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Genetic Association of the CCR5 Region With Lipid Levels in At-Risk Cardiovascular Patients

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***Background*—**There is mounting evidence to suggest that chemokine receptor 5 (CCR5) plays an important role in the development and progression of atherosclerosis. A naturally occurring variant of the *CCR5* gene CCR532, exists at allele frequencies of typically 10% in European populations and results in a nonfunctional CCR5 receptor.

***Methods and Results*—**The CCR5Δ32 deletion and 26 other variants within the chemokine receptor 2-*CCR5*-chemokine receptor-like protein 2 (*CCRL2*) gene cluster spanning 59 kilobases of chromosome 3 were genotyped in 5748 subjects from the Treating to New Targets atorvastatin trial to determine whether genetic associations could be identified with circulating lipid values and cardiovascular disease. Our results demonstrate an association between the CCR5Δ32 deletion and increased plasma high-density lipoprotein cholesterol and decreased plasma triglycerides, both of which are beneficial from a cardiovascular perspective. Three single-nucleotide polymorphisms (rs1154428, rs6808835, and rs6791599) in *CCRL2* in linkage disequilibrium ($r^2 \geq 0.65$) with CCR5Δ32 and located up to 45 kilobases distal to it were associated with high-density lipoprotein cholesterol. The high-density lipoprotein cholesterol and triglycerides findings were replicated in an additional set of >6000 individuals from the Incremental Decrease in Endpoints through Aggressive Lipid Lowering atorvastatin trial.

***Conclusions*—**Our study provides evidence that a locus within the region of the genome encompassing the CCR5-CCRL2 region is associated with lipid levels and suggests that chemokine activity influences lipid levels in populations with preexisting cardiovascular disease.

***Clinical Trial Registration*—**clinicaltrials.gov. Identifier: TNT, NCT00327691; IDEAL, NCT00159835.
(*Circ Cardiovasc Genet*. 2010;3:162-168.)

Key Words: CCR5Δ32 ■ genetics ■ genotype ■ lipids ■ single-nucleotide polymorphism

Atherosclerosis is a chronic inflammatory disease of the vessel wall and underlies clinical complications such as acute myocardial infarction (MI), stroke, and peripheral artery disease that result in cardiovascular morbidity and mortality. Atherosclerosis is characterized by the formation of arterial lesions comprising lipids, fibrous elements, and immune cell infiltrates.[1] Leukocyte, predominantly monocyte, recruitment into the vessel wall is a key step in atherosclerotic lesion formation, and chemokines and their receptors are known to be pivotal in this process.[2,3]

Clinical Perspective on p 168

Chemokine receptor 5 (CCR5) is a heptahelical serpentine G-protein-coupled receptor that is expressed on resting T-lymphocytes, monocytes, macrophages, immature dendritic cells, endothelial cells, and vascular smooth muscle cells[4,5] and mediates the activities of its ligands macrophage inhibitory protein-1α (chemokine ligand 3), macrophage inhibitory protein-1β (chemokine ligand 4), and RANTES (regulated on activation, normal T cell expressed and secreted) (chemokine ligand 5). Mounting evidence suggests that CCR5 plays an important role in the development and progression of atherosclerosis in rodent models. Knockout mouse studies have confirmed a critical role of CCR5 in macrophage accumulation and plaque formation during early[6] and late[7] stages of atherosclerosis in addition to the development of high-fat-diet-induced atherosclerosis.[8] Furthermore, studies using an antagonist of the CCR5 and chemokine receptor 3 receptors[9] or a recombinant RANTES receptor antagonist[10] have demonstrated an attenuation of atherogenesis in low-density lipoprotein (LDL) receptor null mice.

A naturally occurring variant of the *CCR5* gene, known as CCR5Δ32 (dbSNP rs333), exists at allele frequencies of typically 10% in European populations[11] and is defined by a 32-bp deletion that leads to a truncated nonfunctional receptor.[12] Consequently, in individuals homozygous for CCR5Δ32, the

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CCR5 receptor is eliminated from the cell surface,[13] and, in heterozygous individuals, the CCR5 receptor surface expression is reduced by 20% to 30% relative to the wild-type concentrations.[14]

Human genetic association studies investigating the role of *CCR5* in cardiovascular disease have primarily focused on the association between CCR5Δ32 and susceptibility to coronary artery disease and its sequelae, including MI. Several publications have demonstrated a protective effect of the Δ32 allele with cardiovascular disease,[15–18] including reduced C-reactive protein, decreased intima-media thickness, reduced incidence of cardiovascular disease for a 10-year period, and even a greater incidence of longevity. Other studies have demonstrated no relationship with cardiovascular events.[19–23] In contrast, a single study reported an increased risk associated with CCR5Δ32 and an earlier age of onset of MI in women.[24] These inconsistencies in the reported literature may reflect differences in study design, power, or population, and additional data will be helpful to understand the role of *CCR5* polymorphisms.

In the present study, we sought to clarify the role of *CCR5* in risk factors for atherosclerosis and coronary artery disease by comprehensively evaluating a number of genetic variants across the *CCR5* gene, including Δ32, in 2 independent clinical trial populations: the Treating to New Targets (TNT) and the Incremental Decrease in Endpoints through Aggressive Lipid Lowering (IDEAL) trials. In addition, a number of variants in *CCR2* and chemokine receptor-like protein 2 (*CCRL2*) on either side of *CCR5* were included to determine the extent of linkage disequilibrium (LD) in the region and to assess the origin of any association.

Methods

Population and Study Design

In a retrospective approach, we obtained access to DNA samples collected as part of the TNT trial and, for replication purposes, the IDEAL trial. Informed consent for the collection of DNA for genetic analysis was obtained from all participants who were elected to donate a blood sample for this purpose after institutional review committee approval. However, an important distinction is that DNA samples were collected at baseline for each consenting subject in TNT, whereas sample collection in IDEAL did not commence until >2 years after the first subject was enrolled in the study. Hence, subjects in IDEAL with the earliest events had already exited the trial without providing DNA.

The TNT trial was a randomized clinical trial of the possible benefits of moderate versus intensive lowering of LDL cholesterol (LDL-C) achieved with 10 mg/d and 80 mg/d of atorvastatin, respectively. Subjects were patients with clinically evident coronary heart disease, and the study variable was cardiovascular events over a 5-year period.[25] All patients for whom good-quality DNA was available were genotyped. Of those not genotyped, a small fraction gave consent, but the DNA quality was not good enough for high-quality genotyping, whereas the majority of those not genotyped did not provide consent for DNA analysis. The analysis was restricted to whites because of the small number of other ethnicities in the trial. Of the individuals consenting to genetic analysis, 5748 whites had DNA samples of sufficient quality for genotyping. Although there was a slight enrichment for events in ungenotyped subjects, no differences were observed between the genotyped and ungenotyped subpopulations for any of the 3 screening lipid values (all paired differences, <1.5%; P>0.115). CCR5Δ32 and 26 other variants (minor allele frequency, >5%) were genotyped in an initial study. Single-nucleotide polymorphisms (SNPs) were selected from public databases, including dbSNP and the HapMap project, using frequency and HapMap data to exclude SNPs with a minor allele frequency of <5% and SNPs in high LD with one another. Where limited genotyping data were available, SNPs were chosen at a high density. These SNPs mapped to a 59 kilobase genomic region extending into the promoter region of *CCR5* (with 1 SNP in *CCR2*) and beyond the *CCRL2* gene, centromeric to *CCR5* (Figure; Table 1). SNPs were genotyped by using either the Applied Biosystems 5′ nuclease TaqMan technology or Applied Biosystems SNPlex multiplex oligonucleotide ligation/polymerase chain reaction technology. Three SNPs (rs34203322, rs3204849, and rs1015164) had call rates of <90% and were excluded from further analysis. For the included SNPs, 5656 genotype calls were made on CCR5Δ32, whereas only a little more than 5300 were made on some of the subsequent *CCRL2* assays because of sample depletion.

The IDEAL trial was also a randomized clinical trial of moderate versus intensive lowering of LDL-C, in this case obtained with 20 to 40 mg/d of simvastatin and 80 mg/d of atorvastatin, respectively. Subjects were primarily white patients (mean age, 62 years; men, 81%) with a history of MI.[26] Of the 8888 patients enrolled, 6635 whites consented to genetic analysis, provided DNA samples, and were successfully genotyped. As anticipated, given the timing of sample collection ≥2 years into the trial, the differences in outcome for ungenotyped versus genotyped subpopulations exceed a hazard ratio of 2.2 with 95% CI. Hence, the dynamic range for both event outcomes and screening-LDL concentration in the genotyped population does not include the higher risk subjects, which is a limitation on the power to detect genetic associations with each of these 2 end points. However, as reduction in power in itself cannot result in false genetic association, and this should not detract from any positive results reported. The differences between groups merely reflect other factors, irrespective of genotype, namely withdrawal due to cardiovascular events, adverse events, and other reasons. Moreover, the same bias is not present for screening high-density lipoprotein (HDL) and triglyceride levels, each of which had differences of <2.1% between genotyped and ungenotyped subjects (P values of paired comparisons >0.057). Therefore, the only repercussion with respect to delayed sample collection in the IDEAL genotyped subpopulation is that any apparent lack of association between genotype and screening LDL or outcome should not be interpreted as a lack of association in the more general study population. Hence, after the analysis of the data from the TNT genotyping effort, the top findings of association were selected for replication in the IDEAL population. The markers CCR5Δ32 and 3 *CCLR2* (rs11574428, rs6808835, and rs6791599) were genotyped in IDEAL with TaqMan technology. After removing samples missing baseline lipid data or failing in at least 2 of 4 assays, a pool of 6602 white subjects was available for analysis. The actual number analyzed for each assay, as shown in Tables 2 through 4, varies due to samples with single call failures.

Analysis

All polymorphisms genotyped in the TNT population were tested for effect on baseline lipid values (each for LDL-C, HDL cholesterol [HDL-C], and triglycerides), using linear regression on log-transformed concentrations; MI (history or new incidence), using logistic regression; and longitudinal primary cardiovascular events, using Cox proportional hazards regression. All analyses were adjusted for gender and age at baseline. All end points were tested for sex by CCR5Δ32 genotype interaction; none was observed (data not shown). In addition, the MI and primary cardiovascular events analyses were adjusted for log of body mass index at baseline and treatment arm. In this analysis, *baseline* refers to the last visit after statin washout and before the 8-week statin run-in and not the visit at which the subjects already on 10 mg of atorvastatin were randomized to either remain on that dose or increase it to 80 mg, which was the definition of baseline in the trial protocol.

The CCR5Δ32 polymorphism, as the variant of primary interest, was first analyzed, with genotype as a categorical variable. Effect estimates of subjects heterozygotic or homozygotic for the minor allele were reported separately with respect to the mean of subjects homozygotic for the major allele (see Table 2 for a full characterization, including overall P value).

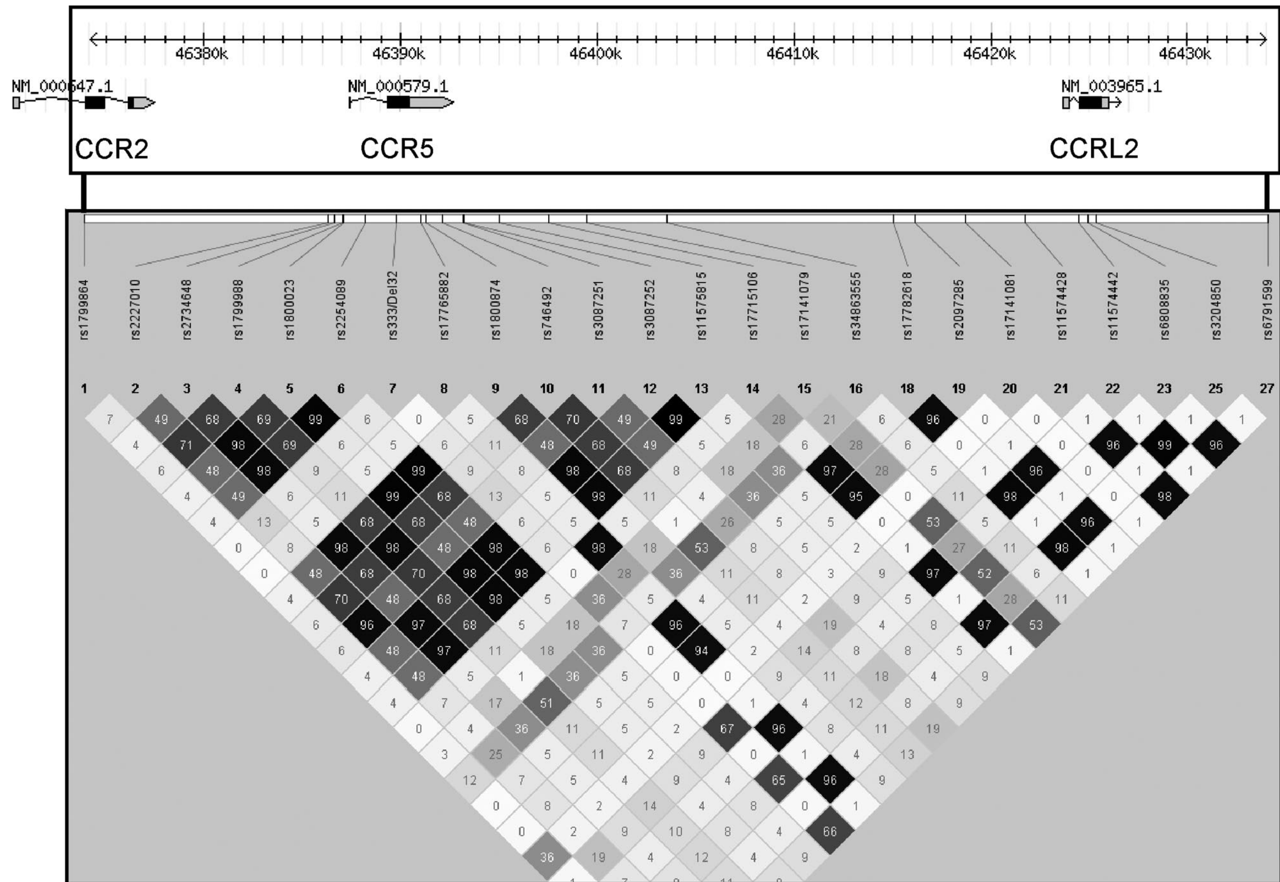

Figure. Overview of *CCR2-CCR5-CCRL2* genomic region on chromosome 3 targeted (using positions from HapMap data release, February 27, 2009). The Haploview version 4.1 figure was generated using the Caucasian genotyping data from the TNT population.

For the full SNP set, the categorical model was believed to have inferior power to models that specify the nature of the mode of action, which is taken here to mean the genetic effects profile that would be observed under a specific mode of inheritance (MOI) (one of dominant, recessive, or additive) were the SNP to be the causative locus. To use the more powerful models while still generating only a single result per SNP, the following scheme was used.

All SNPs (including CCR5Δ32) were analyzed by default under an additive model for the genotypic effect profile; thus, the genotype effect was constrained to a profile that is consistent with what one would observe for a causative SNP presenting an additive MOI. This constraint was implemented by simply coding the genotypes numerically according to the number of minor alleles (see resulting *P* values for CCR5Δ32 in Table 3).

Each SNP also was analyzed under a 2 degrees-of-freedom model, with one term being the additive genotype and the other a potential shift from the line for heterozygous subjects. Where statistical evidence ($P \cong 0.05$ or less) existed that the heterozygotic subjects deviated from the best linear fit (including a flat one) of the response variable against this numeric genotype, the model also was refit using a 2-group categorical genotype variable corresponding to what would be observed by a causative SNP conveying either a dominant or a recessive MOI (according to the direction in which the heterozygotic subjects deviated from the additive line). In such cases, the *P* value of the refit model was recorded as the best MOI *P* value; otherwise, it was left as the additive model *P* value (for CCR5Δ32, see the *P* Value, Best MOI column in Table 3). In this way, only 1 *P* value was selected per SNP, and deviation from the default model was only undertaken if statistically significant ($P \leq 0.05$) evidence for doing so existed. For CCR5Δ32, the *P* value providing evidence for the deviation of the heterozygotes from linear fit also is provided in Table 3 (*P* Value, Nonadditivity column).

Significant associations based on the best MOI *P* value were subsequently tested in the IDEAL population for replication and analyzed as described previously for TNT. The SNPs associated with any of these risk factors in both trials were automatically tested against longitudinal primary cardiovascular events in IDEAL (regardless of the status of the analogous test in TNT) to look for evidence of commensurate alterations in outcomes.

For the *CCRL2* SNPs, none of the fits deviated from an additive/linear genetics effect profile ($P > 0.10$ for deviation in all cases) in either trial; so, for brevity, only the results from the additive genotypic profile are presented here (Table 4). Additionally, note that all lipids were fit under a log transform, but results are reported as percent changes; hence, CIs are asymmetrical.

Results

CCR5Δ32 was significantly associated ($P < 0.05$) with higher fasting plasma HDL-C concentrations and lower fasting plasma triglyceride concentrations in TNT. HDL-C was increased by 6.8% (95% CI, 1.8% to 11.9%) from homozygote wild type (wt/wt) to homozygote deletion (del/del) in TNT ($P = 0.007$). A similar significant effect was observed in IDEAL, with a 3.1% (95% CI, 0.5% to 5.8%) increase in HDL-C ($P = 0.02$), although the effect of the "del" allele was observed to be recessive in TNT while being additive in IDEAL. Similarly, associations with triglycerides also were observed, with del/del showing a 12.5% (95% CI, 3.5 to 20.6%; $P = 0.0075$) reduction for triglycerides in TNT and a 5.1% (95% CI, 0.5 to 9.4%; $P = 0.029$) reduction in IDEAL. These associations are summarized in Table 3.

Table 1. Summary of Polymorphisms Genotyped for the Study

Gene	rs SNP ID	SNP Position	Gene Region	AA Change	TNT Minor Allele Frequency CA	IDEAL Minor Allele Frequency CA
CCR2	rs1799864	46374212	Exon 2	Val64Ile	0.09	
	rs2227010	46386546			0.46	
CCR5	rs2734648	46386844	Intron 1		0.36	
CCR5	rs1799988	46387263	Exon 2		0.45	
CCR5	rs1800023	46387312	Exon 2		0.36	
CCR5	rs2254089	46388422	Intron 2		0.36	
CCR5	rs333/del32	46389951	Exon 3	Truncation	0.11	0.12
CCR5	rs17765882	46391220	Exon 3		0.09	
CCR5	rs1800874	46391474	3′		0.36	
CCR5	rs746492	46392316	3′		0.45	
	rs3087251	46393346			0.46	
	rs3087252	46393421			0.36	
	rs11575815	46395174			0.36	
	rs17715106	46397649			0.09	
	rs17141079	46399573			0.25	
	rs34863555	46403662			0.39	
	rs34203322	46411960			0.09	
	rs17782618	46415049			0.09	
	rs2097285	46416180			0.09	
	rs17141081	46418682			0.05	
	rs11574428	46421725			0.15	0.15
CCRL2	rs11574442	46424395	Intron 1		0.09	
CCRL2	rs6808835	46424868	Exon 2	Gly98Gly	0.16	0.15
CCRL2	rs3204849	46425074	Exon 2	Tyr167Phe	0.46	
CCRL2	rs3204850	46425301	Exon 2	Ile243Val	0.09	
	rs1015164	46426684			0.31	
	rs6791599	46433946			0.16	0.15

AA indicates amino acid; CA, Caucasian.

For the additional 26 SNPs analyzed in the TNT data set, significance at the 0.05 level was determined under a Bonferroni correction for 26 tests, which corresponds here to a nominal $P<0.002$. Under this requirement, 3 SNPs (rs11574428, rs6808835, and rs6791599) exhibited significant P values only for baseline HDL-C (nominal $P<0.00084$ in each case). All 3 are in pairwise LD with $r^2 \geq 0.96$ in the *CCRL2* gene region, and each is in LD with CCR5Δ32 with $r^2 \geq 0.65$ (Figure). These SNPs were then genotyped in IDEAL, and each showed nominal significance against baseline HDL-C ($P<0.0056$ for all 3). Although these SNPs were not significant for triglycerides in TNT (P values between 0.08 and 0.13), the availability of their genotypes from the HDL-C analysis combined with the known correlation between HDL-C and triglycerides justified testing these SNPs against baseline triglycerides in IDEAL, resulting in $P<0.023$ for all 3. These associations are summarized in Table 4.

No significant associations were found for any polymorphisms with LDL-C, MI, or longitudinal primary events in TNT. Summaries of the mean lipid values after covariate adjustment can be found in Tables 5 and 6 for TNT and IDEAL, respectively.

All 4 polymorphisms replicated in IDEAL were in Hardy-Weinberg equilibrium in both trials with $P>0.2$, except

Table 2. Categorical Model CCR5Δ32 Results (TNT Versus IDEAL)

Trial	End Point	n	Minor Allele Frequency	Effect of Heterozygote (95% CI)*	Effect Between Homozygotes (95% CI)*	ANOVA *P*
TNT	HDL-C	5656	0.110	0.78 (−0.64 to 2.22)	6.92 (1.95 to 12.12)	0.015
TNT	Triglycerides	5656	0.110	0.09 (−2.79 to 3.05)	−12.45 (−20.59 to −3.46)	0.028
TNT	Primary events	5599	0.110	1.0 (0.80 to 1.25)	0.73 (0.30 to 1.75)	0.753
IDEAL	HDL-C	6555	0.117	1.31 (−0.15 to 2.79)	4.86 (−0.43 to 10.43)	0.05
IDEAL	Triglycerides	6555	0.117	−2.93 (−5.44 to −0.35)	−2.69 (−11.39 to 6.87)	0.077
IDEAL	Primary events	6535	0.117	1.18 (0.96 to 1.45)	0.32 (0.08 to 1.28)	0.039

Statistical models are all adjusted for age at baseline and sex. In addition, the primary events analysis is adjusted for body mass index at baseline, smoking status, and treatment arm.

*Units are percent change in concentration (from wt/wt) for lipids and hazard rate (relative to wt/wt) for primary events.

Table 3. Best Model CCR5Δ32 Results (TNT Versus IDEAL)

Trial	End Point	n	Minor Allele Frequency	Closest MOI Profile	Effect Between Homozygotes (95% CI)*	*P* Value, Nonadditivity	*P* Value, Best MOI
TNT	HDL-C	5656	0.110	Recessive	6.75 (1.81 to 11.94)	0.062	0.007
TNT	Triglycerides	5656	0.110	Recessive	−12.46 (−20.50 to −3.5)	0.017	0.0075
TNT	Primary events	5599	0.110	Additive	1.09 (0.82 to 1.40)	0.507	0.666
IDEAL	HDL-C	6555	0.117	Additive	3.13 (0.5 to 5.84)	0.467	0.0196
IDEAL	Triglycerides	6555	0.117	Additive	−5.09 (−9.44 to −0.53)	0.546	0.029
IDEAL	Primary Events	6535	0.117	Recessive	3.37 (0.84 to 13.50)	0.013	0.067†

Statistical models are all adjusted for age at baseline and sex. In addition, the primary events analysis is adjusted for body mass index at baseline, smoking status, and treatment arm.

*Units are percent change in concentration (from wt/wt) for lipids and hazard rate (relative to del/del) for primary events.

†Verified using a 10 000-iteration permutation test.

rs6808835, which had a Hardy-Weinberg $P=0.06$ in TNT. As described in the Analysis section, the 4 replicated polymorphisms were analyzed for association with cardiovascular events in each trial. CCR5Δ32 demonstrated some equivocal evidence of a recessive association with primary cardiovascular events in IDEAL (Table 3), where the del/del genotype (present in 86 subjects) exhibited a hazard rate that was 3.37 (95% CI, 0.84 to 13.50) times lower than that for subjects carrying a wild-type allele ($P=0.067$ from a 10 000-iteration permutation test, where the genotype values were permuted while all clinical data were held fixed), using Cox proportional hazards regression. The permutation test was run because of disagreement between the nominal *P* value for the hazard rate contrast ($P=0.09$) and the *P* value obtained comparing the fit likelihoods of the Cox model containing genotype to one not containing genotype ($P=0.033$). Under the permutation test, the former *P* value was reduced to $P=0.067$, whereas the latter remained stable at $P=0.033$. There was no significant association with cardiovascular event rate for CCR5Δ32 in the TNT trial. A breakdown of hazard rates by del32 genotype for each trial can be found in Table 7.

Notably, neither study shows any evidence of benefit in outcomes for del32 heterozygotes. Because of the low number of del32 homozygotes and other limitations outlined previously, both analyses were underpowered to unambiguously demonstrate a significant benefit for del32 homozygotes, despite effect estimates being consistent with such a benefit.

Although not shown, each of the replicated *CCRL2* SNPs also was tested against longitudinal primary events in both trials. All such tests were insignificant, with $P>0.8$ for each SNP in TNT and $P>0.38$ for each SNP in IDEAL.

Discussion

Our results demonstrate an association between the CCR5Δ32 deletion and increased plasma HDL-C and decreased plasma triglycerides in 2 clinical cardiovascular prevention trial populations, both beneficial from a cardiovascular perspective. No significant associations were found with LDL-C or MI. Three SNPs in *CCRL2*, adjacent to *CCR5* and located up to 45 kilobases distal to it, were strongly associated with HDL-C in both trial populations and are associated with triglycerides in 1 trial. To our knowledge, this study is the first to demonstrate an association of this genic region with lipid levels in 2 independent populations. The 3 *CCRL2* SNPs showed no association with beneficial cardiovascular outcomes in either trial; however, the CCR5Δ32 deletion showed a weak association with cardiovascular events in 1 of 2 trials, with benefit evident for the small proportion of CCR5Δ32 deletion homozygotes.

Table 4. Combined Table for *CCRL2* SNP Associations in TNT and IDEAL

Trial	rs SNP ID	End Points	Minor Allele Frequency	n	Effect Between Homozygotes (95% CI)*	*P*
TNT	rs11574428	HDL-C	0.148	5308	4.08 (1.86−6.24)	0.00035
TNT	rs6808835	HDL-C	0.151	5308	4.02 (1.72–6.37)	0.00055
TNT	rs6791599	HDL-C	0.150	5324	3.80 (1.59–5.96)	0.00083
IDEAL	rs11574428	HDL-C	0.148	6583	3.34 (0.96–5.76)	0.00558
IDEAL	rs6808835	HDL-C	0.151	6581	3.45 (1.07–5.88)	0.00421
IDEAL	rs6791599	HDL-C	0.150	6588	3.34 (0.97–5.76)	0.00544
TNT	rs11574428	Triglycerides	0.148	5308	−4.08 (0.69–−9.10)	0.09530
TNT	rs6808835	Triglycerides	0.151	5308	−3.51 (−7.87–1.04)	0.12857
TNT	rs6791599	Triglycerides	0.150	5324	−4.27 (0.49–−9.27)	0.08003
IDEAL	rs11574428	Triglycerides	0.148	6583	−4.90 (−8.82–−0.82)	0.01909
IDEAL	rs6808835	Triglycerides	0.151	6581	−4.75 (−8.68–−0.66)	0.02317
IDEAL	rs6791599	Triglycerides	0.150	6588	−4.79 (−8.70–−0.71)	0.02185

Statistical models are adjusted for age at baseline and sex.

*Units are percent change in concentration from major allele homozygote. All trends showed mode of action consistent with an additive mode of inheritance.

Table 5. TNT Baseline Lipid Summaries* by del32 Genotype

Lipid	Genotype	Counts	Mean (95% CI)
HDL, mg/dL	wt/wt	4446	47.08 (47.07–47.38)
	wt/del	1072	47.47 (47.45–48.08)
	del/del	78	50.32 (50.24–52.73)
LDL, mg/dL	wt/wt	4446	161.42 (161.40–162.11)
	wt/del	1072	161.58 (161.53–162.59)
	del/del	78	163.19 (163.00–169.08)
Triglyceride, mg/dL	wt/wt	4446	183.91 (183.83–186.31)
	wt/del	1072	183.64 (183.49–188.50)
	del/del	78	160.80 (160.38–174.39)

*Group means are adjusted for age at baseline and sex, preserving the grand mean.

These data support evidence that functional changes in the CCR5 region might positively affect lipid levels. However, it is noteworthy that the association with a number of SNPs in the adjacent region also raises the possibility that the functional variants may not be in *CCR5*. Atherosclerosis is known to result from a complex interplay between lipids and inflammation, and it remains to be fully determined how these systems interact. *CCR5* and the neighboring *CCR2* are intimately involved in regulating inflammation but have not been linked previously to lipids. HDL, as a significant carrier of both cholesterol and a variety of inflammatory mediators, has long been suspected to be a key mediator between lipids and inflammation; therefore, an effect of reduced *CCR5* activity on HDL-C and triglycerides would add to this connection.

The evidence for the role of leukocytes, particularly that of the monocyte, in the development of atherosclerosis is well-known.[2,3] In addition to their inhibitory effects on cholesterol biosynthesis, statins have been reported to reduce CCR5 expression on activated CD4+ T lymphocytes[27] and to have antiproliferative effects on U937 monocytic cells in vitro through downregulation of CCR2 and CCR5 expression.[28] However, any direct effect of statin treatment on CCR5 expression and chemokine signaling and cardiovascular outcome could not be assessed in this study because chemokine measurements were not undertaken in either TNT or IDEAL.

In our study, we have suggested that a locus within the CCR5-CCRL2 region is associated with HDL-C and triglyceride levels, an analysis based on screening lipid levels after statin wash out. Nevertheless, all patients received statin treatment, and despite no significant associations with cardiovascular events in either trial, statin effects on chemokine function and signaling cannot be excluded as a potential confounding factor in the outcome analysis.

Table 6. IDEAL Baseline Lipid Summaries* by del32 Genotype

Lipid	Genotype	Counts	Mean (95% CI)
HDL, mg/dL	wt/wt	5077	44.45 (44.44–44.74)
	wt/del	1358	45.04 (45.02–45.63)
	del/del	86	46.60 (46.53–48.98)
LDL, mg/dL	wt/wt	5001	115.72 (115.70–116.61)
	wt/del	1340	115.54 (115.49–117.25)
	del/del	86	115.86 (115.65–122.67)
Triglyceride, mg/dL	wt/wt	5077	134.36 (134.31–136.00)
	wt/del	1358	130.47 (130.37–133.58)
	del/del	86	130.75 (130.40–142.15)

*Group means are adjusted for age at baseline and sex, preserving the grand mean.

Table 7. Hazard Rates by del32 Genotype, IDEAL and TNT

Trial	Genotype	Counts	No. Events	Hazard Rate	Hazard Ratio* (95% CI) Relative to wt/wt
TNT	wt/wt	4449	400	0.0203	. . .
	wt/del	1072	96	0.0203	0.998 (0.798–1.247)
	del/del	78	5	0.0141	0.725 (0.300–1.754)
IDEAL	wt/wt	5077	368	0.0156	. . .
	wt/del	1358	115	0.0184	1.170 (0.949–1.442)
	del/del	86	2	0.0048	0.320 (0.080–1.285)

*Adjusted for age and body mass index at baseline, sex, smoking status, and treatment arm.

The functional relationship of the *CCRL2* polymorphisms to CCR5Δ32 remains unknown, although each has an LD $r^2 \geq 0.67$ with CCR5Δ32. The 59-kilobase span of these SNPs means that unequivocal assignment of the functional SNP will require additional study. The effect size observed in this and other studies suggests a modest proportion of variation in lipid levels is explained by SNPs in this region, consistent with other genetic findings for complex traits. This finding would be particularly striking if the causal variant is CCR5Δ32, and then, the mean difference between wild-type carriers and homozygotic CCR5Δ32 carriers (who produce no CCR5 at all) would be modest (Table 3).

In conclusion, the findings of this study provide evidence that a locus within the region of the genome encompassing the CCR5-CCRL2 region is associated with lipid levels and suggest that chemokine activity influences lipid levels in a population with preexisting coronary heart disease. However, further studies would be required to establish a cause-and-effect relationship for either of these genes on lipid modulation.

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CLINICAL PERSPECTIVE

Evidence suggests a role for the chemokine receptor 5 (CCR5) in the development and progression of atherosclerosis; knockout studies have confirmed a critical role for CCR5 in macrophage accumulation and plaque formation, and attenuation of atherogenesis has been demonstrated in low-density lipoprotein receptor null mice treated with CCR5 and chemokine receptor 3 receptor antagonists. Several human genetic studies have demonstrated a protective effect of the CCR5Δ32 deletion with cardiovascular disease; however, other studies have shown no relationship with cardiovascular events. Differences in study design and relatively small sample sizes may contribute to these inconsistencies. In this investigation, we performed a comprehensive evaluation of 27 genetic variants, including CCR5Δ32, across the chemokine receptor 2-*CCR5*-chemokine receptor-like protein 2 (*CCRL2*) gene region in 5748 white subjects participating in the Treating to New Targets (atorvastatin) cardiovascular events trial. Results demonstrated an association between the CCR5Δ32 deletion and higher plasma high-density lipoprotein cholesterol and lower plasma triglyceride levels as well as an association between 3 CCRL2 single-nucleotide polymorphisms and higher plasma high-density lipoprotein cholesterol. These associations were replicated in a second population-based study (Incremental Decrease in Endpoints through Aggressive Lipid Lowering) in which 6635 white participants were genotyped. Although the findings do not imply a causal effect for the associated single-nucleotide polymorphisms, it is possible that the functional genetic variant underlying the observed associations may reside within *CCR5* or *CCRL2* genes. Overall, our data support the hypothesis that functional changes in the *CCR5* region might positively affect cardiovascular disease in some part by influencing circulating lipid levels. Identification of the functional variant underlying these beneficial associations potentially may lead to the development of a novel and adjunctive approach for the treatment of dyslipidemia and cardiovascular disease, respectively.