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Genetic Risk Factors in Recurrent Venous Thromboembolism: A Multilocus, Population-Based, Prospective Approach

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Abstract

Background—Recurrent venous thromboembolism (VTE) is a common, complex disorder; however, genetic factors have been suggested to play a role in the disease development. We therefore conducted a multi-locus genetic study examining the potential associations of candidate gene variants in inflammation, thrombosis, coagulation, and lipid metabolism pathways, individually or interactively, with risk of recurrent VTE.

Methods—Using DNA samples collected at baseline in the Prevention of Recurrent Venous Thromboembolism trial (PREVENT), we genotyped 86 candidate genes polymorphisms among 43 individuals who subsequently developed recurrent VTE and among 396 individuals who remained free of recurrent event over a mean follow-up period of 2.1 years to prospectively determine whether these gene polymorphisms contribute to the risk of recurrent VTE.

Results—Using a single-marker 'uncorrected' analysis, CCR5 *A(-2459)G* [rs1799864], MMP3 *5A (-1171)6A* [rs3025058] and PON1 *gln192arg* [rs662] gene variants were associated with increased risk, and CETP *C(-629)A* [rs1800775] gene variant with reduced risk of recurrent VTE, respectively. Furthermore, potentially important gene-gene-interactions were detected by the Monte Carlo Markov chain Logic Regression method.

Conclusions—Although the present findings are hypothesis-generating and require confirmation in an independent investigation, our study provides a practical example of detecting epistasis in common, complex diseases.

Keywords

Recurrent VTE; candidate genes; epistasis

Introduction

Although our understanding of the pathophysiology in venous thromboembolism (VTE) and its recurrence has substantially increased, VTE often occurs in patients without conventional

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risk factors, including surgery, trauma, obesity, cancer. Furthermore, a recent meta-analysis questioned the cost-effectiveness of routine testing for established inherited thrombophilic gene variants in patients with a first episode of VTE (1). Moreover, identification of additional (genetic) risk factor(s) has been advocated for the understanding of the underlying pathogenesis (1). Candidate genes associated with lipid metabolism, thrombosis and haemostasis, cell-matrix adhesion, and inflammation have been implicated in the pathogenesis of recurrent VTE (2–7). However, to date, only studies focusing on single-gene variant(s) have been reported. We thus undertook an evaluation of 86 genetic polymorphisms in 56 candidate genes related to these biological pathways in participants from the Prevention of Recurrent Venous Thromboembolism (PREVENT) clinical trial (8) to (i) examine the possible associations of these gene variants with risk of recurrent VTE, and (ii) determine potentially important gene-gene interaction(s) -epistasis- that may be associated with the disease outcome for further investigation.

The candidate genes examined were selected from biochemical pathways that have been implicated in the pathogenesis and/or pathophysiology of thromboembolism. In addition to the biological relevance of the selected candidate genes, the polymorphisms were further selected based on prior evidence of potential functionality, validated allele frequency and heterozygosity, sequence-proven allelic variation, and confirmed mendelian segregation. The selected genetic polymorphisms focused broadly on the genes involved in lipid metabolism, inflammation, cell adhesion, thrombosis and hemostasis, and platelet function.

Materials and Methods

Study Population

We evaluated potential associations between a panel of 86 candidate gene polymorphisms (Supplemental Table 1) and the risk of recurrent VTE by studying prospectively collected DNA samples from the PREVENT trial, a randomized, double-blinded, placebo-controlled trial testing the hypothesis that long-term low intensity warfarin therapy (target INR, 1.5–2.0) might be safe and effective in reducing risk of recurrent VTE in patients with one or more previous idiopathic VTE. The study protocol has previously been described (8). In brief, 508 patients with prior idiopathic VTE were randomized. Confirmation of the endpoint of recurrent VTE included a positive imaging study such as duplex ultrasonography, computed tomography or ventilation perfusion scanning. The trial was terminated in December 2002, due to a large clinical benefit of low-intensity warfarin. The median duration of follow-up at the time of termination of the trial was 2.1 y with a range of 12 days to 4.3 y. A baseline blood sample was collected from each of the participants and subsequently used for genetic analysis of the Factor V Leiden and the prothrombin mutation (8). The present investigation consists of 439 white participants; 43 participants who developed a recurrent VTE (42 idiopathic; 1 due to cancer) and 396 control participants who remained free of recurrent events during follow-up. The overall genotyping completion rate per polymorphism was greater than 95%. The study was approved by the Brigham and Women’s Hospital Institutional Review Board for Human Subjects Research (Boston, MA).

Genotype Determination

Genotyping was performed using previously described and validated linear-array assays for candidate markers of cardiovascular disease, immune response and inflammation (Roche Molecular Systems, Alameda, CA) (9–12). In brief, each DNA sample was amplified in multiplex polymerase chain reactions (PCRs) using biotinylated primers. Each PCR product pool was then hybridized to the corresponding panel of sequence-specific oligonucleotide probes that had been immobilized in a linear array on nylon membrane strips. The colorimetric detection method was based upon the use of streptavidin-horseradish peroxidase conjugate

with hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine as substrates. Genotype assignment was performed using in-house StripScan image processing software (Roche Molecular Systems, Inc., CA). To confirm genotype assignment, scoring was carried out by two independent observers. Overall discordant results (<1% of all scoring) were resolved by a joint reading, and where necessary, a repeat genotyping. In addition, genotypes of factor V Leiden, and the prothrombin 20210G>A mutation determined by the current method were compared to those previously reported using a different genotyping method (8), and we obtained 100% concordance rate.

Statistical approach, and adjustment for multiple comparisons

Marker-by-marker analysis—We examined the association between each of the evaluated polymorphisms and risk of recurrent VTE in a multi-stage procedure. First, Hardy-Weinberg equilibrium was evaluated for each polymorphism using a one-degree of freedom goodness-of-fit test amongst all participants after excluding rare alleles [minor allele frequency (MAF) <5%]. For the present investigation, the prothrombin 20210G>A mutation was excluded from the analysis (owing to its rarity, MAF <5%, which might potentially lead to spurious findings because of loss of power). The association of each of the 86 polymorphisms with risk of recurrent VTE was evaluated using the Cox-proportional hazard regression analysis, assuming an additive or dominant mode of inheritance; the recessive mode was not performed due to potential loss of power. All regression analyses were adjusted for age, gender, and randomized treatment assignment. Hazard ratios (HR) and the corresponding 95% confidence interval (CI) were calculated. A 2-tailed $p<0.05$ was considered a statistically significant result. Because the present study was a hypothesis-generating investigation, no correction for multiple testing was attempted.

Epistasis/Gene-Gene interactions—Potential 2-way, and 2-way gene-gene interactions (epistasis) were examined using the Markov chain Monte Carlo (MCMC) Logic Regression (LR) as described previously (13). In brief, the goal of MCMCLR is to identify all models and combinations of covariables (gene variants) that are potentially associated with the disease outcome and that warrant further investigation, rather than to construct a single model to predict the disease outcome. The method uses Bayesian model selection techniques, with MCMC to explore good-fitting models. Unlike most Bayesian model selection procedures, where the models that were visited in an MCMC run are averaged to construct predictors that are better than individual covariables, summary measures are constructed describing features of all models that were visited. The implementation uses the reversible jump MCMC algorithm of Green (14). The Monte Carlo logic regression software used is freely available from http://bear.fhcrc.org/~ingor/logic.

The MCMC-LR analysis conducted in the present study was performed according to the parameters recommended by the authors (K=3 logic trees, $a=1/\sqrt{2}$, three MCMC chains of 5,000,000 models after a burn-in of 10,000 iterations) (13). The sample population used for this method was 386 subjects (37 cases and 349 controls) who had complete genotypic information and with similar baseline characteristics to those presented in Table 1.

Of note, the prothrombin mutation was excluded from the present investigation due to its rarity (MAF=0.037) in the current sample population, which did not meet the MAF inclusion criterion (0.05) as previously stated.

Results

Baseline characteristics of cohort participants are shown in Table 1. The observed MAF for each of the 86 polymorphisms genotyped are shown in Table 2; all alleles tested demonstrated Hardy-Weinberg equilibrium after Bonferroni correction.

Single-variant approach—As shown in Table 2, in an adjusted Cox regression analysis, assuming an additive model, *PON1* rs662 and *CETP* rs1800775 gene variants were associated with increased (HR=1.79, p=0.023) and reduced (HR=0.63, p=0.041) risk of recurrent VTE, respectively. Furthermore, *MMP3* rs3025058 and *CCR2* rs1799864 gene variants were both found to be associated with increased risk of recurrent VTE, in either an additive (HR=1.66, p=0.015; HR=2.00, p=0.014, respectively) or dominant (HR=6.87, p=0.001; HR=2.50, p=0.008, respectively) model (Table 2).

MCMCLR Gene-Gene interactions—Table 3 shows the top five 2-way, and 3-way interactions, with the top interactions being *MMP3* rs3025058d-*PON1* rs854560r, and *APOA4* rs5110r-*MMP3* rs3025058d-*PON1* rs854560r, respectively. As suggested by the original authors (15) in the interpretation of 2-way interactions, we compared the observed frequency by an estimate of the expected frequency that the 2 variants would occur together if they were selected independently. The magnitude of the ratio suggests the extent to which an interaction between two variants is present. We note from Table 3, the two-variant pair with the highest ratio was *ITGA2 rs1062535 -CCR2 rs1799864*, followed by *ADRB3 rs4994 and TNF rs361525* pair. As also stated previously (15), no expected frequency exists for a 3-way interaction, as there is no simple 'trivariable independence' model based on univariable and bivariable frequencies, other than complete independence, which is no longer appropriate if the covariables are not pairwise.

RYLZ, PMR and RJG conceived the study project. RYLZ conducted the experiments. RYLZ, VB, and SS analyzed the data. All authors interpreted the findings. RYLZ prepared the manuscript. All authors read and approved the manuscript as written. The authors had full access to the data and take full responsibility for its integrity.

Discussion

In this prospective, population-based study, we found an association of gene variant(s) in *CCR2, CETP, MMP3*, and *PON1* with recurrent VTE. In concordance with previous reports, we found little evidence for an association of factor V Leiden, *ACE*, *MTHFR*, nor *SERPINE1* (*PAI1*) gene variation with recurrent VTE (3,5,6,16,17). Whether these differences reflect the play of chance or suggest true differences between populations will require confirmation in future analysis of independent populations. As previously mentioned, prothrombin mutation was excluded from the present analysis due to its rarity. Thus its potential involvement in recurrent VTE risk could not be evaluated in the present context.

In the MCMC logic regression analysis, we found evidence of epistasis: the effect that one gene (locus/variant) may not be detected if the effect of another gene(locus/variant) is not considered. These high-order gene-gene interactions could not be readily detected by traditional statistical methods. Furthermore, the gene-gene interactions detected in the present study demonstrates a trans-chromosomal effect between genes within the same or different candidate biological pathways, as previously suggested by others (15,18).

The strength of our study design is the use of a closed prospective cohort in which the determination of case status was based solely on the subsequent development of disease rather than on any arbitrary selection criteria designed by the investigators. As stated previously, despite the intriguing nature of the present findings, we believe appropriate clinical caution should be used when interpreting results from any association study; epidemiological limitations of association studies potentially leading to false-positive findings include inadequate sample size, failure to ensure that affected and unaffected subjects derive from the same source population, over-reliance on *post-hoc* subgroup analyses, and selective presentation of results without consideration of the chance effects which can arise due to

multiple comparisons. Further, on an *a priori* basis, we present all our data simultaneously and uncorrected for multiple comparisons rather than focusing on any one specific finding. Had we applied correction for multiple testing, none of the observed associations would remain significant. Of a relevant note, the false discovery rate (FDR) (19) is widely used in exploratory genetic-epidemiological studies to correct for multiple hypothesis-testing. The FDR is applied to the adjusted models examining the additive effect of each gene variant. Unlike other common procedures such as the Bonferroni correction, the FDR method does not control the experiment-wise error rate, but instead controls the expected proportion of false positives among all positive results over multiple testing. Furthermore, it remains a challenge for the scientific community to develop and optimize approaches for correction for multiple testing in studies, which examine (equally important) gene-environment/gene-gene interactions.

We recognize that it is also possible that one or more of the observed associations is the result of linkage disequilibrium with a yet-to-be-identified nearby susceptibility locus(i) or gene(s). As such, confirmation of our findings in different populations is encouraged. Furthermore, candidate genes (not examined in the present investigation) such as glycoprotein receptors, endothelial cell receptors, tissue factors, and other coagulation-related genes warrant continuous investigations. In addition, no information on immediate precipitating factors such as medical intervention(s), which might have partially annulled the effects of the gene variants examined in the present investigation was available, and thus this issue could not be evaluated in the current context. Unfortunately, to date, no large genome-wide association investigations have been conducted in relation to (recurrent) VTE, thus, highlighting the need for large-scale, prospective studies in this important clinical condition. Based on our current sample size, and the effect estimates observed, we cannot rule out that a modest risk of recurrent VTE was associated with the polymorphism(s) tested in this study population. Thus, polymorphisms that are potentially false negatives may also be worthy of further investigation.

In conclusion, in this prospective, population-based study, several candidate gene polymorphisms were identified which were independently associated with risk of recurrent VTE. More importantly, the present findings should be viewed as hypothesis-generating/exploratory, and require validation in other prospective studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of white cohort participants.

Variables	All participants (N=439)
Age, y	53.0 [46.0, 65.0]
Male, %	53.5
Current smokers, %	12.1
Bodymass index, kg/m2	29.8 [26.5, 34.1]
Prior malignancy, %	9.8
≥2 pre-enrollment VTE, %	39.0

Median [interquartile range] for continuous variables.

VTE, venous thromboembolism.

Table 2

Estimated effects for polymorphisms selected in risk factor-adjusted analyses.

Gene symbol and dbSNP rs number		MAF	Cox regression (HR; 95%CI; *p*)	
			additive	**dominant**
ACE	*rs1799752*	0.445	1.35; 0.88–2.05; 0.17	1.83, 0.88–3.86, 0.11
ADD1	*rs4961*	0.208	0.73; 0.40–1.32; 0.29	0.70, 0.36–1.36, 0.30
ADRB2	*rs1042713*	0.382	0.89; 0.60–1.40; 0.63	1.09, 0.58–2.07, 0.78
ADRB2	*rs1042714*	0.423	1.37; 0.90–2.09; 0.14	1.69, 0.80–3.56, 0.17
ADRB3	*rs4994*	0.066	0.31; 0.15–1.36; 0.13	0.34, 0.08–1.40, 0.13
AGTR1	*rs5186*	0.255	0.97; 0.60–1.59; 0.91	0.97, 0.52–1.82, 0.93
AGT	*rs699*	0.438	1.28; 0.81–2.00; 0.29	1.25, 0.81–2.57, 0.54
APOA4	*rs675*	0.184	0.97; 0.55–1.72; 0.91	0.90, 0.46–1.78, 0.76
APOA4	*rs5110*	0.068	1.24; 0.54–2.87; 0.62	1.31, 0.55–3.14, 0.54
APOB	*rs1367117*	0.314	0.85; 0.52–1.40; 0.53	0.77, 0.41–1.44, 0.41
APOC3	*rs2542052*	0.382	1.03; 0.67–1.59; 0.89	1.01, 0.53–1.92, 0.97
APOC3	*rs2854117*	0.251	0.91; 0.55–1.51; 0.72	0.96, 0.51–1.80, 0.89
APOC3	*rs2854116*	0.368	1.10; 0.71–1.70; 0.68	1.10, 0.58–2.09, 0.78
APOC3	*rs4520*	0.266	1.11; 0.70–1.78; 0.66	0.93, 0.49–1.76, 0.83
APOC3	*rs5128*	0.098	0.76; 0.33–1.74; 0.51	0.63, 0.25–1.62, 0.34
APOC3	*rs4225*	0.368	1.05; 0.67–1.65; 0.83	0.88, 0.46–1.66, 0.68
APOE	*rs429358*	0.135	1.05; 0.57–1.93; 0.87	1.08, 0.54–2.17, 0.83
APOE	*rs7412*	0.088	1.06; 0.47–2.40; 0.89	1.06, 0.47–2.40, 0.89
C3	*rs2230199*	0.256	0.82; 0.49–1.36; 0.44	0.92, 0.49–1.72, 0.79
C5	*rs17611*	0.435	1.03; 0.64–1.66; 0.90	1.24, 0.60–2.55, 0.56
CCL11	*rs4795895*	0.183	1.38; 0.80–2.38; 0.24	1.66, 0.89–3.10, 0.11
CCL11	*rs3744508*	0.183	0.55; 0.28–1.09; 0.09	0.49, 0.22–1.06, 0.07
CCR2	*rs1799864*	0.088	**2.00; 1.15–3.48; 0.014**	**2.50, 1.26–4.94, 0.008**
CCR5	*rs333*	0.089	0.97; 0.43–2.21; 0.95	1.04, 0.43–2.48, 0.93
CCR5	*rs1799987*	0.457	0.93; 0.61–1.42; 0.75	0.91, 0.47–1.76, 0.77
CD14	*rs2569190*	0.448	0.93; 0.60–1.44; 0.75	0.79, 0.42–1.50, 0.47
CETP	*rs1800775*	0.496	**0.63; 0.40–0.98; 0.041**	0.54, 0.28–1.04, 0.07
CETP	*rs5882*	0.306	1.30; 0.82–2.06; 0.27	1.02, 0.54–1.91, 0.96
CETP	*rs708272*	0.453	1.48; 0.96–2.29; 0.07	1.68, 0.80–3.55, 0.17
CSF2	*rs25882*	0.201	0.75; 0.41–1.38; 0.36	0.74, 0.37–1.48, 0.39
CTLA4	*rs5742909*	0.125	0.91; 0.45–1.86; 0.80	0.95, 0.45–1.99, 0.88
CTLA4	*rs231775*	0.368	0.82; 0.50–1.33; 0.42	0.70, 0.37–1.30, 0.26
F5	*rs6025*	0.137	1.13; 0.60–2.11; 0.71	1.07, 0.55–2.10, 0.84
F7	*rs5742910*	0.121	1.63; 0.93–2.88; 0.09	1.73, 0.91–3.31, 0.10
F7	*rs6046*	0.108	1.79; 0.99–3.23; 0.06	1.82, 0.94–3.51, 0.08
GC	*rs7041*	0.447	1.56; 0.98–2.48; 0.06	1.35, 0.64–2.84, 0.43
GC	*rs4588*	0.297	1.38; 0.87–2.21; 0.17	1.10, 0.58–2.08, 0.76
GNB3	*rs5443*	0.318	0.70; 0.42–1.18; 0.18	0.54, 0.29–1.02, 0.06
ICAM1	*rs1799969*	0.112	1.21; 0.62–2.38; 0.58	0.98, 0.45–2.14, 0.96

Gene symbol and dbSNP rs number		MAF	Cox regression (HR; 95%CI; *p*)	
			additive	dominant
LDLR	*rs5742911*	0.317	0.92; 0.58–1.46; 0.72	0.97, 0.51–1.84, 0.93
IL1A	*rs1800587*	0.340	0.99; 0.60–1.63; 0.95	1.04, 0.54–1.98, 0.90
IL1B	*rs16944*	0.350	0.92; 0.55–1.52; 0.74	1.05, 0.55–2.02, 0.87
IL1B	*rs1143634*	0.229	1.06; 0.65–1.72; 0.83	1.10, 0.59–2.07, 0.76
IL4	*rs2243250*	0.165	0.76; 0.41–1.44; 0.40	0.73, 0.35–1.49, 0.38
IL4R	*rs1801275*	0.201	0.93; 0.54–1.58; 0.78	0.82, 0.42–1.59, 0.56
IL4R	*rs1805015*	0.166	1.04; 0.59–1.86; 0.88	0.86, 0.43–1.72, 0.66
IL5RA	*rs2290608*	0.303	1.01; 0.61–1.70; 0.96	1.20, 0.63–2.25, 0.58
IL6	*rs1800796*	0.074	1.72; 0.87–3.40; 0.12	1.78, 0.88–3.59, 0.11
IL6	*rs1800795*	0.392	0.86; 0.54–1.37; 0.53	0.76, 0.40–1.44, 0.40
IL9	*rs2069885*	0.175	1.16; 0.66–2.02; 0.61	1.00, 0.50–1.96, 0.99
IL13	*rs1295686*	0.205	1.17; 0.70–1.96; 0.55	1.24, 0.66–2.35, 0.50
ITGA2	*rs1062535*	0.399	0.74; 0.47–1.16; 0.19	0.63, 0.34–1.17, 0.14
ITGB3	*rs5918*	0.140	0.92; 0.48–1.75; 0.80	0.87, 0.43–1.78, 0.71
LIPC	*rs1800588*	0.223	1.13; 0.70–1.84; 0.61	1.22, 0.65–2.30, 0.54
LPA	*rs1853021*	0.182	0.97; 0.56–1.70; 0.92	0.73, 0.35–1.50, 0.39
LPA	*rs1800769*	0.164	0.75; 0.38–1.49; 0.41	0.70, 0.32–1.53, 0.37
LPL	*rs328*	0.111	1.16; 0.59–2.28; 0.67	1.09, 0.52–2.31, 0.82
LTA	*rs1041981*	0.343	1.08; 0.72–1.65; 0.70	1.02, 0.55–1.89, 0.95
LTA	*rs909253*	0.344	1.18; 0.73–1.92; 0.51	1.16, 0.61–2.20, 0.66
LTC4S	*rs730012*	0.271	0.96; 0.57–1.59; 0.87	0.83, 0.45–1.56, 0.57
MMP3	*rs3025058*	0.476	**1.66; 1.10–2.49; 0.015**	**6.87, 2.12–22.30, 0.001**
MTHFR	*rs1801133*	0.319	1.26; 0.80–1.98; 0.31	1.12, 0.60–2.08, 0.72
NOS2A	*rs1137933*	0.234	1.01; 0.59–1.75; 0.97	0.87, 0.46–1.64, 0.66
NOS3	*rs1800779*	0.379	0.85; 0.53–1.35; 0.49	0.79, 0.42–1.48, 0.47
NOS3	*rs3918226*	0.073	0.89; 0.36–2.22; 0.80	0.91, 0.36–2.33, 0.85
NOS3	*rs1799983*	0.308	0.82; 0.50–1.37; 0.45	0.70, 0.37–1.30, 0.25
NPPA	*rs5065*	0.161	1.05; 0.60–1.82; 0.88	1.11, 0.58–2.13, 0.74
PON1	*rs854560*	0.375	0.83; 0.52–1.33; 0.44	1.11, 0.58–2.11, 0.76
PON1	*rs662*	0.274	**1.79; 1.08–2.95; 0.023**	1.78, 0.92–3.43, 0.08
PON2	*rs695435*	0.216	1.56; 0.96–2.54; 0.07	1.62, 0.86–3.04, 0.14
PPARG	*rs1801282*	0.124	1.32; 0.67–2.59; 0.42	1.38, 0.69–2.79, 0.36
SCGB1A1	*rs3741240*	0.318	1.02; 0.64–1.63; 0.93	0.90, 0.48–1.68, 0.74
SCNN1A	*rs2228576*	0.257	0.67; 0.38–1.18; 0.17	0.60, 0.31–1.18, 0.14
SDF1	*rs1801157*	0.213	0.75; 0.40–1.37; 0.35	0.72, 0.36–1.42, 0.34
SELE	*rs5361*	0.124	0.71; 0.33–1.54; 0.39	0.74, 0.32–1.66, 0.46
SELP	*rs6133*	0.118	1.06; 0.55–2.05; 0.86	1.08, 0.49–2.34, 0.85
SELP	*rs6131*	0.212	1.35; 0.83–2.20; 0.22	1.78, 0.95–3.31, 0.07
SERPINE1	*rs1799768*	0.542	0.84; 0.55–1.28; 0.42	0.95, 0.46–1.94, 0.89
SERPINE1	*rs7242*	0.413	0.83; 0.52–1.32; 0.43	0.87, 0.46–1.63, 0.66

Gene symbol and dbSNP rs number		**MAF**	**Cox regression (HR; 95%CI; *p*)**	
			additive	**dominant**
TCF7	*rs244656*	0.142	1.00; 0.51–1.96; 1.00	0.94, 0.45–2.00, 0.88
TCF7	*rs5742913*	0.116	1.59; 0.83–3.03; 0.16	1.64, 0.85–3.19, 0.14
TGFB1	*rs1800469*	0.312	1.08; 0.69–1.70; 0.73	1.36, 0.72–2.56, 0.34
TNF	*rs1800629*	0.177	0.72; 0.38–1.35; 0.30	0.77, 0.38–1.54, 0.46
TNF	*rs361525*	0.075	0.71; 0.26–1.95; 0.50	0.72, 0.26–2.03, 0.53
VDR	*rs2228570*	0.420	1.03; 0.66–1.59; 0.91	1.09, 0.56–2.12, 0.80
VDR	*rs1544410*	0.417	1.23; 0.78–1.94; 0.36	1.25, 0.64–2.47, 0.51

MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval.

Significant results (uncorrected) from Cox regression analysis are in boldface.

Table 3

Monte Carlo Markov chain Logic regression analysis

Two-way interactions K=3 trees, a=1/√2					**Three-way interactions K=3 trees, a=1/√2**			
Variant 1	**Variant 2**	**Observed frequency**	**Expected frequency**	**Ratio**	**Variant 1**	**Variant 2**	**Variant 3**	**Observed frequency**
MMP3 rs3025058d	*PON1* rs854560r	0.00846	0.00350	2.42	*APOA4* rs5110r	*MMP3* rs3025058d	*PON1* rs854560r	0.0082
ITGA2 rs1062535d	*CCR2* rs1799864r	0.00564	0.00002	282.00	*CETP* rs708272r	*MMP3* rs3025058d	*PON1* rs854560r	0.0071
ADRB3 rs4994d	*TNF* rs361525d	0.00545	0.00016	34.06	*IL4R* rs1801275r	*MMP3* rs3025058r	*GC* rs7041r	0.0060
CETP rs708272r	*MMP3* rs3025058d	0.00536	0.00130	4.12	*TCF7* rs5742913r	*GC* rs7041r	*CCR5* rs1799987r	0.0059
MMP3 rs3025058d	*IL1A* rs1800587r	0.00517	0.00151	3.42	*CETP* rs708272r	*APOA4* rs5110r	*MMP3* rs3025058d	0.0051

d, dominant model; r, recessive model; Freq, frequency.