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) (60801 cases and 123504 controls), the MIGen and CARDioGRAM Exome consortia (42335 cases and 78240 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.\(^5\)\(^6\)

### 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.\(^3\)\(^9\) 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.\(^12\)\(^3\) 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.\(^1\)\(^2\)\(^4\)\(^-\)\(^16\) 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.\(^1\)\(^2\)\(^4\)\(^-\)\(^16\) Genotypes were imputed using the 1000 Genomes phase 1, version 3 reference panel (Table 1).\(^1\) Variants were filtered on a minor allele frequency (MAF) $\geq 0.05\%$. 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 1 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).\(^1\)\(^6\) 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 $\geq 0.01\%$. Summary-level data were retrieved for polygenic exonic 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).\(^1\)\(^5\)\(^13\) 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,\(^2\)\(^5\)\(^-\)\(^8\)\(^10\) 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.\(^7\)\(^8\)\(^13\) Samples were genotyped on the Illumina 660 and Illumina 770 arrays and imputed using the 1000 Genomes Project phase 1, version 3 reference panel (Table 1).
The PROMIS MI resource is described above.\textsuperscript{17,18} In addition to MI, ratio, lipid and lipid-related traits, and glucose metabolism (Table 1). of glucometabolic traits including type II DM, BMI, weight-to-hip subjects.\textsuperscript{19–24} These resources contain genetic information on a range chemokine receptor variation with glucometabolic traits in European and MAGIC consortia meta-analyses to ascertain the association of Supplement. Briefly, we accessed the DIAGRAM, GIANT, GLGC, published and our specific approach detailed in Table 1 and the Data Detailed descriptions of these meta-analyses have been pub-

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

| Study                                      | Modality                      | Ethnicity               | Trait               | Subjects               | No. of SNPs* |
|--------------------------------------------|-------------------------------|-------------------------|---------------------|------------------------|-------------|
| CAD and MI                                 | 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\textsuperscript{16} | HumanExome BeadChip           | European                | CAD                 | 42,335 cases and 78,240 controls | 20          |
| Exome Sequencing Project and Early-Onset Myocardial Infarction (ESP EOMI) consortium\textsuperscript{17} | Whole-exome sequencing         | 91% European American; 9% African American | MI                  | 4703 cases and 5090 controls | ...         |
| Pakistan Risk of Myocardial Infarction Study (PROMIS)\textsuperscript{17,18} | GWAS, 1000 genomes imputed    | South Asian             | MI                  | 9058 cases and 8379 controls | 181         |

**Glucometabolic traits**

| Study                                      | Modality                      | Ethnicity               | Trait               | Subjects               | No. of SNPs* |
|--------------------------------------------|-------------------------------|-------------------------|---------------------|------------------------|-------------|
| Diabetes Genetics Replication and Meta-analysis (DIAGRAM)\textsuperscript{19} | GWAS, HapMap imputed          | European                | Type 2 DM           | 12,171 cases and 56,862 controls | 53          |
| Genetic Investigation of Anthropometric Traits (GIANT)\textsuperscript{20,21} | GWAS, HapMap imputed          | European                | BMI                 | 123,865                | 53          |
| Global Lipids Genetics Consortium (GLGC)\textsuperscript{22} | GWAS, HapMap imputed          | European                | WHR adjusted for BMI | 77,167                |             |
| Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC)\textsuperscript{23,24} | GWAS, HapMap imputed          | European                | Triglycerides       | 96,598                 | 53          |
|                                           |                                |                         | HDL cholesterol     | 99,900                 |             |
|                                           |                                |                         | 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)\textsuperscript{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).\textsuperscript{1} Individual tests for association were performed on variants with MAF \textgreater{}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 8379 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.\textsuperscript{16–24} 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.\textsuperscript{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.\textsuperscript{1} 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/).\textsuperscript{31} LD structure was visualized in Haploview separately for the 2 populations\textsuperscript{32} with gene structure visualized through the Integrative Genomics Viewer 2.3.67.\textsuperscript{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\times10\textsuperscript{−8}.\textsuperscript{34} 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.\textsuperscript{35}
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 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. 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 meta-analyses, currently the 2 largest genome-wide resources of CAD and MI, 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, 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. 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 5,003 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. 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. Using the ESP EOMI data set, which contains information on rare variation in 4703 EOMI case subjects and 5990 control subjects of European American (90.8%) and black (9.2%) descent, we tested the hypothesis that rare exonic variation in CCR2, CCR5, and CX3CR1 modifies the risk of MI. 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. 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 (r²>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 | Minor Allele | Minor Allele | Minor Allele | Minor Allele | Minor Allele | Minor Allele | Minor Allele | Minor Allele |
|-----------|-------|--------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 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) | 3.70×10⁻³  |

AA indicates amino acid; CARDioGRAMplusC4D, Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDioGRAM) plus The Coronary Artery Disease (CAD) 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).
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 CX3CR1V249I, CX3CR1T280M, nor CCR2V64I 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 (r²>0.97) although bore no relationship to the other variants, one of which was genotyped, are in close LD with one another (r²>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%.

### 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.
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. 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 V249I 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.

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. In the Ludwigshafen Risk of Cardiovascular Health study, a cross-sectional study of 2583 case subjects with angiographic defined CAD and 733 control subjects, neither CX3CR1 T280M nor V249I was significantly associated with CAD or MI (n=1358 subgroup). 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). The common CCR2 variant V64I has been reported to associate with increased risk of early MI although this too has been controversial. 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. 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. 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.

Based on mouse models and small human studies, CCR2, CCR5, and CX3CR1 have been implicated in modulating obesity, insulin resistance, and glucose homeostasis. Both CCR2 and CX3CR1 pathways are reported to modulate monocyte recruitment and macrophage phenotypes in adipose. 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. 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. 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
lacked correction for multiple testing, raising the possibility mice and humans had relatively small sample sizes and often between mouse and human data. First, previous studies in coronary heart disease did not reveal convincing signals for rare variants in human Nevertheless, in our analyses, this seems less that the gene product may be involved in disease, particu- genotypic disease association does not exclude the possibility It is important to recognize, however, that lack of a human gene expression during trauma, burns, and endotoxemia. Human and mouse macrophages have distinct patterns of differences in mouse and human macrophage phenotypes. Human and mouse macrophages have distinct patterns of gene expression during trauma, burns, and endotoxemia. 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 cardiometabolic 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 analy- sis 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 vari- ants 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 cardiometabolic 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.