene region in each race/ethnicity group [40]. A single H-1-*df* omnibus test (H = Number of haplotypes) that jointly tests all haplotype effect was performed first. The omnibus p-values were calculated for each block within each race/ethnicity group and the degrees of significance were plotted across the *RYR3* gene region to

visualize any association hotspots potentially missed by single SNP association analysis. Additionally, since rs2229116, rs7177922 and rs2291734 were previously associated with CCA cIMT among HIV-infected White men and rs2278309 separately associated with CCA cIMT among Mexican Americans in the San Antonio Family Heart Study [24, 30, 31], we performed haplotype-specific association tests for all haploblocks that included these SNPs, using linear regression analyses.

## Results

Characteristics of the 1,230 HIV-infected women included in our study are provided in Table 1. Of these, 58% are non-Hispanic Blacks, 29% are Hispanics and 11% non-Hispanic Whites. The mean age of the participants was 41.7 (± 8.8) years and the mean $\log_{10}$ CCA cIMT measurement was −0.14 mm (± 0.06 mm). Hispanic women had lower mean CCA cIMT value (−0.16 mm ± 0.06 mm) compared to Whites (−0.14 mm ± 0.07 mm, p=0.0005) and Blacks (−0.13 mm ± 0.06 mm, p<0.0001) but there was no significant difference between Whites and Blacks (−0.14 mm ± 0.07 mm vs. (−0.13 mm ± 0.06 mm, p=0.55).

### Single SNP Association Analysis

Single SNP association analysis results are shown in Figure 1. The top associated SNPs in the three separate race/ethnicity groups, in the combined race/ethnicity adjusted analysis, and meta-analysis are shown in Table 2. Specifically, the SNP *rs62012610* (C) (β= −0.037 ± 0.009, p=4.41× $10^{-5}$) among the Hispanic women was significantly associated with CCA cIMT, even after correction for multiple testing. While SNP *rs11856930* (G) (β= 0.032 ± 0.009, p=5.62× $10^{-4}$) was associated with CCA cIMT among Whites, SNP *rs2572204* (A) (β= 0.016 ± 0.005, p=2.45× $10^{-3}$) was the top hit among Blacks. Several additional SNPs were associated in the same direction and of similar magnitude particularly among Blacks and Hispanics (Table 2).

### Haplotype Analysis of Three Regions with Prior Association

There were a total of 80 blocks in the ~600 kb *RYR3* gene region among Whites, 119 among Blacks, and 108 among Hispanics. The extent of haplotype associations, within defined haploblocks, varied across the *RYR3* gene region (Figure 2a). Several blocks of haplotypes were associated with CCA cIMT in Hispanics, more than those observed among Whites and Blacks. Of note, haplotypes in the haploblock region near 33.68 MB were associated with CCA cIMT in all three ethnic groups (Figure 2a).

When considering *RYR3* SNPs highlighted by previous GWAS of CCA cIMT, different haploblock patterns were observed in the three ethnic groups including rs2229116 and/or rs7177922 (Figure 2b). In Whites, a block spanning rs229116 to rs7168848 and also includingrs7177922 was observed. In Blacks, a smaller block spanning rs10519837 to rs58822275 and only including rs7177922 was observed. In Hispanics, a block spanning rs4238566 to rs9806592 and only including rs7177922 was observed (Figure 2b). As shown in Figure 2b (supplement Table 3a), haplotype W-A5 (frequency = 0.16) in Whites and haplotypes H-A7 (frequency = 0.21), H-A9 (frequency =0.11), H-A11 (frequency = 0.10), H-A13 (frequency = 0.06) in Hispanics were associated with CCA cIMT at a significance level of 0.05. As shown in Figure 2c (supplement Table 3b), the SNP rs2291734 did not occur in the haploblock in Blacks, but a haploblock closest to it spanned from rs12148702 to rs10851886. In Whites and Hispanics, the haploblock spanned from rs1495280 to rs10851886 and included rs2291734. Haplotypes H-B2 (frequency = 0.26) and H-B6 (frequency = 0.14) in Hispanics and W-B1 (frequency = 0.13) in Whites were associated with CCA cIMT (Figure 2c). Likewise, rs2278309 occurs in different haploblocks in all three race/ethnicity groups with W-C1 (frequency=0.10) and W-C2 (frequency=0.22)

haplotypes among Whites and H-C1 (frequency=0.37), H-C2 (frequency=0.07) and H-C4 (frequency=0.14) among Hispanics associated with CCA cIMT (Figure 2d, supplement Table 3c).

## Discussion

In this study we assessed variations within the *RYR3* gene and their association with CCA cIMT, a marker for subclinical atherosclerosis, among an ethnically diverse population of HIV-infected women in the WIHS cohort. Several SNPs were associated with CCA cIMT, separately in each race/ethnic group and across the racial/ethnic groups. The top hits/SNPs from the meta-analysis were similar in magnitude and direction of association, particularly among Blacks and Hispanics. With the exception of a small number of SNPs/haplotypes, heterogeneity in the frequency and makeup of the *RYR3* gene region was observed. In tandem, differential associations were observed across the three race/ethnicity groups. While SNPs significantly associated with CCA cIMT in previous studies (i.e., rs2229116, rs7177922, rs2291734, and rs2278309) were not replicated in the single SNP analysis, haplotypes including these SNPs were associated in Whites and Hispanics. Blacks appear to have smaller haploblocks than those observed among Whites and Hispanics and possibly suggest that the susceptibility loci associated with CCA cIMT among Blacks are likely to be different based on LD, particularly if they are not the causal SNP, but only tagging one or more unmeasured causal SNPs.

The RYR3 receptor encoded by the *RYR3* gene is part of the Ryanodine Receptor (RYR) family that contains 3 isoforms (RYR 1–3) [32]. All three isoforms are variably expressed in the cardiac cells and are involved in signal transduction in addition to causing muscle contractions [32]. RYR3 is an intra-cellular calciumion channel attached to the endoplasmic reticulum and is involved in calciumhomeostasis [32]. Recent studies have indicated that *RYR3* gene could play a role in various phenotypes (e.g. breast cancer, Alzheimer's disease) and biological mechanism (e.g. intracellular calcium leakage) [41] [42]. In addition to the association with *RYR3*, SNPs within the *RYR2* gene were also associated with CCA cIMT in the context of HIV [30]. RYR3, when co-expressed with its isoform RYR2 in cell lines has been shown to form heteromeric complexes which are functional and amenable to regulation [43].

Functional studies on RYR3 and its isoforms demonstrate a major role of these receptors in modulating endothelial function and the atherogenic process via calcium signaling pathways [44, 45]. Calcium signaling is known to regulate endothelial cell function through several processes such as synthesis and secretion of vasodilating factors, cell aggregation or cell growth and proliferation [46]. Increased expression of calcium signaling genes, including *RYR*s, was noted in mice with atherosclerosis and in-vitro studies on endothelial cells further demonstrated the role of calcium signaling when induced with oxidized-low density lipoprotein (LDL) cholesterol. Induction by oxidized-LDL increased the endothelial calcium levels and also calcium-mediated production of monocyte chemoattractant protein-1 (MCP-1), [45] which is integral to recruiting inflammatory monocytes into the vascular walls [47]. Furthermore, RYRs are also involved in T-lymphocyte cell activation and expansion through calcium regulation [48, 49]. On the other hand, FK506 binding protein 1A, 12kDa (FKBP12) binds to RYR3 [50] and regulates calciumrelease by stabilizing the closed state of the receptor whereas transforming growth factor beta (TGF-beta) [51] regulates the expression of the RYRs and thereby calcium homeostasis. The association of SNPs in *RYR3* in this HIV cohort, further supports the role of calcium signaling in atherosclerosis [47] and functional studies can help identify mechanisms related to calcium-activated endoplasmic reticulum stress.

The HIV-tat protein has been used to observe the effect of HIV on cell function in several studies. HIV-tat protein was found to activate the RYRs and induce the unfolded protein response through calcium loss from the endoplasmic reticulum [52]. Apoptosis eventually occurs if the unfolded protein response to a trigger is inadequate. Additionally, HIV-tat reduces vasodilatation and release of MCP-1 from endothelial cells which recruit monocytes to the site of injury [53, 54]. These immunological perturbations associated with HIV infection are contributing factors in the atherosclerotic process and corroborate the role of RYRs in HIV associated atherosclerosis. However, HIV-tat levels may be low among HIV-treated patients and therefore other factors in relation to HIV may be driving the RYR3 mediated pathways. Nonetheless, biological mechanisms cannot be understood by association studies alone especially if men and women differ in factors that regulate RYR3 and related pathways or HIV pathogenesis itself.

Our current hypothesis-based analyses to examine variants in RYR3 gene included a predefined population that was a subset of the WIHS cohort with available genotype, but the participants were homogenous with respect to the gender and HIV status. Almost 94% of the HIV-infected women included in this analysis were on HAART, and the rest were taking one or more antiretroviral drugs; therefore, it is difficult to isolate the effect of HAART from HIV infection itself on the RYR3-CCA cIMT association. WIHS has a higher proportion of Black compared to White women and thus the smaller sample size for the Whites coupled with differences in men and women may have limited our ability to detect a statistically significant association with rs2229116 and rs7177922 associated with CCA cIMT among White HIV-infected men [30, 31]. Though the single SNP analysis from our study did not replicate previously reported findings, haplotypes including these SNPs show promise among White and Hispanic populations, but not as much in blacks. While there might be allelic heterogeneity in terms of associations, *RYR3* gene seems to be associated with CCA cIMT in HIV-infected women in WIHS.

Though the findings in our study do not highlight the same variants as previous studies in White, HIV-infected men, results still suggest *RYR3* to be associated with subclinical atherosclerosis in HIV-infected women [30, 31]. Differences may be due to the population being studied in WIHS (i.e. an ethnically diverse group of women demonstrating differences in LD patterns and haploblocks) or the small sample size for some racial groups (especially whites). Finally, HIV-infected women in WIHS appear to have differential risk for CAD due to differences in contributing factors like HIV immunosuppression or duration of antiretroviral therapy compared to HIV-infected men [19, 55] and warrants further investigation of both genetic and environmental factors. In sum, this study continues to demonstrate the potential importance of the *RYR3* gene, although allelic heterogeneity could possibly exist, in atherosclerosis in the context of HIV necessitating future investigations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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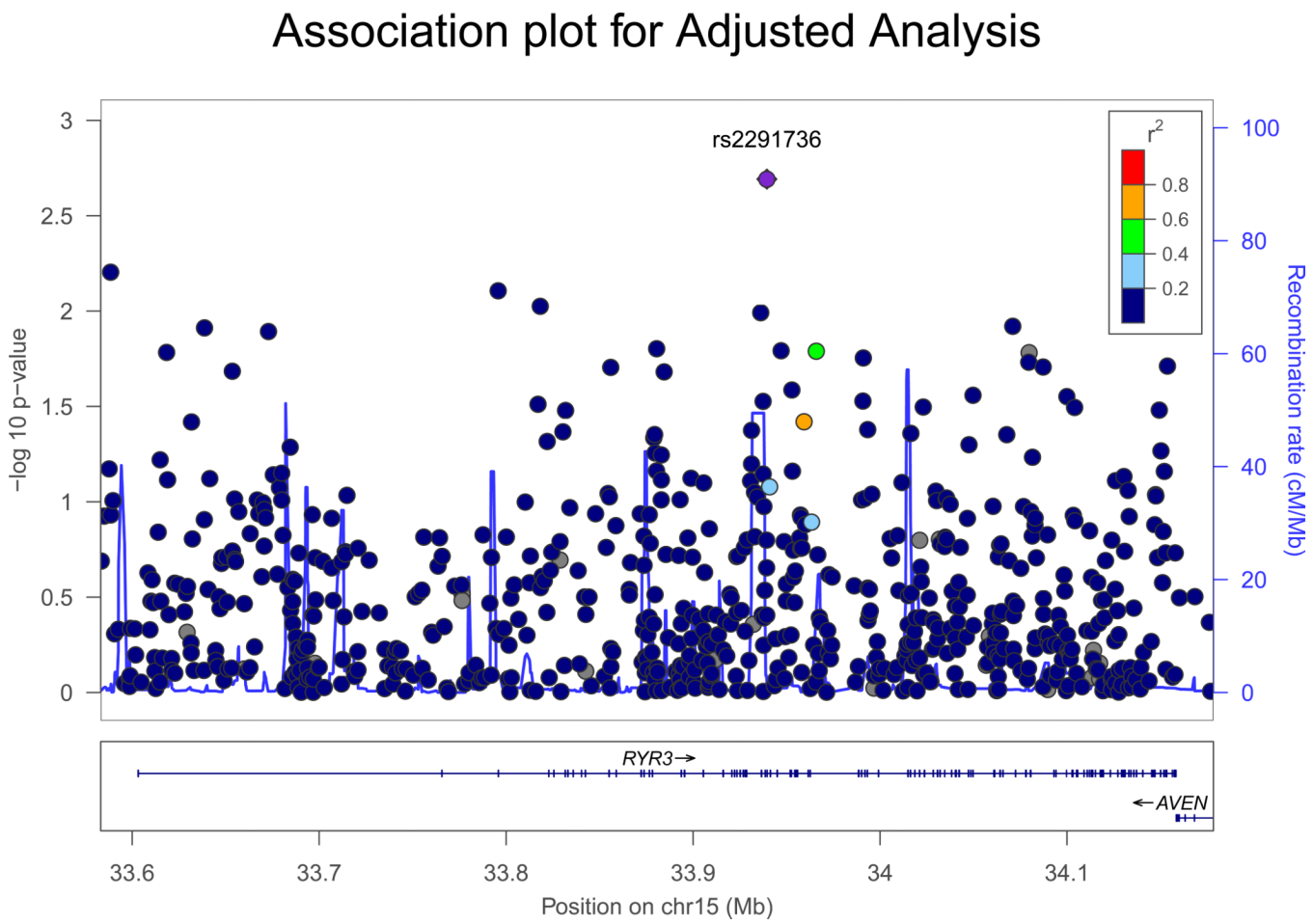
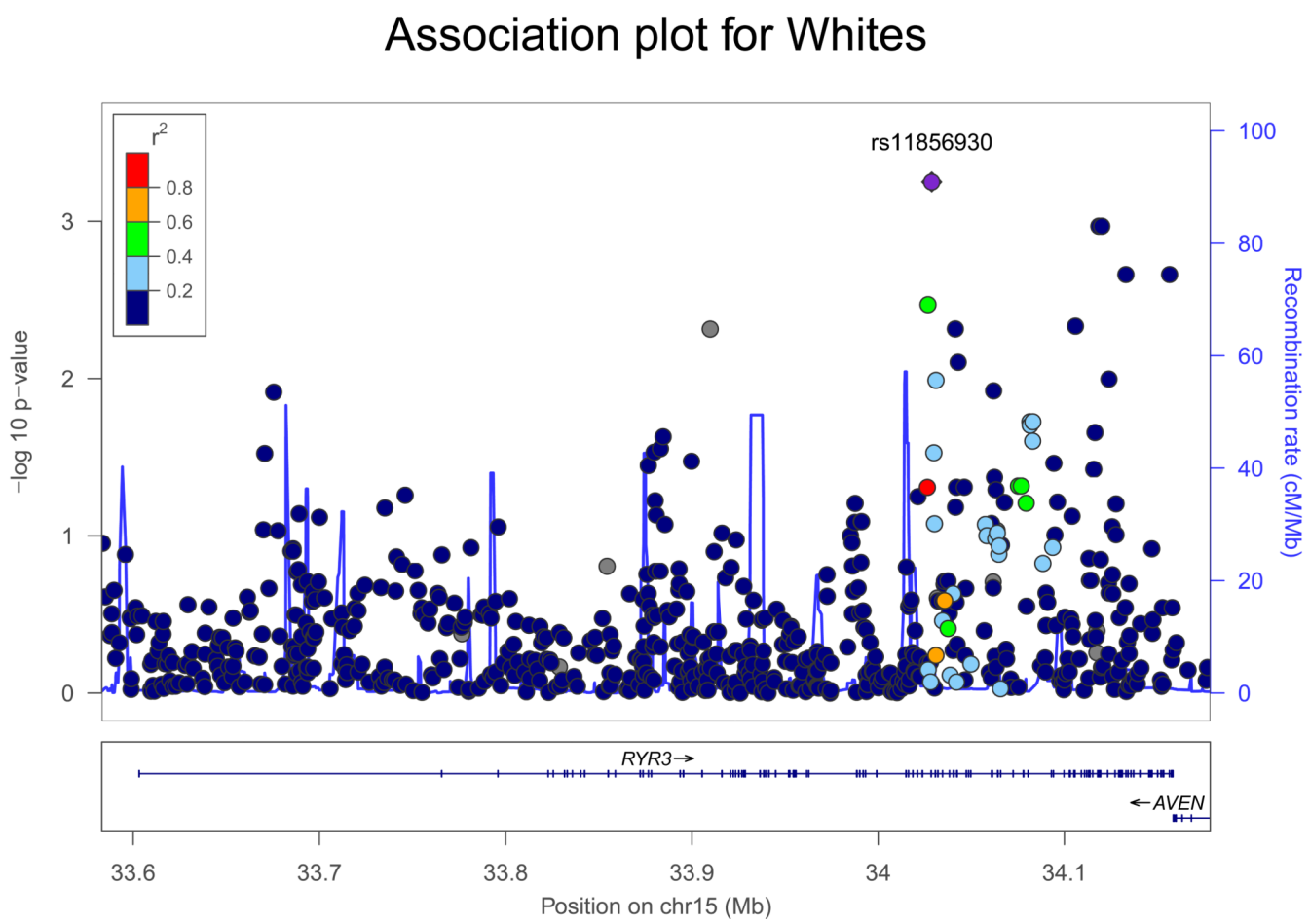
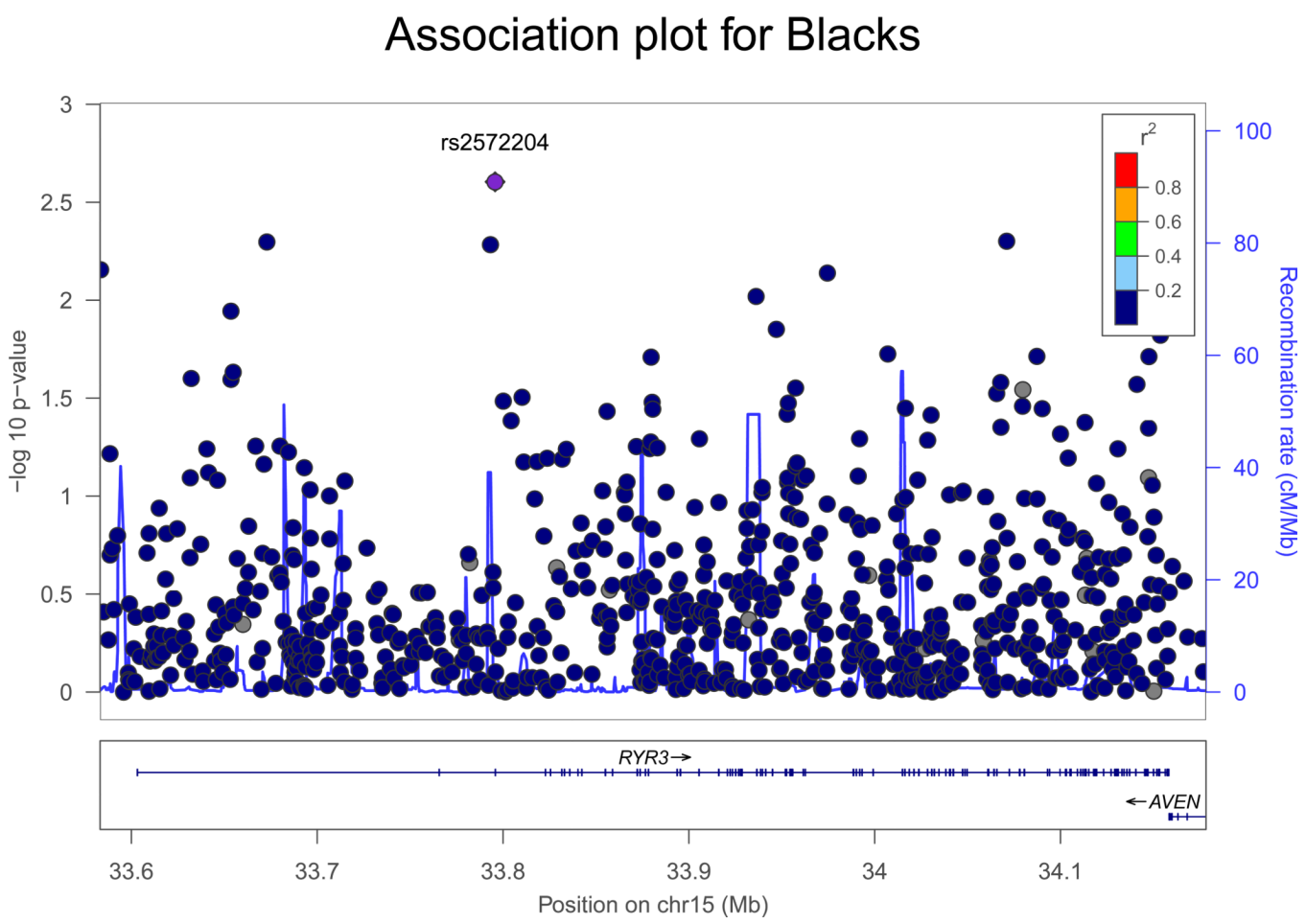
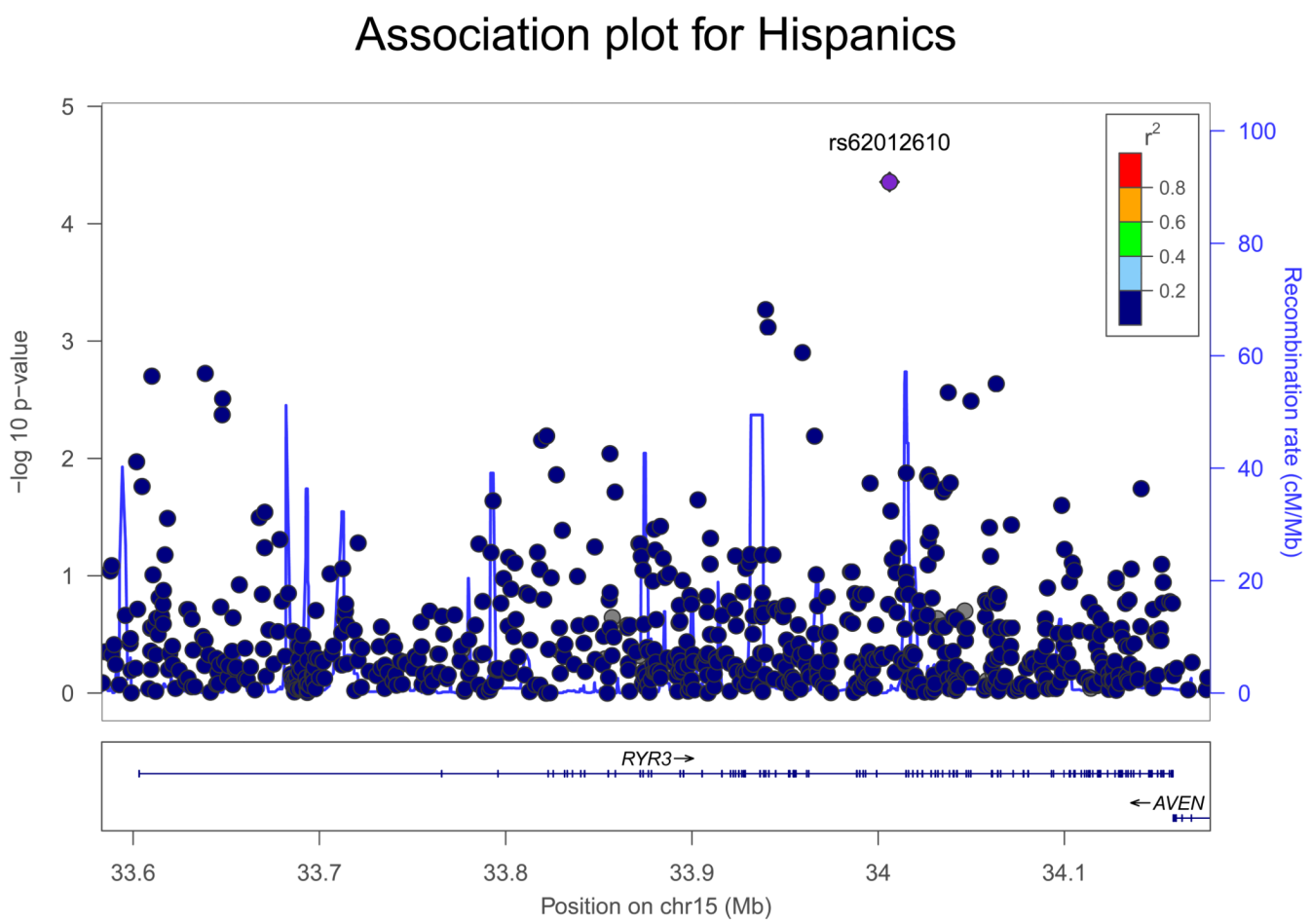
## Highlights

- The *RYR3* gene encodes for the RYR3 receptor which is a calcium channel present on the endoplasmic reticulum of arterial cells, and can play a role in the pathogenesis of subclinical atherosclerosis in both men and women.
- There is differential association of SNPs in the *RYR3* gene with CCA cIMT among racially-diverse HIV-infected women.
- Haploblock patterns within the *RYR3* gene regions vary across three races/ethnicities and various degrees of associations are observed with the haplotypes, specifically in the region encompassing previous significantly associated SNPs.









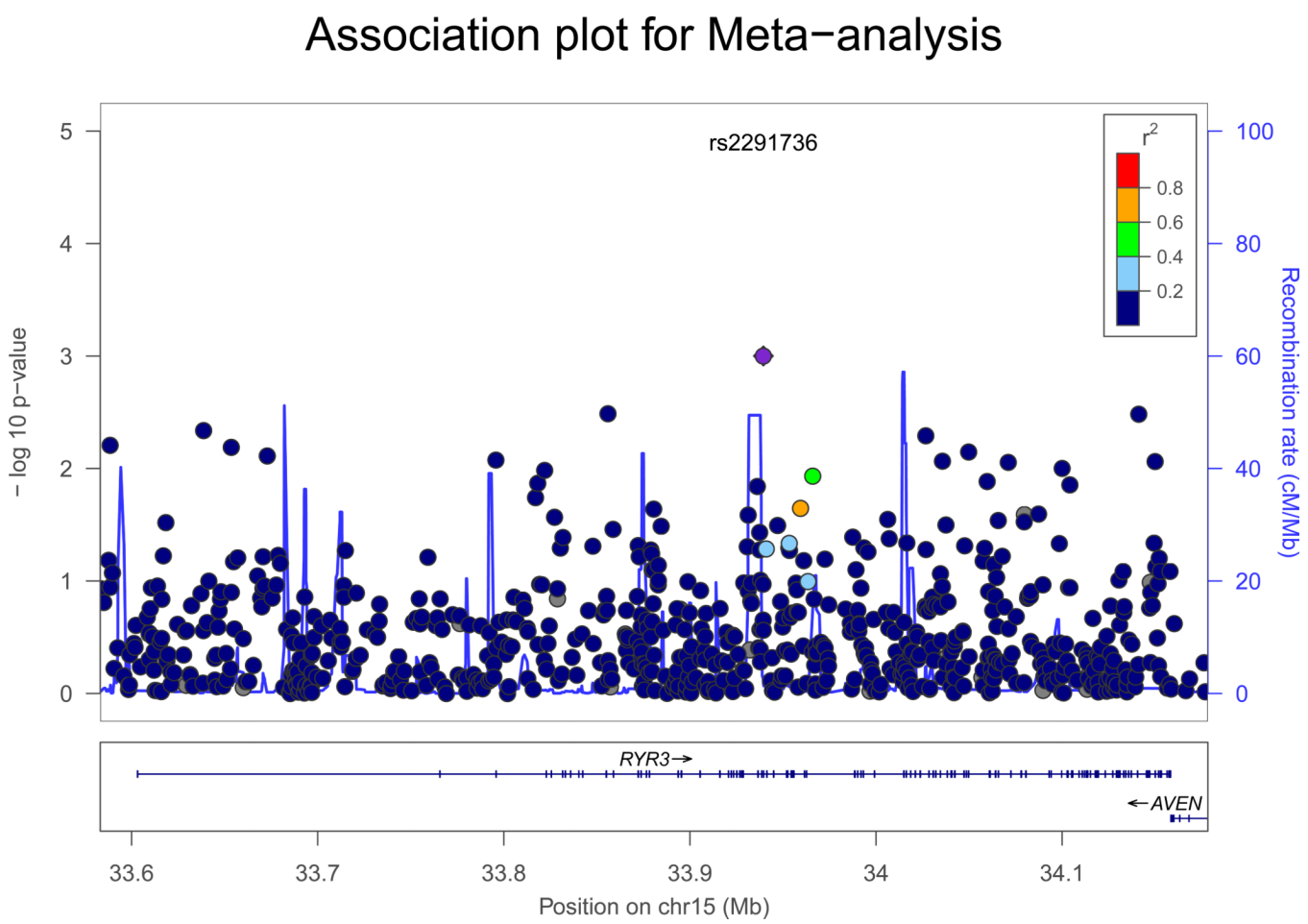



**Figure 1.**

Locus-specific ($-\log_{10}$(p-value)) regional association plots for CCA cIMT in the *RYR3* gene region (bottom) spanning 33603163 to 34158303 base pairs (bp) and ~20 kb flanking region in chromosome 15 (NCBI build 37, hg19) among HIV-infected women in WIHS. The circle in purple indicates the most strongly associated signal. Estimated recombination rates are plotted in light blue graphical lines (labeled in right y-axis) to reflect the local LD structure from the HapMap population. The spectrum of colors indicates LD of each SNP with the most strongly associated signal ($r^2$ values from HapMap where bright red indicates highly correlated, dark blue indicates weakly correlated and grey indicates missing $r^2$ values). The regional association plots of a) race/ethnicity adjusted analysis, b) Whites, c) Blacks, d) Hispanics and e) meta-analysis.

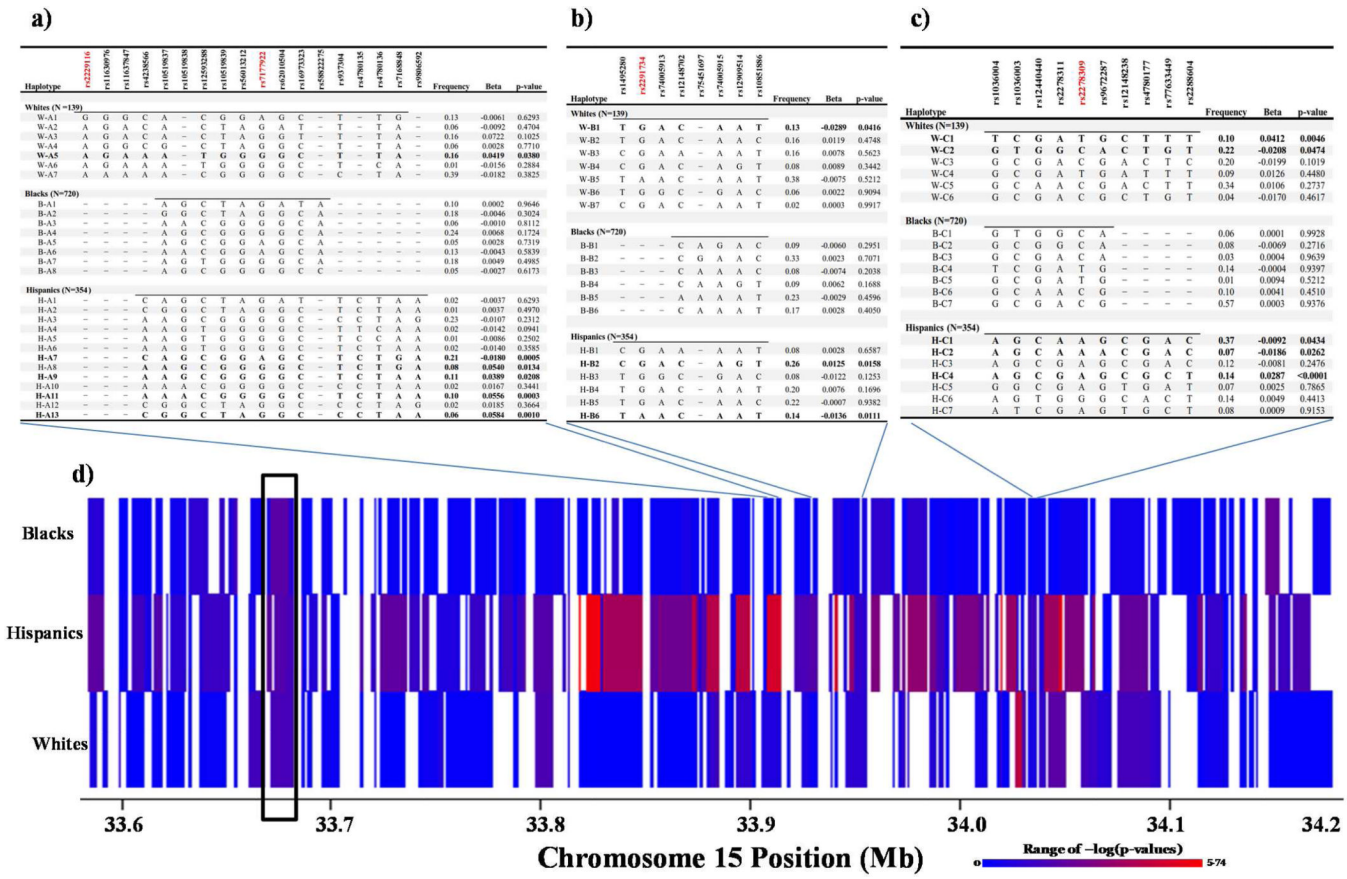



**Figure 2.**

Haploblocks and haplotype analysis of the *RYR3* gene region in Whites, Blacks, and Hispanics. Haploblocks were defined using Gabriel's LD method [40]. The figure shows a) all haploblocks and degree of statistical significance of omnibus haplotype association (−log (pvalues) ranging from least significant (0) in blue to most significant (5.74) in red). The haploblock region that is indicated within a rectangle seems to be associated in all three races; b) haploblocks and haplotype analysis in the region of rs2229116 and rs7177922; c) haploblocks and haplotype analysis in the region of rs2291734; and d) haploblocks and haplotype analysis in the region of rs2278309.

## Table 1

Characteristics of the White, Black and Hispanic HIV-infected women from the Women's Interagency HIV Study (WIHS) included in the genetic study.

| Characteristic | All (N=1230) | Whites (N=140) | Blacks (N=729) | Hispanics (N=361) |
|---|---|---|---|---|
| **Age (years) mean (SD)** | 41.73 (8.85) | 45.8 (9.47) | 41.62 (8.78) | 40.38 (8.28) |
| **Log 10 common carotid IMT (mm) mean (SD)** | −0.14 (0.06) | −0.14 (0.07) | −0.13 (0.06) | −0.16 (0.06) |
| **CD4 cell count, median (IQR)** | 429.00 (365.00) | 444.00 (373.00) | 436.00 (386.00) | 406.50 (338.50) |
| **Negative log 10 viral load mean (SD)** | −2.91 (1.17) | −2.54 (1.03) | −3.02 (1.19) | −2.83 (1.12) |
| **HAART (%)** | 94.04 | 97.97 | 92.51 | 95.55 |

Abbreviations: SD: Standard deviation, IQR: Interquartile range

**Table 2**

Top SNPs significantly associated with CCA cIMT adjusting for HAART and CD4 cell count in Whites, Hispanics and Blacks separately, and in the race/ethnicity (R/E) adjusted and meta-analysis.

| | | Whites (N=139) | | Blacks (N=720) | | Hispanics (N=354) | | R/E adjusted (N=1213) | | Meta-analysis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP**[a] | **Coordinates** | **Beta** | **p-value** | **Beta** | **p-value** | **Beta** | **p-value** | **Beta** | **p-value** | **Beta** | **p-value** |
| **rs2572204 (C/T)** | 33795710 | – | – | **0.0158** | **0.0025** | −0.0061 | 0.6348 | **0.0129** | **0.0078** | **0.0127** | **0.0084** |
| **rs11856930 (A/G)** | 34028778 | **0.0321** | **0.0006** | −0.0038 | 0.1982 | −0.0055 | 0.1547 | 0.0013 | 0.5839 | −0.0004 | 0.8989 |
| **rs62012610 (C/G)** | 34006114 | 0.0055 | 0.7443 | 0.0124 | 0.2667 | **−0.0374** | **<0.0001** | −0.0085 | 0.1966 | **−0.0142** | **0.0284** |
| **rs2291736 (A/G)** | 33939495 | −0.0008 | 0.9366 | 0.0063 | 0.0904 | **0.0179** | **0.0005** | **0.0088** | **0.0020** | **0.0094** | **0.0010** |
| **rs78959515 (C/T)** | 33855915 | −0.0043 | 0.7447 | **0.0149** | **0.0369** | **0.0212** | **0.0091** | **0.0115** | **0.0197** | **0.0146** | **0.0032** |
| **rs12439280 (A/G)** | 34140933 | 0.0050 | 0.6924 | **−0.0066** | **0.0269** | **−0.0131** | **0.0181** | **−0.0069** | **0.0078** | **−0.0076** | **0.0033** |
| **rs17235910 (C/T)** | 33588500 | −0.0173 | 0.3123 | −0.0221 | 0.0607 | −0.0171 | 0.0819 | **−0.0189** | **0.0062** | **−0.0188** | **0.0062** |

[a]The second allele is the minor allele; all SNPs were located in intronic regions of the *RYR3* gene.