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Replication of *RYR3* gene polymorphism association with cIMT among HIV-infected whites

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Abstract

To replicate the association of variants in *RYR3* gene with common carotid intima-media thickness (cIMT), a surrogate marker of atherosclerosis, we genotyped single nucleotide polymorphisms (SNPs) rs2229116 and rs7177922 in a sub-population of 244 HIV-positive and HIV-negative men. SNP rs2229116 was associated with common cIMT in HIV infected white men after adjusting for age and use of stavudine (d4T). The association was more evident at younger ages and decreased among older individuals.

Research letter

Life expectancy of people with HIV infection has been greatly extended with the effective use of highly active antiretroviral therapy (HAART). However, atherosclerotic vascular disease is emerging as an important complication in HIV patients [1–4]. Carotid intima-media thickness (cIMT), a surrogate marker of atherosclerosis, is consistently higher among HIV-positive patients than HIV-negative controls [5–7] and comparable to coronary artery disease patients [8]. There is evidence that genetic variation contributes to variation in cIMT. The initial genome-wide association (GWA) study in 177 HIV^+ white men on HAART [all with history of stavudine (d4T) use] in the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) study suggested that the ryanodine receptor 3 (*RYR3)* gene is linked to cIMT [9]. Two single nucleotide polymorphisms (SNPs), rs2229116 and rs7177922, in tight linkage disequilibrium (LD; $r^2 = 0.97$), were significantly associated with the common cIMT ($P < 3.4 \times 10^{-8}$ and $P < 2.74 \times 10^{-8}$, respectively). The rs2229116 SNP is a nonsynonymous polymorphism, with a residue change of Ile→Val resulting from the A→G nucleotide substitution in the sequence. Individuals with the rs2229116GG genotype showed significantly higher common cIMT than those with AA or AG genotypes [9].

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Conflicts of interest

S.S. supervised the study design, analyses and wrote the manuscript. Q.Y. helped with the analysis. G.J. and J.J.M. designed the genetic assays and performed the genotyping. D.K.A. assisted with the expertise in cardiovascular outcome and L.A.K. assisted with the clinical data of the cohort and is the lead investigator of the cardiovascular substudy in MACS. All the authors participated in the writing and the reading of the article and have seen and approved the final version of the manuscript. The Pittsburgh site of the MACS was funded by the National Institute of Allergy and Infectious Diseases, with supplemental funding from the National Cancer Institute and National Heart Lung and Blood Institute: grant UO1-AI-35041.

To follow-up and replicate the previous study finding, we genotyped rs2229116 and rs7177922 in 244 individuals (117 HIV^+ and 97 HIV^- whites, and 21 HIV^+ and nine HIV^- African–Americans) from the Pittsburgh site of the Multicenter AIDS Cohort Study (MACS) cardiovascular disease sub-study [7]. Briefly, high resolution B-mode carotid artery ultrasound was used to image the far wall of the right common carotid artery, internal carotid artery, and carotid bulb according to the procedure of Hodis *et al.* [10]. Sonographers were trained at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center. cIMT measurements were repeated in 38 healthy volunteers using this method, and the coefficient of variation was 1% (intraclass correlation coefficient = 0.99). The study was restricted to men who were over 40 years and less than 300 lb, with no reported history of coronary heart disease. To replicate the previous GWA study from the FRAM study, we initially restricted the analysis to white men on HAART. Associations of the SNPs with cIMT were assessed using a linear regression model (additive model), adjusting for age and d4T, a nucleoside analog reverse transcriptase inhibitor associated with increased cIMT [11], and other metabolic syndromes [12,13]. We did not adjust for duration of HAART as data was not available for all participants. Further interactions of the genetic variants with both d4T and age were also assessed.

Among whites (median age = 50.05 years), the minor allele frequency was 24.5% for rs2229116(G) and 22.7% for rs7177922(A). Neither SNP deviated from the Hardy–Weinberg equilibrium. Common cIMT was associated with rs2229116(G) ($\beta = 0.27 \pm 0.13$, $P = 0.05$) in an additive model, consistent with the previous study. Further, the association seemed to be strongest earlier and decreased ($\beta = 0.005 \pm 0.002$ per year, $P = 0.045$) as individuals aged (Table 1). The significant interaction of rs2229116 with age is quite important as the prevalence of atherosclerosis is increasing among younger HIV patients on treatment. However, the association of this SNP was not apparent in a smaller set of HIV-negative whites and the analysis was limited among African–Americans (only one individual with GG). The association with rs7177922 was not statistically significant but trended in the same direction. The linkage disequilibrium between the two SNPs was quite high, but slightly lower than in the previous study ($r^2 = 0.90$ vs. 0.97). Of note, LD between these two SNPs differs significantly in various populations, ranging from 0.01 to 1.00 in the 11 HapMap populations, with the highest linkage disequilibrium in Japanese–Asians and lowest in Yoruban–Africans.

RYR3 is a Ca^{2+} channel that mobilizes stored Ca^{2+} to initiate muscle contraction. *RYR3* shares a high amino acid sequence identity (66–70%) with the *RYR1* and *RYR2* genes. The biological function and mechanism of the *RYR3* gene is not as well known as *RYR1* and *RYR2.* Only recently, *RYR3* has been shown to be associated with few other diseases [14,15], but not with cardiovascular outcomes. On the contrary, *RYR1/2* genes have been associated with various cardiovascular and heart-related mechanisms, thus it is very reasonable to speculate that the isoform *RYR3* is biologically associated with cIMT. RYR3 is known to be coexpressed in Ca^{2+} signaling mechanisms in the cardiac and skeletal muscles [14] and studies have shown its role in endothelial vasodilation in the human arterial endothelial cells [16], which is compromised in atherosclerosis. Studies have suggested that Ca^{2+} release through RYR3 is involved in vasodilatation of vascular smooth muscle cells (VSMCs), and any alteration of this release pathway would affect VSMC contractility [17]. RYR is also upregulated in the aorta of atherosclerosis-prone mice compared with atherosclerosis-resistant mice [18]. In terms of HIV, Tat gene in HIV-1 has been shown to cause endothelial dysfunction inducing rapid loss of endoplasmic reticulum Ca^{2+} through the mediation of *RYR*s. It has also been reported that RYR3 is essential in the sustained Ca^{2+} response in T cells [19], the target cells of HIV.

Here, we report a replication study of the previous finding with common cIMT, however, with different measurement methodologies and readers between the two studies [4,7]. Further investigation is required in other ethnic groups and HIV-negative individuals to differentiate whether the mechanism is driven through HIV infection and HAART or independently. One caution is that in other ethnic groups the frequency and the linkage disequilibrium pattern could vary in this large gene with 104 exons in chromosome 15q14–q15 of the human genome. As many as seven alternative spliced variants are known in *RYR3* [20]. Even with our results from this replication study, it is unclear whether this SNP is directly related to cIMT or serves as a surrogate of one or more causal variants in linkage disequilibrium. Further studies cataloguing the complete list of functional variants (rare and common) and how they might be associated with these SNPs are needed to better understand the molecular mechanisms underlying the contribution of *RYR3* to atherosclerosis.

References

1. Pacheco AG, Tuboi SH, Faulhaber JC, Harrison LH, Schechter M. Increase in non-AIDS related conditions as causes of death among HIV-infected individuals in the HAART era in Brazil. PLoS One. 2008; 3:e1531. [PubMed: 18231611]
2. Mehta N, Reilly M. Atherosclerotic cardiovascular disease risk in the HAART-treated HIV-1 population. HIV Clin Trials. 2005; 6:5–24. [PubMed: 15765307]
3. Hsue PY, Lo JC, Franklin A, Bolger AF, Martin JN, Deeks SG, et al. Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection. Circulation. 2004; 109:1603–1608. [PubMed: 15023877]
4. Grunfeld C, Delaney JA, Wanke C, Currier JS, Scherzer R, Biggs ML, et al. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. AIDS. 2009; 23:1841–1849. [PubMed: 19455012]
5. Periard D, Cavassini M, Taffe P, Chevalley M, Senn L, Chapuis-Taillard C, et al. High prevalence of peripheral arterial disease in HIV-infected persons. Clin Infect Dis. 2008; 46:761–767. [PubMed: 18230043]
6. Lorenz MW, Stephan C, Harmjanz A, Staszewski S, Buehler A, Bickel M, et al. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. Atherosclerosis. 2008; 196:720–726. [PubMed: 17275008]
7. Kingsley LA, Cuervo-Rojas J, Munoz A, Palella FJ, Post W, Witt MD, et al. Subclinical coronary atherosclerosis, HIV infection and antiretroviral therapy: Multicenter AIDS Cohort Study. AIDS. 2008; 22:1589–1599. [PubMed: 18670218]
8. Lekakis J, Tsiodras S, Ikonomidis I, Palios J, Poulakou G, Rallidis L, et al. HIV-positive patients treated with protease inhibitors have vascular changes resembling those observed in atherosclerotic cardiovascular disease. Clin Sci (Lond). 2008; 115:189–196. [PubMed: 18251713]
9. Shrestha S, Irvin MR, Taylor KD, Wiener HW, Pajewski NM, Haritunians T, et al. A genome-wide association study of carotid atherosclerosis in HIV-infected men. AIDS. 2010; 24:583–592. [PubMed: 20009918]
10. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, et al. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2001; 135:939–953. [PubMed: 11730394]
11. de Giuliano IC, de Freitas SF, de Souza M, Caramelli B. Subclinic atherosclerosis and cardiovascular risk factors in HIV-infected children: PERI study. Coron Artery Dis. 2008; 19:167–172. [PubMed: 18418233]
12. Nolan D, Reiss P, Mallal S. Adverse effects of antiretroviral therapy for HIV infection: a review of selected topics. Expert Opin Drug Saf. 2005; 4:201–218. [PubMed: 15794714]
13. Domingo P, Cabeza MC, Pruvost A, Salazar J, del Gutierrez MM, Mateo MG, et al. Relationship between HIV/Highly active antiretroviral therapy (HAART)-associated lipodystrophy syndrome and stavudine-triphosphate intracellular levels in patients with stavudine-based antiretroviral regimens. Clin Infect Dis. 2010; 50:1033–1040. [PubMed: 20192724]

14. Zhang L, Liu Y, Song F, Zheng H, Hu L, Lu H, et al. Functional SNP in the microRNA-367 binding site in the 3′UTR of the calcium channel ryanodine receptor gene 3 (RYR3) affects breast cancer risk and calcification. Proc Natl Acad Sci U S A. 2011; 108:13653–13658. [PubMed: 21810988]

15. Stephens SH, Franks A, Berger R, Palionyte M, Fingerlin TE, Wagner B, et al. Multiple genes in the 15q13-q14 chromosomal region are associated with schizophrenia. Psychiatr Genet. 2012; 22:1–14. [PubMed: 21970977]

16. Kohler R, Brakemeier S, Kuhn M, Degenhardt C, Buhr H, Pries A, et al. Expression of ryanodine receptor type 3 and TRP channels in endothelial cells: comparison of in situ and cultured human endothelial cells. Cardiovasc Res. 2001; 51:160–168. [PubMed: 11399258]

17. Massaeli H, Austria JA, Pierce GN. Lesions in ryanodine channels in smooth muscle cells exposed to oxidized low density lipoprotein. Arterioscler Thromb Vasc Biol. 2000; 20:328–334. [PubMed: 10669627]

18. Yuan Z, Miyoshi T, Bao Y, Sheehan JP, Matsumoto AH, Shi W. Microarray analysis of gene expression in mouse aorta reveals role of the calcium signaling pathway in control of atherosclerosis susceptibility. Am J Physiol Heart Circ Physiol. 2009; 296:H1336–H1343. [PubMed: 19304945]

19. Schwarzmann N, Kunerth S, Weber K, Mayr GW, Guse AH. Knock-down of the type 3 ryanodine receptor impairs sustained Ca2+ signaling via the T cell receptor/CD3 complex. J Biol Chem. 2002; 277:50636–50642. [PubMed: 12354756]

20. Jiang D, Xiao B, Li X, Chen SR. Smooth muscle tissues express a major dominant negative splice variant of the type 3 Ca2+ release channel (ryanodine receptor). J Biol Chem. 2003; 278:4763–4769. [PubMed: 12471029]

Table 1

β coefficients (± standard error) and *P*-values from linear regression analysis based on additive model for *RYR3* gene variant and interactions with age associated with cIMT in HIV-positive white men.

	HIV$^+$ whites (*n* = 117)		HIV$^-$ whites (*n* = 97)	
Variable	**β ± standard error**	***P*-value**	**β ± standard error**	***P*-value**
rs2229116[†]	0.27 ± 0.13	0.05	0.007 ± 0.22	0.05
Age	0.004 ± 0.002	0.02	0.003 ± 0.002	0.13
d4T use (yes/no)	−0.016 ± 0.02	0.42		
rs2229116*age	−0.005 ± 0.002	0.04	0.004 ± 0.003	0.25

[†]Additive model coded as 0, 1, 2 for genotypes AA, AG and GG, respectively.