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Genetic Analysis Implicates Resistin in HIV Lipodystrophy

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Abstract

Objectives—To investigate the role of genetic variation in influencing the risk of metabolic complications associated with highly active anti-retroviral therapy (HAART).

Methods—Cluster analysis of metabolic traits of 189 patients enrolled in ACTG5005s, the metabolic sub-study of ACTG384, a clinical trial of HAART, was performed to identify a sub-group of subjects with increased risk of developing a cluster of metabolic abnormalities after exposure to HAART. Almost 300 single nucleotide polymorphisms (SNPs) in 135 candidate genes were evaluated for their association with this sub-group.

Results—A sub-group of patients was identified that had a normal metabolic profile at baseline but developed significantly elevated lipids and insulin resistance on HAART. This high-risk sub-group of patients also experienced significant body composition changes, particularly limb fat loss. Candidate gene analysis revealed that a single nucleotide polymorphism in resistin, a gene previously implicated in obesity and insulin resistance, was associated with this high-risk group ($P = 0.0003$).

Conclusions—Genetic variation in resistin is associated with metabolic complications caused by highly active anti-retroviral therapy.

Keywords

highly active antiretroviral therapy; HAART; dyslipidemia; lipoatrophy; pharmacogenetics; single nucleotide polymorphism; resistin

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Author contributions: PT, KM, WP, SG and MD ran the clinical trial and collected patients. KR, WG, OF, MN designed and executed the genetic analysis. KR, WG, OF, MN, PT, SG, MD drafted the manuscript.

Competing interests

KR, OF, WG are employees of Bristol-Myers Squibb Co. MN is currently employed by GlaxoSmithKline.

Introduction

Highly active anti retroviral (HAART) regimens can be associated with multiple metabolic abnormalities, including elevated lipids, glucose and insulin resistance, which are accompanied by changes in body composition such as visceral obesity and limb fat loss. Typically these abnormalities cluster together in a syndrome called human immunodeficiency virus (HIV) lipodystrophy [1,2]. With the reduction in morbidity and mortality resulting from HAART, these metabolic side-effects are of concern as they are well-known risk factors for future cardiovascular disease [3]. The etiology of HIV lipodystrophy is poorly understood, although some PIs may be more likely to cause metabolic abnormalities [2,4,5]. The genetic basis of HIV lipodystrophy is also unclear. Some studies have implicated nucleotide variation in ApoCIII, the β 3 adrenergic receptor or TNF-alpha [6,7,8]. However, these studies focused only on one trait (e.g. triglycerides or insulin resistance) and examined selected SNPs in a single gene.

In this study, we employed a new approach to analyze metabolic data collected in a clinical trial of HAART to identify a sub-group of patients who developed marked lipid elevations and insulin resistance after exposure to HAART. The clustering approach we used to identify this sub-group is a powerful method for finding patterns in multi-variable data, and has been successfully employed to detect sub-groups of cancer patients based on gene expression profiles [9, 10]. We reasoned that this approach was particularly suited to the metabolic complications caused by HAART because of the apparently syndromic nature of the side-effects [1,2]. Having identified this sub-group of patients, we performed a comprehensive candidate gene analysis to implicate resistin, a gene previously associated with obesity and insulin resistance [11].

Methods

Subjects, genotyping and sequencing

189 individuals who participated in ACTG5005s [12] and who provided written informed consent for genetic analysis were analyzed in this study. Briefly, drug naive HIV+ patients were randomized to a backbone of NRTIs comprised of didanosine/stavudine or zidovudine/lamivudine [13,14]. In addition, subjects were randomized to receive a PI (nelfinavir), an NNRTI (efavirenz) or the combination. Metabolic traits were measured at baseline and then at 16 week intervals up to 64 weeks. Body fat was measured in all consenting patients using dual-energy x-ray absorptiometry at baseline and every 16 weeks on HAART. Genotyping was done using the TaqMan method [15]. The sequence of the entire resistin gene was determined in 47 high-risk individuals, and for comparison, up to 32 individuals from the diversity panel (M44PDR, Coriell repository) to identify SNPs.

Statistical analysis

Insulin resistance by homeostasis-model assessment (HOMA-IR) was calculated using the formula: fasting insulin (μ U/mL) x fasting glucose (mmol/L)/22.5. Traits measured after 32 weeks of HAART were standardized to have a mean of 0 and variance of 1 and then used in the clustering. Traits measured at 32 weeks were selected for clustering because metabolic

side-effects become apparent by this time-point and because later time-points had substantial numbers of missing values. For each individual a profile was made of body mass index, total, LDL, HDL and non-HDL cholesterol, triglyceride, glucose, and HOMA-IR. These profiles were clustered together using “two-step” clustering [16] as implemented in SPSS version 12 (Chicago, Illinois, US). In this clustering method, data are first grouped into sub-clusters using agglomerative clustering and then cluster assignment is refined to determine the optimal number of clusters. The optimal number of clusters was determined in two steps. First, Bayesian information criterion was calculated for the specified number of clusters to obtain an initial estimate of cluster number. Second, this estimate was refined by finding the greatest change in distance between two clusters in each stage of hierarchical clustering. The distance between two clusters was defined as the decrease in log-likelihood resulting from the two clusters being combined into a single cluster. Up to 20 clusters were permitted, but the algorithm repeatedly determined that a two cluster solution was optimal. To assess stability of the cluster solution, multiple runs of clustering (N = 20) were performed using randomly sorted data. All runs produced clusters identical to those described in this study. Differences in means between clusters for metabolic traits and changes in body fat over time were evaluated using Kruskal-Wallis and repeated measures ANOVA, respectively. Total, trunk and limb fat values were natural log transformed to approximate normality and then used in the repeated measures ANOVA. Genetic association between SNPs and clusters was assessed using Fisher’s exact test. Linkage disequilibrium between SNPs and haplotypes were analyzed using Haploview [17].

Results

Clustering of metabolic profiles to identify a sub-group of patients at increased risk of developing metabolic abnormalities after exposure to HAART

We analyzed 189 subjects, enrolled in a HAART clinical trial [13,14], who had longitudinal metabolic measurements, including lipids, glucose and insulin, at baseline and up to 64 weeks of treatment [12]. We applied a clustering approach to the analysis of metabolic traits to identify a sub-group of patients with pronounced changes in their metabolic profiles after treatment with HAART. Because of the stronger phenotypic differences displayed by such subgroups, we reasoned that these subgroups might show greater contrast, and thus increase the likelihood of finding a genetic association [18,19]. In addition, because HIV+ patients on HAART typically present with a cluster of metabolic abnormalities [1,2], it was logical to analyze these traits in unison as opposed to sequential genetic analysis of individual metabolic traits. Furthermore, this approach has the potential to reduce noise, as patients with multiple abnormalities are more likely to be truly affected than those with merely sporadic elevations of a single metabolic trait. Finally, since we analyzed all the metabolic traits at once in this multivariable analytic approach we reduced the number of statistical tests by an order of magnitude, thereby significantly increasing the power of the study, an important consideration given the limited number of samples that were available for genetic analysis.

For each patient, we created a metabolic profile comprised of body mass index, total, LDL, HDL and non-HDL cholesterol, triglycerides, glucose and insulin resistance by homeostasis

model assessment (HOMA-IR), all measured after thirty-two weeks of HAART. We selected this time-point because metabolic abnormalities become apparent at this level of exposure and because later time-points had significant numbers of missing values. We then clustered these metabolic profiles such that individuals with similar metabolic profiles were grouped together. This process was repeated iteratively until all individuals were clustered into two groups. This approach is analogous to the widely used method of clustering gene expression profiles to identify subgroups of tumors or cell-types [9,10].

Examination of mean values of metabolic traits led to labeling one cluster “normal” (N = 142) and the other “high-risk” (N = 47; Table 1). At baseline, lipids and insulin resistance index by HOMA-IR were comparable between the two clusters, although the high-risk group was a little heavier and had higher mean lipid levels. After 32 weeks of HAART, the normal cluster (N = 142) continued to exhibit normal mean total cholesterol (180 ± 29 mg/dl), LDL (109 ± 28 mg/dl), triglycerides (142 ± 77 mg/dl), glucose (90 ± 9 mg/dL) and insulin resistance by HOMA-IR (1.5 ± 0.9). Relative to this subgroup, the high-risk cluster (N = 47) experienced significant increases in mean total cholesterol (261 ± 45 mg/dl, $P < 0.001$), LDL (178 ± 30 mg/dl, $P < 0.001$), triglycerides (310 ± 236 mg/dl, $P < 0.001$), glucose (102 ± 23 mg/dL, $P < 0.001$) and insulin resistance by HOMA-IR (4.3 ± 5.6 , $P < 0.001$). These mean lipid levels and HOMA-IR are considered clinically relevant hyperlipidemia and insulin resistance, respectively [20]. Both clusters experienced only modest and statistically insignificant increases in mean HDL levels after exposure to HAART.

Body composition measurements as determined by dual energy X-ray absorptiometry (DEXA) were available for a subset (N = 76) of the patients evaluated above. DEXA measurements were made at 16 week intervals up to 64 weeks because these time-points were consistent with patient visits planned in the trial. Moreover, at the time of this clinical trial prospective data about DEXA measurements and knowledge of how these measurements change over time were unavailable. Since then however, another study [21] employing only 40 subjects found significant changes in both lean and fat masses as measured by DEXA at 12 week intervals, thereby providing reassurance that the changes in DEXA measurements we note below are clinically relevant and unlikely to be simply fluctuations in DEXA values.

Striking differences in body composition over 64 weeks of HAART (Fig. 1 A-D) were observed between the normal (N = 54) and high-risk (N = 22) clusters described above. The high-risk group had higher mean total and trunk fat at baseline (Fig. 1 A, B), and both groups gained a little over 1 kg of fat up to 32 weeks. Beyond 32 weeks however, the normal cluster stabilized, whereas the high-risk cluster experienced reductions of ~5 kg and ~2.5 kg in mean total (P for interaction with cluster 0.01) and trunk fat (P for interaction with cluster 0.1), respectively. Changes in limb fat over time were even more striking (Fig. 1C). Both clusters had similar mean limb fat levels at baseline, and gained approximately 0.5 kg up to 32 weeks. The high-risk group then experienced a precipitous decline in mean limb fat or lipoatrophy of approximately 2.5 kg, but the normal cluster lost only approximately 0.5 kg of mean limb fat more gradually and returned to baseline by 64 weeks (P for interaction with cluster 0.001). The high-risk cluster had greater mean lean mass at baseline than the normal cluster. This mean lean mass increased by ~4 kg up to 64 weeks of

HAART. In contrast, the normal cluster experienced little gain (0.5 kg) in mean lean mass for the duration of the study (P for interaction with cluster 0.009). This study does not address the mechanistic basis of this interesting difference in lean mass, and future studies will be needed to address this issue.

Taken together, these results demonstrate that the clustering approach successfully classified patients into subgroups that displayed markedly different and clinically relevant metabolic and body composition responses to HAART.

Genetic association with cluster membership

We evaluated age, sex, race, HIV disease severity (baseline HIV copy number and CD4 cell-count) and individual drug treatment arm as potential predictors of high-risk cluster membership. None was significantly predictive (Table 2). Hepatitis C co-infection status or plasma markers of inflammation were not available for most patients, so were not evaluated as potential predictors.

We next examined if nucleotide variation in candidate genes was associated with cluster membership. We genotyped these 189 individuals for 299 SNPs in 135 candidate genes. We selected genes based on their likely involvement in regulating lipid and glucose metabolism, cytokines, drug metabolizing enzymes and transcription factors that regulate expression of these genes. We also selected genes whose expression in cell-culture was significantly perturbed after exposure to protease inhibitors [22]. To reduce genotyping effort and because the HapMap project [23] was not complete when this work was initiated, we concentrated our attention on SNPs that are more likely to affect the activity (e.g. coding sequence changes) or expression (promoter or near splice-sites) of the gene [24].

A SNP (dbSNP ID rs3219177) thirty-nine base-pairs downstream of the second exon of the resistin gene was highly significantly associated with cluster membership (Table 3). The frequency of this SNP was 0.16 in the normal cluster and 0.33 in the high-risk group ($P = 0.0003$; $P = 0.04$ after adjusting for 135 genes that were tested). Heterozygotes and homozygotes were 3 (95% C.I. 1.3–5.3) and 19 (95% C.I. 2–183) times more likely than wild-type to be classified in the high-risk cluster. The association was consistently observed in whites ($P = 0.002$) and blacks ($P = 0.049$), but because of the small number of hispanics ($N = 27$), the association was not significant in this race (Table 4); nonetheless heterozygotes were at consistently increased risk in whites (odds ratio, OR = 2.3), blacks (OR = 3.3) and hispanics (OR = 2.3) and there was no evidence of heterogeneity among the three races ($P = 0.9$). As noted below in the Discussion, resistin is a strong candidate for the metabolic changes observed in the high-risk cluster. For this reason, we decided to further investigate genetic variation at this locus.

To examine if other SNPs in the resistin gene were also associated with cluster membership we determined the sequence of the entire resistin gene in all 47 individuals classified as high-risk and, for comparison, up to 32 individuals from a human diversity panel. In addition, from dbSNP we selected SNPs that are located in phylogenetically conserved regions in the vicinity of the resistin gene. We genotyped the entire cohort for eight SNPs in resistin, including four newly identified by sequencing and two from phylogenetically

conserved regions. Although three other SNPs were also associated with cluster membership ($P < 0.05$), none was as significantly associated as the SNP in intron 2 described above (Table 3). Furthermore, haplotype analysis revealed that only haplotypes bearing the intron 2 SNP, rs3219177, were significantly associated with cluster membership (data not shown). Taken together these results indicate that this SNP is potentially causative.

Discussion

By grouping patients with similar metabolic profiles after exposure to HAART using a powerful clustering approach, we identified a sub-group of patients that was especially vulnerable to the metabolic side-effects caused by HAART. This sub-group of patients had slightly elevated lipids at baseline, which were made worse after exposure to HAART, suggesting that HAART exacerbated underlying pre-disposition to dyslipidemia and insulin resistance.

As has been noted by others [25], “The idea that complex data can be grouped into clusters or categories is central to our understanding of the world, and this structure arises in many diverse contexts.” Similar clustering approaches have been employed widely in the analysis of whole genome expression arrays [9,10] to identify patterns in data that would not be apparent in univariate analysis. We believe such an approach is particularly suited to complex datasets with limited number of patients, such as the current study, to increase statistical power.

By performing a comprehensive candidate gene analysis we implicated resistin in the susceptibility to HIV lipodystrophy. Resistin was cloned as an adipocyte hormone that links obesity and insulin resistance [11]. Increased resistin resulted in mice with abnormal glucose tolerance [11]. Conversely, resistin knockout mice had lower fasting blood glucose [26]. SNPs in resistin have also been variably associated with metabolic traits captured by the clusters described above including body mass index, fat mass, insulin resistance and diabetes [27–31]. Engert et al. [27] examined SNPs in the 5' flanking region of the resistin gene and found significant association between SNP rs1862513 and increased body mass index in non-diabetic obese subjects. Although this particular SNP was not significantly associated with cluster membership in this study, two flanking SNPs, rs321975 and rs3760678, which are 62 and 673 basepairs respectively from rs1862513 were significantly associated with high-risk cluster (Table 3). Wang et al. [28] demonstrated significant interaction between rs3219177 and BMI and insulin sensitivity, with the common CC genotype demonstrating greater mean insulin sensitivity. The association between this SNP and clusters which show significant differences in mean insulin resistance is consistent with this observation (Tables 1, 3). Sentinelli [29] did not find significant association between one SNP that they tested and diabetes status in a small case-control study. We did not detect this SNP in our population. Coneely et al. [30] found significant association between SNP rs3219177 and increased weight in elderly non-diabetic subjects, an observation that is completely consistent with the highly significant association we found between the same SNP and high-risk cluster, which also had increased mean BMI (Table 1). Osawa et al. [31] found significant association between a SNP in the promoter, rs1862513, and diabetes status. As noted above, we did not find significant association with this particular SNP but

two SNPs in proximity to this one were significantly associated with cluster membership in this study. More recently, the same group also found significant association between this SNP and plasma resistin levels, which in turn, were correlated with C-reactive protein in a Japanese population [32]. Taken together with the highly significant genetic association that we found, these genetic studies in non-HIV subjects support a strong link between genetic variation in resistin and the metabolic side-effects observed in the high-risk cluster. Because this SNP is located in non-coding sequence we hypothesize that it could influence expression of resistin, perhaps by affecting its splicing or transcription. Sequence comparison revealed that this SNP is located in a consensus binding-site for the T-cell transcription factor, TCF7L2, which has been previously implicated in susceptibility to Type II diabetes [33].

Additional work will be needed to understand how genetic variation in resistin might predispose individuals to HIV lipodystrophy. One possibility is that HIV+ patients on HAART who are in a chronic inflammatory state [34], might have increased resistin, as resistin gene expression can be induced by the pro-inflammatory molecules lipopolysaccharide and tumor necrosis factor alpha [35,36]. Individuals carrying the associated SNP in resistin might be particularly vulnerable to such changes in resistin gene expression. Given the link between inflammation and resistin levels and genotypes, it will be important in future studies of patients treated with HAART to determine circulating levels of inflammatory molecules including TNF- α , its soluble receptor and C-reactive protein in addition to resistin.

Our work suggests a potential basis for managing lipodystrophy in high-risk individuals. Since increased resistin is associated with obesity and insulin resistance, agents that reduce resistin including the marketed peroxisome proliferator-activated receptor agonists pioglitazone and rosiglitazone could potentially have a beneficial impact on HAART-induced lipodystrophy. In fact, consistent with this idea, rosiglitazone can reduce elevated circulating resistin levels in subjects with HIV lipodystrophy [37]. Another study did not find correlation between circulating serum resistin and lipodystrophy [38]; possibly because the different diagnostic criteria used to define lipodystrophy in that study led to subjects with normal lipid levels being classified as lipodystrophic. It is also possible that the paracrine effects of resistin are crucial to HIV lipodystrophy, and circulating resistin levels as measured in plasma in these studies do not accurately capture resistin levels at the site of its action.

In conclusion, we have used a clustering approach to identify a sub-group of patients with increased susceptibility to the metabolic side-effects cause by HAART. Genetic analysis revealed highly significant association between SNPs in the resistin gene and this subgroup of patients. Additional studies with larger cohorts exposed to other HAART regimens will be needed to determine the general applicability of these findings.

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Figure 1A:

Figure 1B:

Figure 1C:

Figure 1D:

Figure 1.

Normal (N = 54) and high-risk (N = 22) clusters show markedly different body composition responses to HAART. Changes in body composition over time and interaction with clusters were analyzed using repeated measures ANOVA. Solid and dashed lines indicate values for

the normal and high-risk clusters respectively. Error bars represent standard error of the mean. Although natural log transformed total, trunk and limb fat values were used in the repeated measures ANOVA to determine significance, for clarity untransformed values are provided in the graph (A) Total fat (P value for interaction with clusters = 0.01) (B) Trunk fat (P = 0.11) (C) Limb fat (P = 0.001) (D) Lean mass (P = 0.009).

Figure 1A: Change in total fat by cluster

Figure 1B: Change in trunk fat by cluster

Figure 1C: Change in limb fat by cluster

Figure 1D: Change in lean mass by cluster

Table 1

Metabolic traits at baseline and after 32 weeks of HAART by cluster

Trait	Mean $\pm$ SD		P value*
	Normal cluster N = 142	High-risk cluster N = 47	
Total cholesterol, mg/dL	159 $\pm$ 31	182 $\pm$ 33	<0.001
	180 $\pm$ 29	261 $\pm$ 45	<0.001
LDL cholesterol, mg/dL	98 $\pm$ 28	120 $\pm$ 30	<0.001
	109 $\pm$ 28	178 $\pm$ 30	<0.001
Non-HDL cholesterol, mg/dL	125 $\pm$ 30	148 $\pm$ 30	<0.001
	138 $\pm$ 28	221 $\pm$ 44	<0.001
HDL cholesterol, mg/dL	34 $\pm$ 10	34 $\pm$ 9	0.98
	42 $\pm$ 14	40 $\pm$ 13	0.34
Triglycerides, mg/dL	145 $\pm$ 121	176 $\pm$ 128	0.04
	142 $\pm$ 77	310 $\pm$ 236	<0.001
HOMA-IR	2.1 $\pm$ 2.7	2.0 $\pm$ 1.5	0.52
	1.5 $\pm$ 0.9	4.3 $\pm$ 5.6	<0.001
Glucose, mg/dL	85 $\pm$ 11	89 $\pm$ 32	0.94
	90 $\pm$ 9	102 $\pm$ 23	<0.001
Body mass index, kg/m ²	24.4 $\pm$ 4.9	26.3 $\pm$ 5.1	0.01
	25.3 $\pm$ 5.5	27.3 $\pm$ 5.5	0.01

For each trait the baseline and week 32 values are indicated in the first and second row, respectively. Univariate P value from Kruskal-Wallis test

Table 2

No association between demographics, HIV disease severity, drug treatment arm and cluster membership

Trait	Mean $\pm$ SD (Percent)		P value
	Normal cluster N = 142	High-risk cluster N = 47	
Age, years	38 $\pm$ 9	38 $\pm$ 10	0.9
Sex, % men	87	92	0.6
Race, N (%)			0.4
White	81 (57)	24 (51)	
Black	37 (26)	16 (34)	
Hispanic	20 (14)	7 (15)	
Asian/PI	4 (3)	0	
CD4 count, cells/mm ³	257 $\pm$ 206	289 $\pm$ 208	0.4
HIV copy number, log copies/mL	5.1 $\pm$ 0.8	5.0 $\pm$ 0.8	0.6
Intravenous drug use, never N (%)	129 (91)	44 (94)	0.5
Lipid-lowering treatment, N (%)	1 (0.7)	0	0.5
Diabetes, N (%)	1 (0.7)	0	0.5
Hypertension, N (%)	8 (6)	3 (6)	0.9
Anti-retroviral treatment arm, N (%)			0.3
Efavirenz + Didanosine + Stavudine	30 (21)	7 (15)	
Efavirenz + Zidovudine + Lamivudine	24 (17)	4 (9)	
Nelfinavir + Didanosine + Stavudine	26 (18)	6 (13)	
Nelfinavir + Zidovudine + Lamivudine	17 (12)	9 (19)	
Nelfinavir + Efavirenz + Didanosine + Stavudine	26 (18)	14 (30)	
Nelfinavir + Efavirenz + Zidovudine + Lamivudine	19 (13)	7 (15)	
Didanosine + Stavudine vs. Zidovudine + Lamivudine			1
Didanosine + Stavudine	82 (58)	27 (57)	
Zidovudine + Lamivudine	60 (42)	20 (43)	
Efavirenz vs. Nelfinavir vs. Efavirenz + Nelfinavir			0.1
Efavirenz	54 (38)	11 (23)	
Nelfinavir	43 (30)	15 (32)	
Efavirenz + Nelfinavir	45 (32)	21 (45)	

Table 3

Association between Resistin SNPs and Cluster Membership

Resistin SNP genotypes	SNP Location ^a	Normal Cluster	High-risk Cluster	Genotype frequency difference P^b	Allele frequency difference P^b
rs3760678	7639120			0.03	0.01
A/A		114 (81)	29 (64)		
A/G		23 (16)	13 (29)		
G/G		3 (2)	3 (7)		
rs1862513	7639793			0.5	0.6
C/C		59 (42)	16 (34)		
C/G		63 (45)	26 (55)		
G/G		17 (12)	5 (11)		
rs3219175	7639855			0.09	0.03
G/G		122 (87)	46 (98)		
G/A		15 (11)	1 (2)		
A/A		3 (2)	0 (0)		
5'UTR	7640204			0.06	0.01
G/G		139 (100)	45 (96)		
G/A		0 (0)	2 (4)		
rs3219177	7640369			0.001	0.0003
C/C		97 (69)	20 (43)		
C/T		42 (30)	23 (49)		
T/T		1 (1)	4 (9)		
INT1_NEW	7640573			0.3	0.08
A/A		141 (100)	45 (98)		
A/G		0 (0.8)	1 (2)		
IVS3+30C/T	7640814			0.4	0.3
C/C		119 (87)	36 (80)		
C/T		17 (12)	9 (20)		
T/T		1 (1)	0 (0)		
rs3219178	7640951			0.7	0.6
C/C		63 (47)	25 (53)		

Resistin SNP genotypes	SNP Location ^a	Number, (%)		Genotype frequency difference P^b	Allele frequency difference P^b
		Normal Cluster	High-risk Cluster		
C/G		61 (45)	18 (38)		
G/G		11 (8)	4 (9)		

^a Number indicates genomic nucleotide location of SNP based on reference sequence NT077812.

^b P values were determined from Fisher's exact tests.

Table 4

Association between resistin SNP rs3219177 and cluster membership by ethnicity

Ethnicity	Number, (%)		P value ^a
	Normal Cluster	High-risk Cluster	
Caucasians			0.002
C/C	55 (69)	10 (42)	
C/T	24 (30)	10 (42)	
T/T	1 (1)	4 (16)	
African-American			0.049
C/C	24 (67)	6 (38)	
C/T	12 (33)	10 (62)	
T/T	0 (0)	0 (0)	
Hispanics			0.332
C/C	15 (75)	4 (57)	
C/T	5 (25)	3 (43)	
T/T	0 (0)	0 (0)	

^a P value from Fisher's exact test