Original Article

Common and Rare Genetic Variation in *CCR2*, *CCR5*, or *CX3CR1* and Risk of Atherosclerotic Coronary Heart Disease and Glucometabolic Traits

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Background**—**The chemokine receptors CCR2, CCR5, and CX3CR1 coordinate monocyte trafficking in homeostatic and inflammatory states. Multiple small human genetic studies have variably linked single nucleotide polymorphisms in these genes to cardiometabolic disease. We interrogated genome-wide association, exome sequencing, and exome array genotyping studies to ascertain the relationship between variation in these genes and coronary artery disease (CAD), myocardial infarction (MI), and glucometabolic traits.

Methods and Results**—**We interrogated the CARDIoGRAMplusC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) (60 801 cases and 123 504 controls), the MIGen and CARDIoGRAM Exome consortia (42 335 cases and 78 240 controls), and Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI; 4703 cases and 5090 controls) data sets to ascertain the relationship between common, low frequency, and rare variation in *CCR2*, *CCR5*, or *CX3CR1* with CAD and MI. We did not identify any variant associated with CAD or MI. We then explored common and low-frequency variation in South Asians through Pakistan Risk of Myocardial Infarction Study (PROMIS; 9058 cases and 8379 controls), identifying 6 variants associated with MI including *CX3CR1* V249I. Finally, reanalysis of the European HapMap imputed Diabetes Genetics Replication and Meta-Analysis (DIAGRAM), Global Lipids Genetics Consortium (GLGC), Genetic Investigation of Anthropometric Traits (GIANT), and Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC) data sets revealed no association with glucometabolic traits although 3 single nucleotide polymorphisms in PROMIS were associated with type II diabetes mellitus.

Conclusions**—**No chemokine receptor variant was associated with CAD, MI, or glucometabolic traits in large European ancestry cohorts. In a South Asian cohort, we identified single nucleotide polymorphism associations with MI and type II diabetes mellitus but these did not meet significance in cohorts of European ancestry. These findings suggest the need for larger studies in South Asians but exclude clinically meaningful associations with CAD and glucometabolic traits in Europeans. **(*Circ Cardiovasc Genet*. 2016;9:250-258. DOI: 10.1161/CIRCGENETICS.115.001374.)**

Key Words: atherosclerosis ■ diabetes mellitus ■ genetics ■ genome-wide association study ■ myocardial infarction

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Despite advances in the diagnosis and treatment of cardiometabolic diseases, the genetic basis of atherosclerosis and glucometabolic traits remains only partially understood. Multiple genome-wide association studies (GWAS) have begun to elucidate the genetics of complex cardiometabolic diseases, yet the majority of its heritability remains unknown.[1,2] Initial GWAS evolved to HapMap-based meta-analyses focused on detecting common variation at the population level. More recently, imputation, using data from the 1000 Genomes project and exome sequencing projects, has allowed capture of additional information on low-frequency and rare variation.[3,4] Gains in our understanding of complex traits through these approaches suggest that multiple variants with small effect sizes drive complex diseases and that a variety of unbiased, targeted, and functional strategies are required to elucidate the full genetic contributions to cardiometabolic disease.[1,5]

Clinical Perspective on p 258

The chemokine receptors *CCR2*, *CCR5*, and *CX3CR1* are potential modifiers of both atherosclerosis and glucometabolic traits.[6] These receptors are expressed on leukocyte populations and vascular cells in both homeostatic and inflammatory states. Mice lacking any of these receptors have attenuated atherosclerosis with combinations of multiple receptor knockouts demonstrating a more pronounced phenotype, supporting the idea that these chemokine pathways act in an independent and additive manner.[7] In vitro studies have demonstrated that cells carrying the human *CX3CR1* variants V249I and T280M have a reduced number of fractalkine binding sites and reduced affinity for fractalkine on peripheral blood mononuclear cells.[8,9] Before the GWAS era, a series of small case–control studies provided inconsistent and at times conflicting data for association of these single nucleotide polymorphisms (SNPs) with coronary artery disease (CAD), myocardial infarction (MI), and glucometabolic traits.[9–11] Similarly, a handful of small studies have explored the relationship of the *CCR2 V64I* variant to CAD with inconsistent findings.[12,13] With the advent of large-scale human genetic databases, we are now able to ascertain whether the findings observed in knockout mouse models are transferable to humans. This question is of broad and general importance to translational studies of atherosclerosis particularly for innate and adaptive immune pathways where there has been limited clinical translation despite convincing evidence of disease modulation in mouse models.

We thus interrogated large contemporary data sets of common, low-frequency, and rare genetic variation at *CCR2*, *CCR5*, and *CX3CR1* for CAD, MI, and glucometabolic traits. Briefly, our focus was first on the V249I and T280M *CX3CR1* and V64I *CCR2* variants previously reported to associate with cardiometabolic traits. We then interrogated all common and low-frequency variation in and around each gene. Finally, when available, we examined in composite rare exonic variants in each gene for trait association. Specifically, we accessed the CARDIoGRAMplusC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) and Myocardial Infarction Genetics (MIGen) and CARDIoGRAM Exome array meta-analyses for common and low-frequency variants in CAD as well as the Exome Sequencing Project (ESP) Early Onset Myocardial Infarction (EOMI) consortium data for rare variation in MI. We then performed a focused interrogation of summary data from the MAGIC, DIAGRAM, GLGC, and GIANT consortia GWAS meta-analyses, which assess common variation in subjects with a range of glucometabolic phenotypes. Pakistan Risk of Myocardial Infarction Study (PROMIS) case–control studies were leveraged to explore low-frequency and common variation in *CCR2, CCR5*, and *CX3CR1* in a South Asian population in which CAD, MI, and type II diabetes mellitus (DM) are enriched.

Methods

Studies of CAD and MI

We leveraged the sample sizes and statistical power of the CARDIoGRAMplusC4D, MIGen, and CARDIoGRAM Exome array, and ESP EOMI studies, all described in detail in the Data Supplement.[1,2,4,14–16] The CARDIoGRAMplusC4D meta-analysis includes merged data from the classic CARDIoGRAM and C4D GWAS, consolidating genetic information from 60 801 CAD and MI case subjects and 123 504 control subjects of mixed ancestry across 48 studies.[1,2,5,14] Genotypes were imputed using the 1000 Genomes phase 1, version 3 reference panel (Table 1).[3] Variants were filtered on a minor allele frequency (MAF) >0.5%. Genomic control was applied before inclusion in the meta-analysis, and subsequently a second correction for genomic control was repeated after inclusion in the meta-analysis. Association testing was performed using logistic regression on additive, recessive, and dominant models of disease susceptibility. Studies were combined using a fixed-effects, inverse-variance–weighted meta-analysis. Summary-level data from additive models were extracted for variants within 5000 bps of the start and end positions of *CCR2*, *CCR5*, and *CX3CR1* (Table I in the Data Supplement).

The MIGen and CARDIoGRAM Exome array consortia meta-analyzed data from 19 studies totaling 42 335 MI case subjects and 78 240 control subjects of European ancestry (Table 1) all genotyped on the Illumina HumanExome BeadChip (Illumina, San Diego, CA).[16] The individual studies performed logistic regression on an additive model using the principal components of ancestry as covariates, and study level data were combined using an inverse-variance–weighted meta-analysis. Variants were restricted to those with a MAF ≥0.01%. Summary-level data were retrieved for polymorphic exomic variants in *CCR2*, *CCR5*, and *CX3CR1.* Although 8 studies in the data set overlapped completely or partially with the CARDIoGRAMplusC4D meta-analysis, the focus of the MIGen and CARDIoGRAM Exome array consortia differs substantively from that of CARDIoGRAMplusC4D given its specific focus on low-frequency variation.

The ESP EOMI consortium merged exome sequence data from 14 studies, 11 initial studies and 3 follow-up studies, totaling 4703 case subjects and 5090 control subjects of European ancestry (Table 1).[4,15] Association testing for genetic variation in *CCR2*, *CCR5*, and *CX3CR1* was performed by aggregating a burden of rare variants (SNPs and indels present at a MAF <1%) for each gene. The predicted functional impact of each rare variant was annotated using 7 algorithms,[25–30] and we tested for an association separately for 3 classes of variants: (1) nonsynonymous variants, (2) disruptive variants (nonsense, splice-site, and indel frameshift variants), and (3) deleterious variants, defined as disruptive variants in combination with missense variants damaging by at least 5 of the 7 aforementioned algorithms.

PROMIS is a retrospective case–control study of subjects with an acute first MI in urban Pakistan.[17,18] Samples were genotyped on the Illumina 660 and Illumina 770 arrays and imputed using the 1000

Table 1. Genome-Wide Association Study and Genome-Wide Sequencing Study Resources

	Study	Modality	Ethnicity	Trait	Subjects	No. of SNPs*
CAD and MI	CARDIoGRAMplusC4D consortium[5]	GWAS, 1000 genomes imputed	77% European; 13% South Asian; 6% East Asian	CAD	60 801 cases and 123 504 controls	220
	Myocardial Infarction Genetics (MIGen) and CARDIoGRAM Exome array consortia[16]	HumanExome BeadChip	European	CAD	42 335 cases and 78 240 controls	20
	Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI) consortium[4]	Whole-exome sequencing	91% European American; 9% African American	MI	4703 cases and 5090 controls	…
	Pakistan Risk of Myocardial Infarction Study (PROMIS)[17,18]	GWAS, 1000 genomes imputed	South Asian	MI	9058 cases and 8379 controls	181
Glucometabolic traits	Diabetes Genetics Replication and Meta-analysis (DIAGRAM)[19]	GWAS, HapMap imputed	European	Type 2 DM	12 171 cases and 56 862 controls	53
	Genetic Investigation of Anthropometric Traits (GIANT)[20,21]	GWAS, HapMap imputed	European	BMI	123 865	53
				WHR adjusted for BMI	77 167	
	Global Lipids Genetics Consortium (GLGC)[22]	GWAS, HapMap imputed	European	Triglycerides	96 598	53
				HDL cholesterol	99 900	
	Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC)[23,24]	GWAS, HapMap imputed	European	Fasting glucose	46 186	53
				HgbA1c	46 368	
				Fasting insulin	38 238	
				HOMA-IR	37 037	
				HOMA-B	36 466	
	Pakistan Risk of Myocardial Infarction Study (PROMIS)[17,18]	GWAS, 1000 genomes imputed	South Asian	HDL cholesterol	16 328	181
				Triglycerides	16 194	
				Type 2 DM	10 310 cases and 7038 controls	

BMI indicates body mass index; CAD, coronary artery disease; CARDIoGRAMplusC4D, Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics; DM, diabetes mellitus; GWAS, genome-wide associated study; HDL, high-density lipoprotein; HgA1c, glycated hemoglobin; HOMA-B, Homeostasis Model Assessment-B score; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance; MI, myocardial infarction; SNP, single nucleotide polymorphism; and WHR, waist:hip ratio.

*Refers to the number of SNPs within *CCR2*, *CCR5*, and *CX3CR1* in each data set. P-values Bonferroni corrected for the number of SNPs tested.

genomes phase I integrated reference panel (March 2012).[3] Individual tests for association were performed on variants with MAF >1% adjusting for the first 4 principal components. Summary-level data were examined for SNPs within 5000 bps of *CCR2*, *CCR5*, and *CX3CR1* on 9058 MI case subjects and 8378 control subjects (Table 1; Table I in the Data Supplement). Although PROMIS data are nested in full within the CARDIoGRAMplusC4D database, we focused on it separately to interrogate ethnic-specific differences potentially obscured by the larger CARDIoGRAMplusC4D cohort.

Studies of Glucometabolic Traits

Detailed descriptions of these meta-analyses have been published and our specific approach detailed in Table 1 and the Data Supplement. Briefly, we accessed the DIAGRAM, GIANT, GLGC, and MAGIC consortia meta-analyses to ascertain the association of chemokine receptor variation with glucometabolic traits in European subjects.[19–24] These resources contain genetic information on a range of glucometabolic traits including type II DM, BMI, weight-to-hip ratio, lipid and lipid-related traits, and glucose metabolism (Table 1). The PROMIS MI resource is described above.[17,18] In addition to MI, association tests were performed for type II DM and lipid levels (Table 1), and summary data extracted for SNPs within 5000 bps of *CCR2*, *CCR5*, and *CX3CR1*.

Statistical Analysis

For our chemokine receptor focus, unadjusted summary association *P* values were Bonferroni corrected for the number of SNPs tested in each study (Table 1). For the ESP EOMI consortium, unadjusted *P* values are reported. Linkage disequilibrium (LD) for European subjects was taken from the 1000 genomes phase 3 reference panel available through the 1000 genomes browser.[3] LD for South Asian subjects was calculated from the 1000 genomes phase 3, version 5 SAS reference panel using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/).[31] LD structure was visualized in Haploview separately for the 2 populations[32] with gene structure visualized through the Integrative Genomics Viewer 2.3.67.[33]

To calculate power, risk allele frequencies from CARDIoGRAMplusC4D were tested against a range of risk allele frequency differences under a genome-wide significance threshold of 5×10^{-8}.[5] Sample sizes were taken from the CARDIoGRAMplusC4D and PROMIS data sets. The power calculation formula was modified from Skol to incorporate unequal numbers of cases and controls.[34]

Results

CAD and MI

Common and Low-Frequency Variation in CCR2, CCR5, and CX3CR1 Lacks Association With CAD or MI in Large Predominantly European Ancestry Samples

In the pre-GWAS era, the V249I and T280M variants in *CX3CR1* and V64I in *CCR2* were ascribed functional effects although found to have conflicting findings for association with CAD.[8–13,35] We extracted CAD and MI association *P* values for these SNPs from the CARDIoGRAMplusC4D and MIGen and CARDIoGRAM Exome array consortia meta-analyses, currently the 2 largest genome-wide resources of CAD and MI.[5,16] Neither *CX3CR1* V249I, *CX3CR1* T280M nor *CCR2* V64I reached statistical significance in either data set (Table 2).

Next, because complete deletion of these chemokine receptor genes in mouse models attenuates atherosclerosis,[7,36,37] we more comprehensively surveyed association signals in these loci by examining whether any common or low-frequency SNPs in *CCR2*, *CCR5*, or *CX3CR1* relate to CAD or MI. To address this, we used the CARDIoGRAMplusC4D 1000 genomes imputed summary data set that contains information on common and low-frequency variation in 60 801 CAD subjects and 123 504 control subjects.[5] Of the 220 variants interrogated, 5 SNPs in *CCR5* and 3 SNPs in *CX3CR1* met unadjusted *P* value significance thresholds of 0.05 but none approximated statistical significance after Bonferroni correction for 220 variants (Table I in the Data Supplement). In the MIGen and CARDIoGRAM Exome array consortia, which contains genetic information for 54 003 low-frequency and common, nonsynonymous, autosomal variants in 120 575 individuals of European ancestry, 42 335 of which have CAD, we extracted association data for the 20 polymorphic SNPs in *CCR2*, *CCR5*, and *CX3CR1*.[16] Three SNPs in *CCR5* and 1 in *CX3CR1* had unadjusted $P<0.05$, but none met statistical significance after Bonferroni correction for 20 variants tested (Table II in the Data Supplement).

Rare Variation in CX3CR1, CCR2, and CCR5 and Risk of MI

Although large GWAS have systematically evaluated the genetic underpinnings of CAD and MI, they have not been designed to assess trait-associations with rare variants.[1] Using the ESP EOMI data set, which contains information on rare variation in 4703 EOMI case subjects and 5090 control subjects of European American (90.8%) and black (9.2%) descent, we tested the hypothesis that rare exomic variation in *CCR2*, *CCR5*, and *CX3CR1* modifies the risk of MI.[4] Given the high baseline rate of rare neutral mutations, we systematically aggregated variants using a computational approach in an effort to enrich for pathogenic alleles, deriving sets of nonsynonymous, disruptive, and deleterious variants. Despite this approach, we failed to find an association between rare variants predicted to be functionally deleterious in these chemokine receptors and MI (Table 3). Although we noted a potential signal in *CX3CR1* emerging for disruptive variants damaging by 5 of 7 (P=0.09; odds ratio [OR], 2.71) and 6 of 7 (P=0.13; OR, 2.89) prediction algorithms, this trend lacked consistency across all prediction algorithms (eg, PolyPhen-2: P=0.32; OR, 0.86) and failed to meet significance even without correction for multiple testing.

Association of Common Variation in CX3CR1 With MI in South Asians

To extend our investigation to a distinct ethnic setting in which the risk of MI is increased, we leveraged summary-level data from the 1000 genomes imputed PROMIS data set, which contains SNPs at a MAF >1% in this Pakistani South Asian sample.[17,18] The *CX3CR1* variant V249I met significance after correction for all SNPs tested, but neither *CX3CR1* T280M nor *CCR2* V64I were significant in adjusted analyses (Table 2). In interrogation of all 181 low-frequency and common SNPs in *CCR2*, *CCR5,* and *CX3CR1*, 5 additional noncoding variants in *CX3CR1* were significantly associated with MI after Bonferroni correction (Table 2; Table III in the Data Supplement). These variants, 4 of which were genotyped, are present in the population at a frequency of 12.8% and in PROMIS are in close LD with one another and with *CX3CR1* V249I and T280M (r^2>0.8; Figure I and Table III in the Data Supplement). Given that these variants are present but not associated with MI in the larger predominantly European ancestry CARDIoGRAMplusC4D meta-analysis, the clinical significance of these associations with respect to MI remains uncertain and requires specific follow-up in larger South Asian cohorts.

Table 2. Genome-Wide Association Study Findings for Variants With Prior Reports for Association With Coronary Artery Disease/MI

AA Change	Gene	rs no.	Minor Allele	MAF (%)* EUR	MAF (%)* SAS	CARDIoGRAMplusC4D (n=184 305) β (SE)	CARDIoGRAMplusC4D Unadjusted *P* Value	MIGen Exome array (n=120 575) β	MIGen Exome array Unadjusted *P* Value	PROMIS MI (n=17 437) β (SE)	PROMIS MI Unadjusted *P* Value
V249I	*CX3CR1*	rs3732379	T	28.53	12.78	−0.002 (0.01)	0.88	0.01	0.48	−0.11 (0.03)	2.64×10^{-4}
T280M	*CX3CR1*	rs3732378	A	17.20	10.94	−0.01 (0.01)	0.63	0.01	0.45	−0.12 (0.04)	5.54×10^{-4}
V64I	*CCR2*	rs1799864	A	8.65	9.82	−0.0001 (0.02)	0.99	0.01	0.43	−0.09 (0.05)	3.70×10^{-2}

AA indicates amino acid; CARDIoGRAMplusC4D, Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics; Eur, European; MAF, minor allele frequency; MI, myocardial infarction; MIGen, Myocardial Infarction Genetics; PROMIS, Pakistan Risk of Myocardial Infarction Study; and SAS, South Asian.

*MAF per the 1000 genomes, phase 3 reference panel. *CX3CR1* V249I met significance in PROMIS alone following Bonferroni correction. *P* values significant in PROMIS at a Bonferroni correction threshold of 2.76×10^{-4} (n=181).

Table 3. Gene Burden Testing on Rare Variants in 9703 Subjects Fails to Show an Association With Myocardial Infarction

Gene	Variant Set	T1 Cases (n=4703)	T1 Controls (n=5090)	*P* Value	Odds Ratio
CCR2	7 of 7	4	3	0.58	1.44
CCR2	6 of 7	15	12	0.35	1.35
CCR2	5 of 7	17	13	0.30	1.42
CCR2	PolyPhen-2	18	15	0.39	1.30
CCR2	Nonsynonymous	47	51	0.30	1.00
CX3CR1	7 of 7	1	0	0.35	NA
CX3CR1	6 of 7	8	3	0.13	2.89
CX3CR1	5 of 7	10	4	0.09	2.71
CX3CR1	PolyPhen-2	32	40	0.32	0.86
CX3CR1	Nonsynonymous	149	146	0.58	1.11
CCR5	7 of 7	20	17	0.45	1.27
CCR5	6 of 7	20	17	0.45	1.27
CCR5	5 of 7	47	49	0.38	1.04
CCR5	PolyPhen-2	51	49	0.52	1.13
CCR5	Nonsynonymous	138	193	0.36	0.77

Glucometabolic Traits

Common Variation in CX3CR1, CCR2, and CCR5 Lacks Association With Glucometabolic Traits in Cohorts of European Ancestry

Although multiple mouse and human studies have suggested a role for chemokine receptor variation in atherosclerosis, a smaller number of rodent and human studies have implicated the *Ccr2*, *Cx3cr1*, and *Ccr5* pathways in the development of obesity, insulin resistance, and glucose homeostasis.[6,11,38–40] Therefore, we performed a focused reanalysis of HapMap imputed DIAGRAM, GLGC, GIANT, and MAGIC data sets that contain information on genetic associations for a range of glucometabolic and anthropometric traits.[19–24] We first interrogated *CX3CR1* V249I and T280M as well as *CCR2* V64I in GWAS of glucometabolic traits. Neither *CX3CR1* variant approximated significance in the HapMap-based GWAS MAGIC, DIAGRAM, GIANT, or GLGC data sets for any phenotype interrogated (Table 4), whereas *CCR2* V64I was not included in these GWAS meta-analyses. We then extended our examination to all available variation at these loci. Of the 53 HapMap-imputed variants within 5000 bps of *CCR2*, *CCR5*, and *CX3CR1*, none approached statistical significance after correction for multiple testing.

Association of Common and Low-Frequency Variation in CX3CR1 With Glucometabolic Traits in South Asians

We interrogated the 1000 genomes imputed PROMIS data set that contains trait-association information on type II DM, high-density lipoprotein-cholesterol, and triglyceride levels on up to 17 348 individuals in this South Asian cohort. Neither *CX3CR1* V249I, *CX3CR1* T280M, nor *CCR2* V64I approximated statistical significance for type II DM or lipid levels (Tables 4). Of the 181 SNPs within 5000 bps of *CCR2*, *CCR5*, and *CX3CR1*, 3 low-frequency, noncoding *CX3CR1* variants were associated with type II DM after correction for multiple testing (Table IV in the Data Supplement). These variants, one of which was genotyped, are in close LD with one another (r^2>0.97) although bore no relationship to the *CX3CR1* variants V249I and T280M (Figure I in the Data Supplement). Of note, 2 of the variants (rs17038647 and rs17038663) are included in the European MAGIC and DIAGRAM meta-analyses. Although these had a trend toward association with Homeostasis Model Assessment-Insulin resistance (uncorrected *P*=0.038; *P*=0.029), a measure of insulin resistance, in MAGIC, these variants were not significant after correction for multiple testing. Furthermore, there was no association between these SNPs and type II DM in DIAGRAM (uncorrected *P*=0.92; *P*=0.94). Finally, none of the 181 SNPs were associated with plasma lipid levels in PROMIS.

CARDIoGRAMplusC4D but Not PROMIS Has Ample Power to Detect Genetic Variation at a Range of Allele Frequencies and ORs

To ascertain whether significant variation in PROMIS is likely to represent biologically relevant variation as opposed to false-positive findings, we performed a post hoc power calculation based on the CARDIoGRAMplusC4D and PROMIS databases using a range of allele frequencies and allele frequency differences (Table V in the Data Supplement). At each allele frequency surveyed in CARDIoGRAMplusC4D, we had >95% power to detect an allele frequency difference as small as 0.1%. In contrast, in PROMIS, we had 95% power to detect allele frequency differences only when these were >5%.

Table 4. Genome-Wide Association Studies Findings for Variants With Prior Reports for Association With Glucometabolic Traits

AA Change	Gene	rs no.	Minor Allele	MAF (%)*		HDL		Triglycerides		Type II DM	
				EUR	SAS	GLGC, *P* Value	PROMIS, *P* Value	GLGC, *P* Value	PROMIS, *P* Value	DIAGRAM, *P* Value	PROMIS, *P* Value
V249I	*CX3CR1*	rs3732379	T	28.53	12.78	0.57	1.00	0.76	0.70	0.46	0.30
T280M	*CX3CR1*	rs3732378	A	17.20	10.94	0.38	0.53	0.82	0.47	0.48	0.59
V64I	*CCR2*	rs1799864	A	8.65	9.82	…	0.70	…	0.07	…	0.48

AA indicates amino acid; DIAGRAM, Diabetes Genetics Replication and Meta-analysis; DM, diabetes mellitus; GLGC, Global Lipids Genetics Consortium; HDL, high-density lipoprotein; MAF, minor allele frequency; and PROMIS, Pakistan Risk of Myocardial Infarction Study.

*MAF per the 1000 genomes, phase 3 reference panel. No variant met significance following Bonferroni correction. Presented *P* values are not corrected for multiple testing.

Discussion

Experimental and clinical studies have attempted to elucidate the role of several chemokines and their receptors in the development of atherosclerosis and glucometabolic disorders. Rodent studies provide convincing data supporting a role, both independent and additive, for the chemokine receptors CCR2, CCR5, and CX3CR1 in the development of experimental atherosclerosis, insulin resistance, and cardiometabolic disorders through their modulation of monocyte recruitment and macrophage phenotypes. As a paradigm for exploring the consistency of human genetic data with mouse models of disease, we interrogated large contemporary data sets of common, low-frequency, and rare genetic variation in these chemokine receptor genes for association with CAD, MI, and glucometabolic traits. We failed to find evidence of an association between genetic variation in *CCR2*, *CCR5*, and *CX3CR1* and any of the traits studied in European ancestry data sets. In South Asians, we identified SNPs in *CX3CR1* with suggestive MI and type II DM associations, yet these same variants did not meet statistical significance in much larger predominantly European data sets. Our findings exclude clinically meaningful associations with CAD and glucometabolic traits in Europeans but suggest a need for larger studies in South Asians and other ethnicities.

Mouse data suggest a role for CCR2, CCR5, and CX3CR1 in atherogenesis. In hypercholesterolemic, atherosclerosis-susceptible apolipoprotein E–deficient mice, combined inhibition of *Ccl2*, *Cx3cr1*, and *Ccr5*, led to abrogation of bone marrow monocytosis and to an additive reduction in circulating monocytes in the setting of persistent hypercholesterolemia.[7] This was associated with a marked and additive 90% reduction in atherosclerosis. Ablation of individual chemokine receptors each modulated specific monocyte subpopulations and had significant but lesser impact on mouse atherosclerosis than combined inhibition. The common *CX3CR1* coding polymorphisms V249I and T280M, which are in strong LD, are reported to reduce cellular adhesion in vitro under conditions of physiological shear-stress and to impair chemotaxis and cell signaling.[8]

Despite convincing studies in mice and evidence for functional impact of human genetic variation on monocytes, the role of these genes in human atherosclerosis and CAD has not been well established. Many small genetic studies have looked for associations between chemokine receptor polymorphisms and CAD and MI with conflicting results.[9,10,41,42] In the Ludwigshafen Risk of Cardiovascular Health study, a cross-sectional study of 2583 case subjects with angiographically defined CAD and 733 control subjects, neither *CX3CR1* T280M nor V249I, was significantly associated with CAD or MI (n=1358 subgroup).[10] This study contrasts with a 7-study meta-analysis of 2000 CAD subjects and 2841 controls in which the V249I-T280M haplotype was found to be protective (OR, 0.81; 95% CI, 0.71–0.92; *P*=0.001).[42] The common *CCR2* variant V64I has been reported to associate with increased risk of early MI although this too has been controversial.[41,43,44] Similarly, CCR5delta32 has been linked in small studies to reduced susceptibility to CAD and protection against MI.[41,45]

Here, we shed light on this issue by interrogating the largest human data sets of common and low-frequency genetic variation for CAD and MI—the CARDIoGRAMplusC4D GWAS consortium in which we focus on common variation, and the MIGen and CARDIoGRAM Exome array consortia in which our focus is on low-frequency exonic variation.[5,16] These overlapping data sets contain information on 42 335 and 60 801 CAD subjects and 123 504 and 78 240 control subjects respectively, all of predominantly European descent. First, we examined *CX3CR1* V249I, *CX3CR1* T280M, and *CCR2* V64I given their putative functional effects and purported CAD associations, but failed to identify any significant associations with CAD or MI. Next, we broadened our search to look at all common and low-frequency variation within 5000 bps of these genes. Again, we did not identify any variants significantly associated with CAD or MI.

In the absence of CAD associations for common and low-frequency variants, it remains possible that rare coding variation and mutations in *CCR2*, *CCR5*, or *CX3CR1* have a clinically important impact on disease. Therefore, we interrogated the ESP EOMI data set that contains exome sequencing data on 4703 EOMI subjects and 5090 control subjects.[4] We hypothesized that rare alleles in aggregate in each gene might contribute to the risk of MI. When T1 allele count testing was applied, no gene-based signal for any of the chemokine receptors deviated from what was expected by chance though larger exome-seq data sets are required to exclude more modest impact of rare variation.[4]

Based on mouse models and small human studies, CCR2, CCR5, and CX3CR1 have been implicated in modulating obesity, insulin resistance, and glucose homeostasis.[11,38–40] Both CCR2 and CX3CR1 pathways are reported to modulate monocyte recruitment and macrophage phenotypes in adipose.[38,40,46] Multiple small studies have examined the association of obesity with the *CX3CR1* variants V249I and T280M, demonstrating an association with increased waist circumference, higher levels of Homeostasis Model Assessment-Insulin resistance, and a trend toward association with type II DM and metabolic syndrome.[46,47] Despite these previous trends, we did not find any association between common variation in *CCR2*, *CCR5*, or *CX3CR1* and any glucometabolic traits in the large GIANT, DIAGRAM, MAGIC, and GLGC GWAS resources.

The burden of CAD and type II DM is increasing at a greater rate in South Asia than in any other global region.[17] Nevertheless, little is known about the genetic determinants of disease in this population. Although PROMIS is contained in full within the CARDIoGRAMplusC4D meta-analysis, we chose to interrogate PROMIS separately given the distinct genetic background and increased risk of coronary heart disease in this Pakistani sample. We focused our initial investigation on the 1000 genomes imputed PROMIS data set that contains genetic information on 9058 subjects with CAD and 10 310 subjects with type II DM. After correction for multiple testing, we identified 6 variants in PROMIS associated with MI, including *CX3CR1* V249I, and 3 low-frequency, noncoding variants associated with type II DM. All variants associated with MI were present in the combined CARDIoGRAMplusC4D meta-analysis of CAD, none of which approached statistical significance. Similarly, 2 of the 3 PROMIS DM-associated variants (rs17038647 and rs17038663) were in MAGIC and DIAGRAM, and neither associated with type II DM nor glucose metabolism in these resources.

The significance of these associations with MI and type II DM in South Asians is unclear. In an earlier analysis of PROMIS data, Saleheen et al[18] showed that the genetic determinants of plasma lipid levels in PROMIS were broadly comparable with those of German subjects in the Ludwigshafen Risk of Cardiovascular Health study, yet the allelic frequencies and magnitude of association differed between the 2 ethnic groups. Similar to our analyses, differences were observed between PROMIS and CARDIoGRAMplusC4D in the allele frequencies (Table 4; Table III in the Data Supplement) and LD structures (Figure I in the Data Supplement). Given these ethnic patterns, these *CX3CR1* variants may deserve further follow-up in larger South Asian studies although false-positive findings are also possible given smaller sample size in PROMIS.

We illustrate here the challenge of extrapolating mouse models to human disease. Discrepancies may be because of differences in the molecular basis and pathophysiology of disease in mouse models versus humans. Alternatively, true loss- or gain-of-function human mutations may not be present in candidate genes, limiting a direct comparison between mouse genetic models and human genetic data. Molecular and pathophysiological heterogeneity may be of particular concern in studies of innate and adaptive immunity given differences in mouse and human macrophage phenotypes.[48] Human and mouse macrophages have distinct patterns of gene expression during trauma, burns, and endotoxemia.[49] It is important to recognize, however, that lack of a human genetic disease association does not exclude the possibility that the gene product may be involved in disease, particularly if loss- or gain-of-function mutations are not present in humans. Nevertheless, in our analyses, this seems less likely because exome sequencing and exome chip analyses did not reveal convincing signals for rare variants in human coronary heart disease.

There are other potential contributors to discrepancies between mouse and human data. First, previous studies in mice and humans had relatively small sample sizes and often lacked correction for multiple testing, raising the possibility of false-positive results. Second, our analyses may be underpowered in non-European ancestry to detect variants of small to moderate effect sizes (Table V in the Data Supplement). Previous analyses in PROMIS, however, have detected many loci with modest effects on MI suggesting that any association signals at *CCR2*, *CCR5*, or *CX3CR1*, if undetected, must be small if present.

This work has several strengths yet questions remain to be addressed. This is the largest systematic interrogation of cardiometabolic phenotypes for genetic variation in *CCR2*, *CCR5*, and *CX3CR1*. Multiple traits were examined, large data sets for common, low-frequency, and rare variants at these loci were available, and multiple ethnicities were included. Yet, we lacked low-frequency and rare variant data for glucometabolic traits, sample sizes for non-European ancestry were modest, and statistical power for detection of rare variant effects in MI cannot exclude small effects of true mutations. We applied Bonferroni correction for multiple testing, yet this assumes independence across SNPs tested, raising the possibility that we could have missed variants with true small effect sizes. This correction, however, is not conservative in terms of the total number of potential genome-wide tests, and we did not correct for the number of traits examined. A sensitivity analysis also excludes significant effects of more distant regulatory variation within 50 000 bps of each gene (data not shown). Suggestive evidence for associations of variants in *CX3CR1* with MI and type II DM only within South Asians requires larger follow-up.

In conclusion, in a comprehensive survey of common, low-frequency, and rare *CCR2*, *CCR5*, and *CX3CR1* genetic variation in cardiometabolic traits across multiple populations, we failed to find evidence of significant associations in predominantly European ancestry. Although *CX3CR1* variants were significantly associated with MI and type II DM in PROMIS, these associations were not significant in the larger CARDIoGRAMplusC4D meta-analysis of CAD or in the MAGIC or DIAGRAM meta-analyses of DM and glycemic traits. This suggests ethnic-specific effects or false-positive findings in PROMIS. Despite convincing rodent model data, our findings fail to support a clinically important role for *CCR2*, *CCR5*, or *CX3CR1* in the pathogenesis of atherosclerosis or glucometabolic traits in populations of European ancestry.

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Disclosures

None.

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CLINICAL PERSPECTIVE

In an effort to identify novel therapeutic targets, experimental and clinical studies have attempted to elucidate the role of several chemokines and their receptors in the development of atherosclerosis and glucometabolic disorders. Mouse data have suggested a role for *CCR2*, *CCR5*, and *CX3CR1* in atherogenesis and glucose metabolism although the role of these genes in human disease has not been well established. We performed a comprehensive survey of common, low-frequency, and rare *CCR2*, *CCR5*, and *CX3CR1* genetic variation in cardiometabolic traits across multiple populations, including a separate analysis of South Asian subjects, a population enriched for cardiometabolic disease. We failed to find disease associations in large primarily European cohorts. In a South Asian cohort, we identified *CX3CR1* variants associated with myocardial infarction and type 2 diabetes mellitus, suggesting ethnic-specific effects or possibly false-positive findings. Our data thus exclude clinically important association of genetic variation in *CCR2*, *CCR5*, and *CX3CR1* with cardiometabolic traits and suggest the need for further studies to identify whether there are ethnic-specific differences in *CX3CR1* that may be relevant to cardiometabolic disease pathogenesis and treatment.

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Common and Rare Genetic Variation in *CCR2*, *CCR5*, or *CX3CR1* and Risk of Atherosclerotic Coronary Heart Disease and Glucometabolic Traits

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on behalf of CARDIoGRAMplusC4D, Myocardial Infarction Genetics (MIGen), Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI), and the Pakistan Risk of Myocardial Infarction Study (PROMIS) Consortia*

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SUPPLEMENTAL MATERIALS

Supplemental methods:

Studies of coronary artery disease and myocardial infarction:

CARDIoGRAMplusC4D consortium: The details of the CARDIoGRAMplusC4D, 1000 genomes imputed dataset have already been published.[1] In brief, the CARDIoGRAMplusC4D dataset consists of merged data from the classic genome-wide association studies (GWAS) CARDIoGRAM and C4D, combining genotype information on 60,801 case subjects and 123,504 control subjects from 48 studies then imputed using the 1000 Genomes phase 1, version 3 reference panel.[1-4] As described, case subjects were defined by an inclusive coronary artery disease (CAD) diagnosis including myocardial infarction (MI), acute coronary syndrome, chronic stable angina, or coronary stenosis >50%. Association data for each contributing study were individually filtered for MAF > 0.5% and an imputation quality metric. For each study, ancestry-informative or other study-specific covariates were included as necessary which was confirmed on submission by review of the study-specific genomic control lambda. Variants that were retained in at least 60% of the studies were submitted for analysis. Following an inverse variance–weighted, fixed-effects meta-analysis, heterogeneity was assessed by Cochran's Q statistic[5] and the I^2 inconsistency index[6] and variants showing marked heterogeneity were reanalyzed using a random-effects model.[7] Overdispersion in the resulting meta-analysis was adjusted for by a second application of the genomic control procedure. 8.6 million single nucleotide polymorphisms (SNPs) and 836,000 insertions/deletions (indels) were included in the analysis. Association testing was performed by logistic regression on additive, recessive, and dominant models of disease susceptibility. Individual studies were combined using a fixed-effects, inverse-variance weighted meta-analysis.

Myocardial Infarction Genetics (MIGen) and CARDIoGRAM Exome consortia: The MIGen and CARDIoGRAM Exome array consortia consists of merged data from 19 studies totaling 42,335 MI case subjects and 78,240 control subjects of European ancestry.[8] Subjects were genotyped for 220,231 non-synonymous autosomal variants on the Illumina HumanExome BeadChip v1.0 (Illumina, San Diego, Ca). Quality control filters were applied before and after implementation of a zCall algorithm as described.[9] For variants that passed quality control procedures, individual tests for association with CAD were performed within each study. For variants with a MAF greater than 0% in both cases and controls, logistic regression was run on an additive model with the principal components of ancestry as covariates. Individual studies were combined using an inverse-variance weighted meta-analysis. Variants were functionally annotated as published and than restricted to those with a MAF $\geq$ 0.01%, leaving 54,003 variants with reported association testing. While 8 studies in the MIGen and CARDIoGRAM Exome array consortia dataset overlapped completely or partially with the CARDIoGRAMplusC4D meta-analysis, our focus here differs substantively from that of CARDIoGRAMplusC4D given its specific focus on low-frequency variation.

Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI) consortium: Details of the National Heart, Lung and Blood Institute's GO exome sequencing project (NHLBI ESP) and the ESP early-onset myocardial infarction (ESP EOMI) study have been published.[10,11] Briefly, the ESP EOMI was conducted using 4,703 EOMI case subjects and 5,090 control subjects. EOMI cases were defined as individuals who had an MI at age $\leq$ 50 years for men and at age $\leq$ 60 years for women. Control subjects were selected from individuals without a history of MI at baseline or whom did not have an MI during follow-up surveillance to a pre-specified age. Initial exome sequencing on subjects from 11 studies was performed at the Broad Institute with sequencing, exome capture, read mapping, variant analysis and quality control as published previously.[11] Follow-up sequencing was subsequently performed on samples from three additional studies. These samples were similarly sequenced at the Broad Institute with processing and quality control as published.[11] To test whether rare mutations contribute to EOMI, burden of rare variant association testing was performed on SNPs and indels present in *CCR2, CCR5,* and *CX3CR1.* The analysis was performed using the Efficient Mixed-Model Association eXpedited (EMMAX) Combined Multivariate and Collapsing (CMC) test.[12] The analysis was restricted to variants with a MAF < 1% as calculated using all sequenced samples in the study. Variants were analyzed using seven algorithms: LRT score, MutationTaster, PolyPhen-2 HumVar, PolyPhen-2 HumDiv, SIFT, MutationAssessor, and FATHMM.[13-18] To enrich for harmful alleles, different iterations of rare variant testing were performed using (1) non-synonymous variants; (2) disruptive variants (nonsense, slice-site, and indel frameshift variants); and (3) deleterious variants, defined as disruptive variants in combination with missense variants damaging by five, six, or seven of the aforementioned algorithms. Reported P-values were calculated using the EMMAX CMC test.

The Pakistan Risk of Myocardial Infarction Study (PROMIS): PROMIS is a retrospective case-control study of acute, first MIs in 6 centers in urban Pakistan combining data from 9,058 subjects with an acute MI and 8,378 control subjects.[19,20] Cases were defined as subjects presenting within 24 hours of symptom onset with typical ECG changes and a positive troponin-I. Control subjects were drawn from individuals without self-reported cardiovascular disease identified in the same hospitals as index cases. For each participant, information was collected on demographic factors, lifestyle, personal and family history. Non-fasting blood samples were collected from each participant to allow for measurement of serum biomarkers.

Samples were genotyped on the Illumina 660 and Illumina 770 arrays. Genotypes were imputed using the 1000 phase I integrated reference panel (March, 2012) using SHAPEIT and IMPUTE2.[21-23] SNPs were filtered for HWE $<1x10^{-5}$, imputation quality score (INFO) <0.7, and MAF $\leq$ 1%. Individual tests for association were performed with respect to MI adjusting for the first four principal components. The genomic inflation factor was 1.09.

Studies of glucometabolic traits:

Diabetes Genetics Replication and Meta-analysis (DIAGRAM): DIAGRAM contains information on 12,171 case subjects with type II Diabetes Mellitus (DM) and 56,862 control subjects of European descent combined across 12 GWAS. The details of the study have been published.[24] Sample and SNP quality control were undertaken within each study. Each GWAS was imputed using the phase II CEU HapMap reference panel. SNPs with a MAF>1% passing quality control criteria were tested for association with type II DM under an additive model after adjustment for study-specific covariates. Association summary statistics were combined via a fixed-effects, inverse-variance weighted meta-analysis.

The Genetic Investigation of ANthropometric Traits (GIANT) consortium: The GIANT meta-analysis contains genetic information on 123,865 subjects of European ancestry combined from across 46 studies.[25] All samples were genotyped using the Affymetrix (Affymetrix, Santa Clara, Ca) and Illumina (Illumina, San Diego, CA) whole genome genotyping arrays. Polymorphic SNPs were imputed using the HapMap CEU reference panel.

Association analysis with Body Mass Index (BMI): The GWAS on BMI includes genetic information from subjects across all 46 studies.[25] Each study performed single marker association analyses with BMI under an additive genetic model. BMI was adjusted for age, age^2, and principal components as deemed appropriate and than inverse normally transformed. SNPs with poor imputation quality and a minor allele count less than 3 in each sex- and case-specific stratum were excluded. The meta-analysis was performed in METAL using both the inverse variance method and the weighted *z*-score method.

Association analysis with Waist-Hip Ratio (WHR): The GWAS on WHR includes information on a subset of 77,167 subjects from 32 GWAS.[26] For each cohort, age-adjusted residuals were calculated for men and women separately with BMI adjustment then inverse normally transformed to ensure comparability across studies. SNP associations for WHR adjusted for BMI were computed by linear regression separately for men and women though these were combined to account for relatedness when appropriate. In addition to study-specific quality control measures, SNPs were excluded for low imputation quality and if the MAF times the number of subjects for a SNP in one study was less than 3.[26] A fixed-effects, inverse-variance weighted model was used to pool β estimates. P-values and standard errors for each study were genomic control corrected and a second genomic control correction was applied to meta-analyzed results.

Global Lipids Genetics Consortium (GLGC): The 2010 GLGC meta-analysis includes information on 100,184 individuals of European descent from 46 GWAS of lipids and lipid-related traits.[27] Each study performed genotype imputation with respect to the phase II CEU HapMap reference panel. Residual lipoprotein concentrations were determined after regression adjustment for the covariates age, age^2, and sex. Each genotyped or imputed SNP was tested for association with each trait assuming an additive genetic model. Linear regression was employed for studies of unrelated individuals and linear mixed effects models were used to account for family structure in family-based studies. SNPs with a MAF < 0.01 and poor imputation quality were excluded. Results were combined using a fixed effects meta-analysis in METAL for each of the lipid traits.

Meta-Analysis of glucose and Insulin-related traits consortium (MAGIC): The MAGIC consortium contains information on glycemic traits from non-diabetic individuals of European descent. The results have been published and are freely available online.[28,29] Polymorphic SNPs were imputed using the HapMap CEU reference panel. HgbA1c association results were available for 46,368 non-diabetic adults of European descent from 23 GWAS. The fasting insulin and fasting glucose datasets were generated by performing a meta-analysis of up to 21 GWAS informative for fasting glucose, fasting insulin and indices of β-cell function (HOMA-B) and insulin resistance (HOMA-IR) in 46,186 non-diabetic participants.[29] Trait values for fasting insulin, HOMA-IR, and HOMA-B were naturally log transformed. Datasets were adjusted for age, sex and study-specific covariates and then combined using a fixed-effects, inverse-variance approach.

The Pakistan Risk of Myocardial Infarction Study (PROMIS): The PROMIS resource is described above.[19,20] In addition to MI, association tests were performed for type II DM and lipid levels and summary data extracted for SNPs within 5,000bps of the start and end positions of *CCR2, CCR5,* and *CX3CR1*.

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Gene	Position (hg19)	rs#	Location	Alternate Allele	EAF	Beta	P-value
CX3CR1	3:39300023	rs140374643	Intergenic	A	0.98	-0.02	0.63
CX3CR1	3:39300157	rs17038640	Intergenic	C	0.65	0.004	0.71
CX3CR1	3:39300476	rs75540383	Intergenic	C	0.95	-0.02	0.34
CX3CR1	3:39300686	rs62244210	Intergenic	G	0.95	0.04	0.15
CX3CR1	3:39300845	rs73060520	Intergenic	C	0.91	0.01	0.59
CX3CR1	3:39301123	rs73060524	Intergenic	A	0.76	0.002	0.83
CX3CR1	3:39301287	rs151027349	Intergenic	G	0.98	-0.05	0.17
CX3CR1	3:39302355	rs11711922	Intergenic	A	0.69	-0.01	0.56
CX3CR1	3:39302415	rs112580659	Intergenic	G	0.99	-0.05	0.25
CX3CR1	3:39302659	rs79659083	Intergenic	A	0.94	0.02	0.43
CX3CR1	3:39303147	rs4676487	Intergenic	T	0.56	-0.01	0.45
CX3CR1	3:39304464	rs1877563	Downstream	A	0.88	-0.004	0.76
CX3CR1	3:39304549	rs11713282	Downstream	C	0.76	0.002	0.84
CX3CR1	3:39304570	rs17038645	Downstream	A	0.96	-0.02	0.30
CX3CR1	3:39304588	rs17038647	Downstream	C	0.94	-0.02	0.28
CX3CR1	3:39304602	rs11129819	Downstream	T	0.76	0.003	0.77
CX3CR1	3:39304794	rs11129820	Downstream	T	0.69	0.0003	0.98
CX3CR1	3:39304818	rs9826296	Downstream	A	0.86	0.001	0.96
CX3CR1	3:39305304	rs17038663	3' UTR	T	0.95	-0.02	0.37
CX3CR1	3:39306134	rs76874165	3' UTR	T	0.98	-0.05	0.18
CX3CR1	3:39306219	rs11710546	3' UTR	A	0.76	0.002	0.84
CX3CR1	3:39306605	rs17038674	3' UTR	T	0.96	-0.02	0.29
CX3CR1	3:39306784	rs1050592	3' UTR	G	0.76	0.003	0.79
CX3CR1	3:39307162	rs3732378	Exonic (Nonsynonymous)	A	0.85	0.01	0.63
CX3CR1	3:39307256	rs3732379	Exonic (Nonsynonymous)	T	0.76	0.002	0.88
CX3CR1	3:39307962	rs41535248	Exonic (Nonsynonymous)	A	0.99	-0.05	0.24
CX3CR1	3:39308205	rs55955702	Intronic	A	0.99	-0.05	0.26
CX3CR1	3:39308293	rs4271863	Intronic	T	0.50	0.01	0.49
CX3CR1	3:39308298	rs141909558	Intronic	T	0.99	0.01	0.77
CX3CR1	3:39309108	rs2669850	Intronic	C	0.58	-0.01	0.47
CX3CR1	3:39309215	rs17793056	Intronic	C	0.52	-0.01	0.16
CX3CR1	3:39310269	rs57345776	Intronic	T	0.50	-0.01	0.57
CX3CR1	3:39310764	rs116395001	Intronic	C	0.98	0.005	0.90
CX3CR1	3:39310793	rs145883535	Intronic	G	0.99	0.03	0.65
CX3CR1	3:39311087	rs140133131	Intronic	T	0.99	-0.02	0.79
CX3CR1	3:39311345	rs56379504	Intronic	G	0.81	-0.01	0.42
CX3CR1	3:39311583	rs9862876	Intronic	G	0.78	-0.01	0.48
CX3CR1	3:39311657	rs7615733	Intronic	G	0.69	-0.003	0.78
CX3CR1	3:39311666	rs13077357	Intronic	T	0.61	0.001	0.92
CX3CR1	3:39312017	rs78796740	Intronic	A	0.99	-0.08	0.07
CX3CR1	3:39312941	rs9868689	Intronic	T	0.81	-0.01	0.35
CX3CR1	3:39313391	rs35660161	Intronic	G	0.98	-0.02	0.54
CX3CR1	3:39313443	rs56110221	Intronic	G	0.01	-0.11	0.07
CX3CR1	3:39313524	rs34808142	Intronic	T	0.97	-0.06	0.04

CX3CR1	3:39314574	rs4423707	Intronic	T	0.91	0.01	0.57
CX3CR1	3:39315319	rs55675170	Intronic	G	0.99	-0.03	0.50
CX3CR1	3:39315901	rs56391246	Intronic	T	0.90	-0.001	0.97
CX3CR1	3:39316416	rs4676624	Intronic	C	0.94	-0.07	0.05
CX3CR1	3:39316828	rs12636547	Intronic	C	0.91	0.01	0.50
CX3CR1	3:39316976	rs4676625	Intronic	A	0.68	-0.01	0.17
CX3CR1	3:39317913	rs13062158	Intronic	C	0.69	0.01	0.23
CX3CR1	3:39318001	rs56095464	Intronic	G	0.59	-0.01	0.52
CX3CR1	3:39318192	rs56039226	Intronic	A	0.96	-0.02	0.32
CX3CR1	3:39318238	rs56386815	Intronic	T	0.99	-0.04	0.45
CX3CR1	3:39318288	rs2853712	Intronic	C	0.57	-0.01	0.38
CX3CR1	3:39318704	rs2669841	Intronic	T	0.73	-0.003	0.80
CX3CR1	3:39318797	rs2853711	Intronic	T	0.73	-0.003	0.80
CX3CR1	3:39319037	rs72865917	Intronic	G	0.91	0.01	0.49
CX3CR1	3:39319197	rs6796033	Intronic	G	0.57	-0.01	0.36
CX3CR1	3:39319288	rs56239258	Intronic	G	0.99	-0.06	0.26
CX3CR1	3:39319510	rs56035529	Intronic	T	0.91	0.003	0.86
CX3CR1	3:39320000	rs11720041	Intronic	T	0.82	-0.01	0.64
CX3CR1	3:39320055	rs2669843	Intronic	G	0.87	-0.01	0.70
CX3CR1	3:39320511	rs7622254	Intronic	T	0.98	-0.03	0.41
CX3CR1	3:39320598	rs116583694	Intronic	A	0.90	-0.01	0.74
CX3CR1	3:39320644	rs75098903	Intronic	T	0.97	-0.06	0.03
CX3CR1	3:39321218	rs2669845	Intronic	T	0.87	-0.01	0.70
CX3CR1	3:39321373	rs41376750	Intronic	A	0.98	-0.04	0.39
CX3CR1	3:39321412	rs41336745	Intronic	T	0.97	0.03	0.49
CX3CR1	3:39321516	rs36230801	5' UTR	A	0.98	-0.03	0.42
CX3CR1	3:39321710	rs35500272	Intronic	T	0.89	0.005	0.74
CX3CR1	3:39321770	rs9813187	5' UTR	A	0.83	-0.01	0.69
CX3CR1	3:39321805	rs36230797	5' UTR	C	0.89	-0.002	0.91
CX3CR1	3:39321867	rs871610	5' UTR	T	0.72	0.01	0.16
CX3CR1	3:39322466	rs871144	Intronic	T	0.68	-0.01	0.23
CX3CR1	3:39322483	rs55695898	Intronic	T	0.99	-0.04	0.37
CX3CR1	3:39322542	rs56156211	5' UTR	A	0.99	-0.04	0.36
CX3CR1	3:39322665	rs938203	5' UTR	A	0.84	-0.003	0.84
CX3CR1	3:39322826	rs2669846	Intronic	T	0.66	0.01	0.15
CX3CR1	3:39322843	rs2853708	Intronic	C	0.60	-0.01	0.29
CX3CR1	3:39323163	rs11715522	Exonic (Nonsynonymous)	C	0.61	0.01	0.14
CX3CR1	3:39323177	rs147724093	Exonic (Nonsynonymous)	C	0.99	-0.02	0.81
CX3CR1	3:39323423	rs11917223	Upstream	G	0.71	0.01	0.21
CX3CR1	3:39323542	rs62244246	Upstream	A	0.97	0.06	0.11
CX3CR1	3:39323765	rs11716530	Upstream	T	0.78	0.01	0.61
CX3CR1	3:39323843	rs13098237	Upstream	C	0.72	0.01	0.39
CX3CR1	3:39323847	rs13098239	Upstream	C	0.71	0.01	0.42
CX3CR1	3:39323992	rs9861437	Upstream	G	0.89	0.001	0.92
CX3CR1	3:39324065	rs6783639	Upstream	G	0.61	0.01	0.20
CX3CR1	3:39324246	rs76897474	Intergenic	C	0.89	0.01	0.58
CX3CR1	3:39324283	rs2853707	Intergenic	G	0.77	-0.02	0.13

CX3CR1	3:39325104	rs10865886	Intergenic	T	0.67	0.01	0.32
CX3CR1	3:39325126	rs192343698	Intergenic	C	0.02	0.02	0.56
CX3CR1	3:39325128	rs149810846	Intergenic	G	0.88	0.01	0.60
CX3CR1	3:39325227	rs188646763	Intergenic	T	0.99	0.01	0.92
CX3CR1	3:39325489	rs4256069	Intergenic	G	0.45	-0.01	0.32
CX3CR1	3:39325523	rs3020453	Intergenic	C	0.77	-0.02	0.10
CX3CR1	3:39325614	rs2965057	Intergenic	G	0.82	0.01	0.44
CX3CR1	3:39325677	rs3926044	Intergenic	T	0.59	-0.01	0.27
CX3CR1	3:39326084	rs11720953	Intergenic	A	0.90	-0.003	0.86
CX3CR1	3:39326283	rs1014638	Intergenic	G	0.67	0.01	0.50
CX3CR1	3:39326317	rs111791069	Intergenic	A	0.99	0.05	0.29
CX3CR1	3:39326511	rs938200	Intergenic	A	0.62	-0.02	0.10
CX3CR1	3:39327174	rs938199	Intergenic	A	0.62	-0.02	0.10
CX3CR1	3:39327376	rs13062901	Intergenic	T	0.67	0.01	0.50
CX3CR1	3:39327449	rs12486535	Intergenic	T	0.67	0.01	0.52
CX3CR1	3:39327556	rs187302965	Intergenic	G	0.99	-0.04	0.57
CX3CR1	3:39327676	rs4270454	Intergenic	T	0.67	0.01	0.50
CX3CR1	3:39327736	rs4271864	Intergenic	C	0.98	-0.07	0.07
CX3CR1	3:39327784	rs4271865	Intergenic	A	0.67	0.01	0.52
CX3CR1	3:39328082	rs190087508	Intergenic	A	0.99	-0.03	0.63
CCR2	3:46390228	rs35728689	Intergenic	A	0.89	-0.001	0.96
CCR2	3:46391071	rs6441971	Intergenic	C	0.76	-0.004	0.70
CCR2	3:46391390	rs6441972	Intergenic	A	0.67	-0.01	0.24
CCR2	3:46391648	rs35943069	Intergenic	G	0.91	-0.02	0.23
CCR2	3:46391788	rs17141006	Intergenic	G	0.89	-0.001	0.97
CCR2	3:46392060	rs17141010	Intergenic	T	0.89	-0.001	0.97
CCR2	3:46392089	rs1894387	Intergenic	T	0.91	-0.02	0.17
CCR2	3:46392131	rs1894388	Intergenic	T	0.91	-0.02	0.18
CCR2	3:46392162	rs62242995	Intergenic	A	0.89	-0.0001	1.00
CCR2	3:46392265	rs768539	Intergenic	A	0.76	-0.004	0.70
CCR2	3:46392976	rs34473395	Intergenic	T	0.91	-0.02	0.17
CCR2	3:46393463	rs3918354	Intergenic	G	0.91	-0.02	0.17
CCR2	3:46393827	rs3918355	Intergenic	T	0.96	0.003	0.92
CCR2	3:46393970	rs3918357	Intergenic	A	0.89	0.00001	1.00
CCR2	3:46394419	rs3918358	Upstream	C	0.67	-0.01	0.24
CCR2	3:46394680	rs3918359	Upstream	A	0.76	-0.004	0.70
CCR2	3:46395313	rs3749461	5' UTR	G	0.91	-0.02	0.16
CCR2	3:46395585	rs3092964	5' UTR	G	0.76	-0.004	0.71
CCR2	3:46395615	rs3918376	5' UTR	C	0.99	-0.02	0.76
CCR2	3:46395786	rs3918361	Intronic	A	0.78	0.002	0.83
CCR2	3:46395930	rs3918362	Intronic	T	0.91	-0.02	0.16
CCR2	3:46396616	rs3762823	Intronic	A	0.78	0.002	0.83
CCR2	3:46396938	rs3092963	Intronic	G	0.68	-0.01	0.49
CCR2	3:46397039	rs3092962	Intronic	A	0.78	0.003	0.82
CCR2	3:46397440	rs3092961	Intronic	A	0.43	0.01	0.45
CCR2	3:46398159	rs3918363	Intronic	T	0.89	-0.0003	0.99
CCR2	3:46398291	rs3918364	Intronic	T	0.89	-0.0003	0.99
CCR2	3:46398364	rs3918365	Intronic	G	0.91	-0.02	0.16
CCR2	3:46399174	rs3918367	Exonic	T	0.99	-0.08	0.20

			(Synonymous)				
CCR2	3:46399208	rs1799864	Exonic (Nonsynonymous)	A	0.90	0.0001	0.99
CCR2	3:46399798	rs1799865	Exonic (Synonymous)	C	0.68	-0.01	0.48
CCR2	3:46400062	rs3092960	Exonic (Synonymous)	A	0.88	-0.01	0.39
CCR2	3:46401032	rs3138042	Intronic	G	0.68	-0.01	0.47
CCR2	3:46401606	rs140253702	3' UTR	G	0.99	-0.01	0.86
CCR2	3:46402018	rs743660	3' UTR	A	0.78	0.002	0.85
CCR2	3:46402053	rs34138562	3' UTR	G	0.89	-0.001	0.94
CCR2	3:46402431	rs11575062	Downstream	T	0.91	-0.02	0.16
CCR2	3:46402564	rs762788	Downstream	T	0.78	0.002	0.88
CCR2	3:46402627	rs762789	Downstream	A	0.66	-0.01	0.56
CCR2	3:46402645	rs71327057	Downstream	C	0.91	-0.02	0.16
CCR2	3:46402688	rs762790	Downstream	G	0.87	-0.01	0.44
CCR2	3:46402734	rs34041956	Downstream	A	0.91	-0.02	0.16
CCR2	3:46403240	rs3918368	Downstream	A	0.91	-0.02	0.20
CCR2	3:46403315	rs6441973	Downstream	A	0.43	0.01	0.44
CCR2	3:46403401	rs1034382	Downstream	T	0.77	0.002	0.89
CCR2	3:46403468	rs3092959	Intergenic	A	0.91	-0.02	0.16
CCR2	3:46403681	rs3092958	Intergenic	A	0.91	-0.02	0.18
CCR2	3:46403961	rs3092957	Intergenic	A	0.91	-0.02	0.16
CCR2	3:46404163	rs2373226	Intergenic	T	0.77	0.003	0.81
CCR2	3:46404270	rs150203971	Intergenic	G	0.99	-0.04	0.51
CCR2	3:46404740	rs34944500	Intergenic	T	0.84	0.005	0.72
CCR2	3:46404742	rs34030880	Intergenic	T	0.64	-0.003	0.81
CCR2	3:46404744	rs35893284	Intergenic	A	0.47	0.001	0.89
CCR2	3:46404897	rs139885889	Intergenic	A	0.96	0.02	0.43
CCR2	3:46406367	rs2213290	Intergenic	T	0.60	0.01	0.17
CCR2	3:46406546	rs143226343	Intergenic	T	0.99	0.004	0.94
CCR2	3:46406578	rs2373227	Intergenic	C	0.83	0.02	0.12
CCR5	3:46408180	rs35513549	Intergenic	A	0.91	-0.02	0.14
CCR5	3:46408731	rs2040388	Intergenic	G	0.59	0.003	0.75
CCR5	3:46409113	rs3136535	Intergenic	A	0.87	-0.01	0.42
CCR5	3:46410036	rs7637813	Intergenic	G	0.70	0.01	0.51
CCR5	3:46410137	rs41490645	Intergenic	C	0.88	-0.01	0.36
CCR5	3:46410494	rs2856757	Intergenic	C	0.62	0.02	0.11
CCR5	3:46410936	rs2734225	Upstream	T	0.64	0.02	0.07
CCR5	3:46411542	rs2227010	Upstream	G	0.57	0.0005	0.96
CCR5	3:46411661	rs2856758	5' UTR	G	0.87	-0.01	0.42
CCR5	3:46411840	rs2734648	Intronic	T	0.63	0.02	0.09
CCR5	3:46411935	rs1799987	Intronic	A	0.47	-0.01	0.55
CCR5	3:46412259	rs1799988	5' UTR	C	0.47	-0.01	0.48
CCR5	3:46412308	rs1800023	5' UTR	G	0.64	0.02	0.06
CCR5	3:46412559	rs1800024	Intronic	T	0.89	0.0003	0.99
CCR5	3:46413334	rs2856762	Intronic	T	0.90	-0.02	0.12
CCR5	3:46413418	rs2254089	Intronic	T	0.64	0.02	0.06
CCR5	3:46413676	rs181867134	Intronic	T	0.98	-0.05	0.41

CCR5	3:46413743	rs2856764	Intronic	T	0.64	0.02	0.06
CCR5	3:46413950	rs2856765	Intronic	A	0.64	0.02	0.06
CCR5	3:46414035	rs41515644	Intronic	G	0.66	0.02	0.10
CCR5	3:46414557	rs1799863	Exonic (Nonsynonymous)	A	0.98	-0.003	0.94
CCR5	3:46414975	rs62625034	Exonic (Nonsynonymous)	T	0.96	-0.04	0.23
CCR5	3:46416216	rs17765882	3' UTR	T	0.90	-0.02	0.11
CCR5	3:46416470	rs1800874	3' UTR	T	0.64	0.02	0.05
CCR5	3:46416686	rs41526948	3' UTR	G	0.98	0.02	0.62
CCR5	3:46417069	rs41442546	3' UTR	A	0.97	0.02	0.64
CCR5	3:46417312	rs746492	3' UTR	G	0.48	-0.01	0.35
CCR5	3:46418342	rs3087251	Downstream	A	0.57	-0.002	0.85
CCR5	3:46418417	rs3087252	Downstream	T	0.64	0.02	0.03
CCR5	3:46418689	rs3087253	Downstream	C	0.57	-0.002	0.84
CCR5	3:46420104	rs11575816	Intergenic	T	0.64	0.02	0.04
CCR5	3:46420170	rs11575815	Intergenic	T	0.64	0.02	0.03
CCR5	3:46420618	rs181392199	Intergenic	G	0.99	-0.06	0.25
CCR5	3:46420781	rs71327059	Intergenic	T	0.91	-0.02	0.14
CCR5	3:46420799	rs3181038	Intergenic	C	0.91	-0.03	0.11
CCR5	3:46421838	rs3181039	Intergenic	C	0.64	0.02	0.04
CCR5	3:46422355	rs11575821	Intergenic	A	0.86	-0.01	0.45
CCR5	3:46422645	rs17715106	Intergenic	T	0.90	-0.02	0.11

Supplemental Table 1: No SNPs within *CCR2, CCR5*, or *CX3CR1* were significantly associated with CAD in the CARDIoGRAMplusC4D meta-analysis. Presented are the 206 SNPs within *CCR2, CCR5*, and *CX3CR1* captured in the CARDIoGRAMplusC4D meta-analysis. None of these SNPs nor the 14 indels in the corresponding genes were significantly associated with CAD after correction for multiple testing. Key: SNP = Single nucleotide polymorphism; CAD = Coronary artery disease; EAF = Effect Allele Frequency.

Gene	Position (hg19)	Change	AA Change	Minor Allele (MAF %)	P-value	Subjects
CCR2	3:46399208	Missense	V64I	A (9.0)	0.43	120565
CCR2	3:46399158	Missense	P47L	T (0.3)	0.82	68833
CCR5	3:46414947	Missense	S185I	T (11.1)	0.012	120557
CCR5	3:46414975	Missense	Q194H	T (11.1)	0.013	120573
CCR5	3:46414696	Nonsense	C101X	A (0.2)	0.032	112293
CCR5	3:46414557	Missense	L55Q	A (2.3)	0.14	120555
CCR5	3:46414573	Missense	R60S	T (0.2)	0.19	105867
CCR5	3:46414611	Missense	A73V	T (0.2)	0.43	119401
CCR5	3:46415066	Nonsense	R225X	T (0.1)	0.75	38938
CCR5	3:46415255	Missense	T288A	G (0.4)	0.78	47885
CCR5	3:46415061	Missense	R223Q	A (0.2)	0.88	108979
CX3CR1	3:39307832	Missense	T57A	C (0.5)	0.009	119734
CX3CR1	3:39323163	Missense	F8L	C (38.5)	0.39	120570
CX3CR1	3:39307962	Missense	E13D	A (1.2)	0.44	120573
CX3CR1	3:39307162	Missense	T280M	A (17.1)	0.45	120575
CX3CR1	3:39307256	Missense	V249I	T (27.8)	0.48	120558
CX3CR1	3:39307927	Missense	D25G	C (0.2)	0.49	114667
CX3CR1	3:39307637	Missense	V122I	T (0.1)	0.60	112828
CX3CR1	3:39307125	Missense	I292M	C (0.1)	0.84	77082
CX3CR1	3:39323177	Missense	P4A	C (0.9)	0.99	120516

Supplemental Table 2: No SNPs within *CCR2, CCR5*, or *CX3CR1* captured in the MIGen and CARDIoGRAM Exome array meta-analysis were significantly associated with CAD. Of the 20 polymorphic SNPs with MAF $\geq$ 0.1% in *CCR2, CCR5*, and *CX3CR1* captured in the MIGen and CARDIoGRAM Exome array dataset, none were significantly associated with CAD after correction for multiple testing. Key: CAD= Coronary artery disease; AA = Amino acid; MAF = Minor Allele Frequency; SNP = Single nucleotide polymorphism.

rs#	Location	Minor allele	CARDIoGRAMplusC4D (n=184,305)			PROMIS (n=17,437)			LD with V249I (R^2)		LD with T280M (R^2)	
			MAF (%)*	Beta	P-Value	MAF (%)*	Beta	P-Value	CEU	SAS	EUR	SAS
rs1050592†	3' UTR	G	28.63	-0.003	0.79	12.78	-0.12	1.59×10^{-4}	1.00	1.00	0.58	0.82
rs11129819†	Downstream	T	28.53	-0.003	0.77	12.78	-0.11	2.49×10^{-4}	1.00	1.00	0.58	0.82
rs11713282	Downstream	C	28.63	-0.002	0.84	12.88	-0.11	2.56×10^{-4}	1.00	0.99	0.58	0.81
rs3732379†	Exon	T	28.53	-0.002	0.88	12.78	-0.11	2.64×10^{-4}	-	-	0.58	0.82
rs11710546†	3' UTR	A	28.53	-0.002	0.84	12.78	-0.11	2.67×10^{-4}	1.00	1.00	0.58	0.82
rs73060524	Downstream	A	28.63	-0.002	0.83	12.88	-0.11	2.69×10^{-4}	1.00	0.98	0.58	0.80

* MAF per the 1000 genomes phase 3 EUR and SAS reference panels respectively.
† Variant genotyped in PROMIS.

Supplemental Table 3. *CX3CR1* variants significantly associated with MI in PROMIS: Values significant at a Bonferroni correction threshold of 2.76×10^{-4} (n=181). Key: MI = Myocardial infarction; MAF = Minor allele frequency; LD = Linkage disequilibrium; UTR= Untranslated region.

rs#	Location	Minor allele	PROMIS			LD with V249I (R^2)		LD with T280M (R^2)	
			MAF (%)*	Beta	P-Value	CEU	SAS	CEU	SAS
rs17038647	Downstream	C	4.70	0.22	1.61×10^{-6}	0.00	0.02	0.00	0.02
rs75540383	Downstream	C	4.70	0.22	3.15×10^{-6}	0.00	0.02	0.00	0.02
rs17038663†	3' UTR	T	4.60	0.22	3.29×10^{-6}	0.00	0.02	0.00	0.02

* MAF per 1000 genomes phase 3, version 5 SAS reference panel.
† Variant genotyped in PROMIS.

Supplemental Table 4. *CX3CR1* variants significantly associated with type II DM in PROMIS: Values significant at a Bonferroni correction threshold of 2.76×10^{-4} (n=181). The three variants are in near perfect LD with one another (r^2 >0.97) though not with the *CX3CR1* variants V249I and T280M. Key: DM = Diabetes mellitus; MAF = Minor allele frequency; LD = Linkage disequilibrium; UTR= Untranslated region.

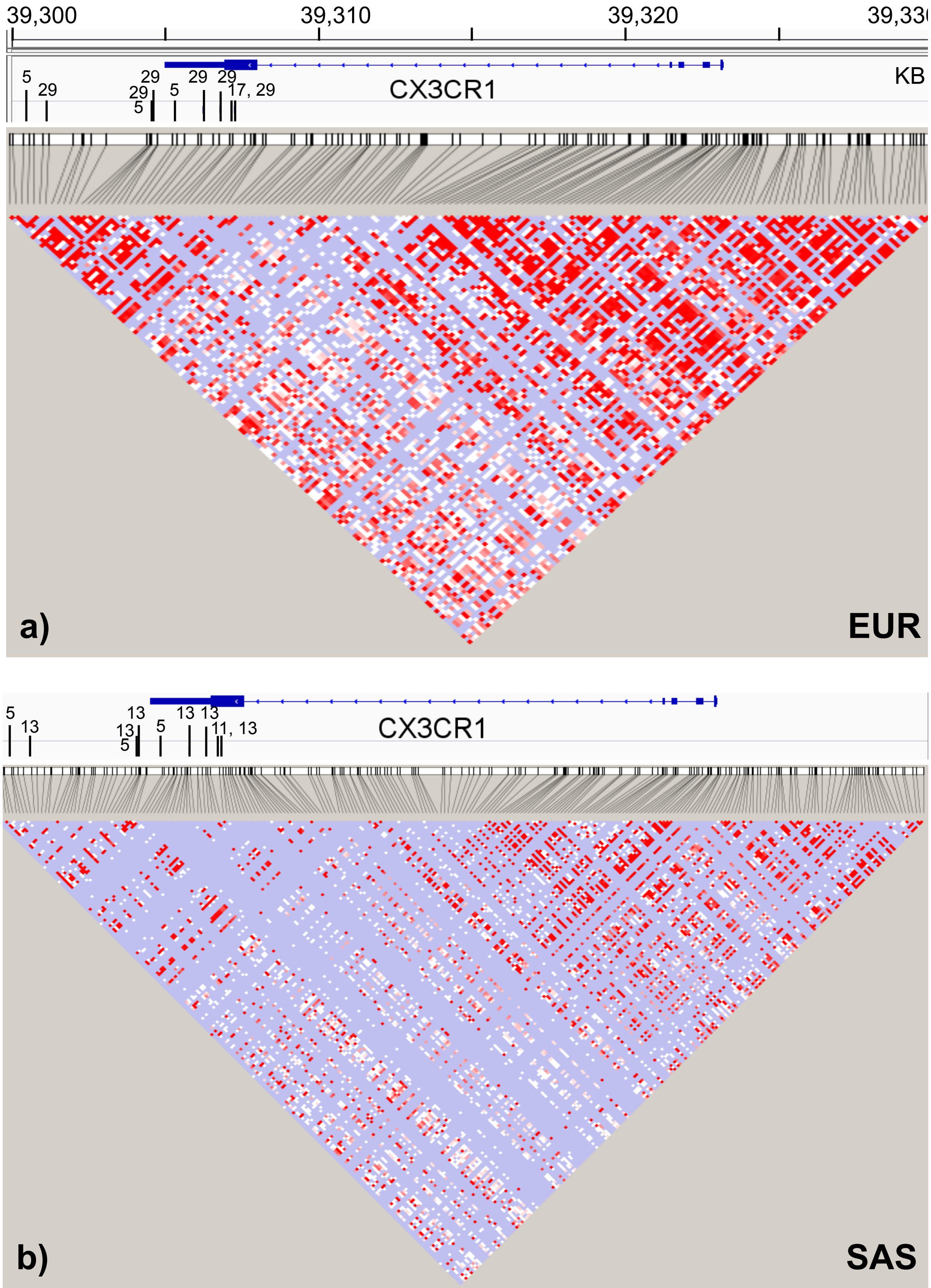
a) CARDIoGRAMplusC4D, 1000 genomes imputed (based on GWAS of 60,801 case subjects and 123,504 control subjects)

Risk AF	AF 0.05	AF 0.1	AF 0.15	AF 0.2	AF 0.25	AF 0.3	AF 0.35	AF 0.4	AF 0.45	AF 0.5
0.054	0.033	1	1	1	1	1	1	1	1	1
0.103	1	0.001	1	1	1	1	1	1	1	1
0.158	1	1	0.229	1	1	1	1	1	1	1
0.216	1	1	1	0.989	1	1	1	1	1	1
0.266	1	1	1	1	0.97	1	1	1	1	1
0.307	1	1	1	1	1	0.01	1	1	1	1
0.380	1	1	1	1	1	1	1	0.998	1	1
0.403	1	1	1	1	1	1	1	0	1	1
0.444	1	1	1	1	1	1	1	1	0.002	1
0.501	1	1	1	1	1	1	1	1	1	0

b) PROMIS (based on GWAS of 9,058 case subjects and 8,379 control subjects)

Risk AF	AF 0.05	AF 0.1	AF 0.15	AF 0.2	AF 0.25	AF 0.3	AF 0.35	AF 0.4	AF 0.45	AF 0.5
0.054	0	1	1	1	1	1	1	1	1	1
0.103	1	0	1	1	1	1	1	1	1	1
0.158	1	1	0	0.958	1	1	1	1	1	1
0.216	1	1	1	0.002	0.477	1	1	1	1	1
0.266	1	1	1	1	0.001	0.33	1	1	1	1
0.307	1	1	1	1	0.998	0	0.719	1	1	1
0.380	1	1	1	1	1	1	0.086	0.003	1	1
0.403	1	1	1	1	1	1	0.962	0	0.796	1
0.444	1	1	1	1	1	1	1	0.656	0	0.977
0.501	1	1	1	1	1	1	1	1	0.899	0

Supplemental Table 5: CARDIoGRAMplusC4D but not PROMIS has ample power to detect genetic variation at a range of allele frequencies. Displayed is the power calculated using actual risk allele frequencies taken from CARDIoGRAMplusC4D tested against a range of theoretical allele frequency differences (i.e. odds ratios) under a genome-wide significance threshold of $5x10^{-8}$. Key: AF= Allele frequency.



Supplemental Figure 1: LD plots for European and South Asian subjects. Displayed are the LD plots for European (a) and South Asian (b) subjects with respect to *CX3CR1.* Hash marks above figures correspond to the nine significant variants in PROMIS as well to the V249I and T280M variants. Approximate MAFs (%) are denoted above the hash marks. Key: LD = linkage disequilibrium; MAF = Minor allele frequency.

<u>Appendix:</u> Data presented on behalf of CARDIoGRAMplusCD, Myocardial Infarction Genetics (MIGen) and CARDIoGRAM Exome, Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI), and the Pakistan Risk of Myocardial Infarction Study (PROMIS) consortia.

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