Published in final edited form as:
Genes Immun. 2006 June ; 7(4): 310–315. doi:10.1038/sj.gene.6364299.

Multifactor dimensionality reduction reveals gene–gene interactions associated with multiple sclerosis susceptibility in African Americans

D Brassat[1,6], **AA Motsinger**[2,6], **SJ Caillier**[1], **HA Erlich**[3], **K Walker**[3], **LL Steiner**[3], **BAC Cree**[1], **LF Barcellos**[4], **MA Pericak-Vance**[5], **S Schmidt**[5], **S Gregory**[5], **SL Hauser**[1], **JL Haines**[2], **JR Oksenberg**[1], and **MD Ritchie**[2]

[1]Department of Neurology and Center for Human Genetics, School of Medicine, University of California at San Francisco, San Francisco, CA, USA

[2]Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA

[3]Department of Human Genetics, Roche Molecular Systems, Alameda, CA, USA

[4]Division of Epidemiology, School of Public Health, UC Berkeley, Berkeley, CA, USA

[5]Center for Human Genetics, Duke University Medical Center, Durham, NC, USA

Abstract

Multiple sclerosis (MS) is a common disease of the central nervous system characterized by inflammation, myelin loss, gliosis, varying degrees of axonal pathology, and progressive neurological dysfunction. Multiple sclerosis exhibits many of the characteristics that distinguish complex genetic disorders including polygenic inheritance and environmental exposure risks. Here, we used a highly efficient multilocus genotyping assay representing variation in 34 genes associated with inflammatory pathways to explore gene–gene interactions and disease susceptibility in a well-characterized African-American case–control MS data set. We applied the multifactor dimensionality reduction (MDR) test to detect epistasis, and identified single-IL4R(Q576R)- and three-IL4R(Q576R), IL5RA(-80), CD14(-260)- locus association models that predict MS risk with 75–76% accuracy ($P < 0.01$). These results demonstrate the importance of exploring both main effects and gene–gene interactions in the study of complex diseases.

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system that affects nearly two and a half million people worldwide. Histologically, it is characterized by extensive perivascular inflammation and the presence of areas of demyelination, axonal loss and gliosis. Clinical manifestations vary from a benign illness to a rapidly evolving and incapacitating disease. The exact etiology is unknown, but MS reveals the general

Correspondence: Dr M Ritchie, Alison Motsinger, Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, 519 Light Hall, Vanderbilt University Medical School, Nashville, TN 37232-0700, USA. alison.a.motsinger@vanderbilt.edu.
[6]Both these authors contributed equally to this work.

characteristics of an autoimmune disorder, including immune dysregulation, polygenic inheritance and environmental exposure risks.[1] The genetic component in MS is indicated primarily by an increased relative risk to siblings compared to the general population, an increased concordance rate in monozygotic compared to dizygotic twins, and an increased prevalence in certain ethnic groups independent of geographic location.[2]

The genetics of MS has been extensively investigated although with modest replication of results. The one consistent finding is the partial susceptibility conferred by human leukocyte antigen (HLA) genes located within the major histocompatibility complex (MHC) on chromosome 6p21. This region has shown both linkage and association in multiple genomic screens and candidate gene studies.[3–5] The majority of evidence for this effect has been found in high-prevalence white populations of northern European descent, but association has also been shown in African-Americans (AA),[6] who develop MS less frequently.[7] In fact, the unique patterns of linkage disequilibrium (LD) in the AA genome have proven to be a remarkable resource in this disease for the fine mapping of regions of interest, characterization of allelic and clinical heterogeneity, and identification of new susceptibility loci.[6,8–11]

It is estimated that the *HLA* region accounts for 10–50% of genetic susceptibility in MS.[12] Even at the upper bound of this estimate, much of the complex inheritance of MS remains unexplained. However, the traditional search for main effects by individual genes has been largely unsuccessful, suggesting the need to test for epistatic interactions. To this end, a candidate gene approach was applied to a case–control study design. Several candidate genes were chosen based on the current understanding of inflammatory pathways involved in autoimmunity, and gene–gene interactions were evaluated. The dimensionality involved in the evaluation of combinations of many such variables (candidate genes) quickly diminishes the usefulness of traditional, parametric statistical methods.[13] Logistic regression, for example, relates one or more independent variables, such as single nucleotide polymorphisms (SNPs), to a binary outcome event (cases or controls). When the sample size is small, logistic regression can result in biased estimates on the regression coefficients, large standard errors, and spurious associations.[14,15] Logistic regression modeling fails to characterize epistasis models in the absence of main effects owing to the hierarchical model building process. This leads to an increase of type II error and decreased power, especially in the case of small sample sizes.[16] In fact, simulation studies have demonstrated that 10 outcome events per independent variable are required for each parameter estimate.[15] Thus for 49 unlinked SNPs, one would need to estimate 99 main effect parameters and require at least 990 cases or 990 controls. The number of parameters increases exponentially as the number of independent variables increases and interaction terms are added to the model. Even if an exhaustive (and not hierarchical) approach was taken to detecting interactions, multiple testing limits the power of logistic regression. To test all single locus through four locus interactions (as performed in this study), a *P*-value less than 1.99×10^{-7} would be required for a model to reach statistical significance.

To resolve some of these important issues, a novel computational approach for the detection of complex gene–gene and gene–risk factor interactions was developed. Multifactor dimensionality reduction (MDR) is a model-free, non-parametric data reduction method for

detecting multilocus genotype combinations that predict disease risk for common, complex disease. Empirical studies with simulated data indicate that MDR has good power to identify high-order gene-gene interactions,[17] and has been proven to be maximally efficient at discriminating between clinical end points using multilocus genotype data.[18] Studies with simulated data (with multiple models of different allele frequencies and heritability) have also shown that MDR has high power to identify interactions in the presence of many types of noise commonly found in real data sets – including missing data and genotyping error.[17] Additionally, MDR has been shown to have better power to detect interactions than classification and regression trees and stepwise logistic regression (unpublished results).

In this study, MDR was applied to a study of MS susceptibility in an informative and well-characterized AA population. A significant effect of the interleukin (*IL)4R(Q576R)* polymorphism was observed. In addition, a statistically significant three-locus interaction between *IL4R(Q576R)*, *IL5RA(−80)* and *CD14(−260)* was detected. The results provide evidence of complex genomic patterns associated with MS and demonstrate the value of exploring both main effects and gene–gene interactions in the context of complex disease etiology.

Results

A screen of 735 individuals, including 442 well-characterized MS patients, was performed for 48 SNPs and one deletion representing 34 genes involved in inflammatory pathways (Table 1). Single nucleotide polymorphisms were selected for investigation based on the hypothesis that variation in one or more loci mediating an inflammatory response might contribute to disease predisposition. Single nucleotide polymorphism selection was based on two pragmatic premises: (1) relative high minor allele frequencies in the population, (2) amenability of flanking frequencies to multiplex polymerase chain reaction (PCR) in a single reaction, recognizing that none of the tested genes could be definitively excluded. Human leukocyte antigen was not included in this list of candidates for two reasons. First, the role of HLA in this data set is described in detail in Oksenberg *et al.*[6] Secondly, MDR is sensitive to the inclusion of genes of major effect in the analysis, so inclusion of an SNP with a large main effect could prevent the discovery of novel interactions.

Table 2 shows the most significant results of the MDR analysis conducted in our sample. We observed a statistically significant main effect of the *IL4R(Q576R)* polymorphism with an average prediction error (PE) of 24.39% ($P<0.01$). This indicates that the *IL4R(Q576R)* polymorphism can correctly predict disease status 75.61% of the time. In addition, a statistically significant three-locus interaction between *IL4R(Q576R)*, *IL5RA(-80)* and *CD14(-260)* was detected, which predicts disease status correctly 76.1% of the time ($P<0.01$), slightly better than the main effect alone.

The four next best three-locus models yielded nonsignificant triples and included: *UGB, CD14, IL4R* (PE = 36.98%, $P = 0.5$); *UGB, TGFB1, IL4R* (PE = 39.08%, $P = 0.69$); *UGB, TGFB1, IL1A* (PE = 38.32%, $P = 0.62$); and *IL1A, CCR5, IL4R* (PE = 39.12%, $P = 0.69$). Surprisingly, the 6p21 markers in LD with the MHC (*LTF* and *TNFA*) were not included in any of the predictive combinations.

Traditional statistical methods were applied to these two models to aid in interpretation, not for additional statistical testing. The single locus model of *IL4R(Q576R)* had an odds ratio of 9.7856 (95% confidence interval (CI): 6.2498,15.3217). The χ^2 value of this model was 119.2111 (P<0.0001). The sensitivity of this model is 0.9081 and the specificity is 0.4976. The three-locus interaction between *IL4R(Q576R)*, *IL5RA(−80)* and *CD14(−260)* had an odds ratio of 8.5585 (95% CI: 5.7318,12.7793) and a χ^2 value of 124.6602 (P<0.0001). The sensitivity of this model is 0.8524 and the specificity is 0.5972.

Figures 1 and 2 show the MDR models for the main effect and interactive effect. The number of cases and controls are shown for each genotype combination, with high-risk status indicated by dark gray shading. Figure 1 clearly demonstrates a main effect, with one genotype value clearly conferring disease risk. Figure 2 again shows this main effect, as one entire column is high risk (for the same genotype as shown in Figure 1). Figure 2 also demonstrates an interactive effect, with high risk cells scattered throughout N-dimensional space, not demonstrating any marginal effect, as would be indicated by a solid column or row being all high risk.

Discussion

Multiple sclerosis clusters with the so-called complex genetic diseases, a group of disorders characterized by modest disease risk heritability and multifaceted gene–gene and gene–environment interactions. In this study, we investigated the potential associations between candidate genes in inflammatory pathways and risk of MS using a case–control study design and applying novel statistics to identify epistasis. The candidate genes were chosen because of their biological relevance, and MDR was implemented to reduce the genes of interest to a number that could manageably be investigated for functional importance in the disease state. We detected a significant three-locus interaction among *IL4R(Q576R)*, *IL5RA(−80)* and *CD14(−260)*. This model was able to accurately predict disease status ∼ 76% of the time. The *IL4R* polymorphism appears to confer a main effect within the interaction. Interestingly, we have recently reported the transmission distortion and significant association of a 10-SNP *IL4R* haplotype with MS in *HLA-DR1*5-negative white nuclear families.[19] Transmission distortion of *IL4R* alleles were also reported in a Belgian trio families MS data set.[20] Taken together, the latter and current reports provide accumulating evidence for a potential role for the IL4 receptor and its ligand, IL-4, in MS susceptibility and pathogenesis. The three-locus model reinforces the potential main effect of the *IL4R* along with demonstrating the importance of considering gene–gene interactions. In fact, the *IL4R* polymorphism is included in every *n*-level model produced by the analysis, further supporting its main effect in disease susceptibility.

The *IL4R* gene at 16p11–p12 encodes a subunit of the receptor heterodimer, a molecule critical to T helper cell differentiation and commitment.[21] Both the IL4 receptor and its ligand localize to immune cells at the site of active MS lesions in the brain, suggesting a role for these molecules in disease progression.[22] Changes to amino-acid residues, including the Gln576Arg polymorphism, are functionally important and impact the signaling cascade by the engaged receptor,[23] and *IL4R* SNPs are associated with susceptibility to immune-related diseases including asthma, atopy and type I diabetes.[24–26]

One consideration in this study is that we made no attempts to correct for multiple comparisons, although we conducted several tests. First, MDR itself is protected from multiple comparisons as permutation testing is conducted at the end of an MDR analysis, only on the best model at each level of interaction. This limits the number of hypothesis tests that were actually conducted. Therefore, when interpreting an MDR analysis, there is no need to perform further correction. Second, MDR is used for exploratory analysis rather than hypothesis testing. Although *P*-values are generated to compare the observed PE to the PE under the null hypothesis of no association, these *P*-values are meant to indicate interesting models that should be followed up in subsequent studies. With hypothesis generation as the goal of this study, no corrections for multiple testing were performed. Also, traditional statistics were only performed on the models generated by MDR to aid in interpretation, not with the purpose of additional statistical testing.

It is difficult to interpret from MDR results alone the possible functional effect of the *IL4R* and what role an interaction with the additional genes translates into biologically. Deciphering the results is part of the challenge in exploring gene–gene interactions in complex disease. In some ways, this challenge is even greater than the statistical limitations. Currently MDR methodology cannot provide further guidance in interpreting these models beyond indicating that they are statistically significant, and the best models detected in these data sets. The goal of the MDR method can be summed up as hypothesis generation, even though a form of hypothesis testing (permutation testing) is implemented to ascribe significance to the final model/hypothesis. Despite the challenges of interpretation, the results of this study demonstrate the ability of MDR to generate hypothesis that are biologically interesting.

Nevertheless, it is noteworthy that the final MDR models included genes very specific to a particular immune cell – dendritic cells (DCs). Dendritic cells are potent antigen-presenting cells, playing a crucial role in the decision between T-cell activation or anergy. They participate in the initiation and regulation of adaptive immunity against pathogens and in triggering autoimmunity. Several lines of evidence indicate an important role of DCs in MS. First, elevated levels of circulating dendritic cells have been associated with MS.[27] Also, there is evidence of the unusual presence of DCs in the brain lesions of MS patients.[28,29] Dendritic cells develop from CD14+ monocyte precursor cells. This development is dependent on signaling through the IL4 receptor supporting the observed interactions in the MDR models. The expression of the IL-5 receptor on DCs also was reported as well.[30]

In summary, we have evaluated inflammatory candidate genes and detected an interesting single-locus and gene–gene interaction effects in AAs, which is an understudied population in MS. Previous laboratory data emphasize the potential biological relevance of the models generated by the MDR analysis. We have demonstrated a main effect and epistasis model associated with risk of MS. The sample size of the data set was modest and it would be beneficial to conduct these complex, high-order gene–gene interaction analyses in larger sample sizes, but this study is an example where MDR can reveal epistasis effects. We also demonstrate the utility of MDR as a hypothesis generating method, producing results with interesting biological plausibility. The *IL4R* polymorphism, as well as the *IL4R(Q576R),*

IL5RA(−80), CD14(−260) interaction, should be followed up in subsequent association studies, as well as investigated for potential biological and mechanistic explanations.

Subjects and methods

Sample population

The data set comprised 442 unrelated MS patients and 293 unrelated controls, including 82 spouses. Controls were usually recruited by the patients and screened by experienced interviewers to assess compliance with rigorous inclusion criteria. All study participants were self-reported AAs, but European ancestry in patients and controls was documented based on genotyping results of 186 SNPs. Based upon the use of two parent populations, West Africans and Europeans, these SNPs have a mean 54% allele frequency difference between the parental populations and are spaced at least 10cM from each other across the genome.[31] Global estimation of European ancestry in the data set indicated 21.7±2.8% (s.e.) average admixture in patients and 21.6±2.8% (s.e.) in controls. Further, the frequencies of predominantly African DRB1*03021-DQB1*0402 and DRB1*1304-DQB1*0301 and Northern European- DRB1*0800-DQB1*0402 haplotypes in patient and control groups were statistically indistinguishable (6.6–5.2% $P = 0.57$, 0.5–1.2%, $P = 0.28$, and 0.5–0.9%, $P = 0.64$, respectively). These observations, together with the SNP genotyping results mentioned earlier, indicate that the MS patients and controls used in this study were adequately matched.[6] Medical records of all patients were reviewed by one of the authors (SLH or BACC) and in all cases diagnosis was confirmed using McDonald criteria.[32] Ascertainment protocols, and clinical and demographic characteristics are summarized elsewhere.[6,8] IRB approval and informed consent from all study participants were obtained.

Laboratory techniques

Table 1 displays the list of candidate genes from inflammatory pathways selected for this study. Fortynine 5′ biotinylated primer pairs were designed and optimized for a single megaplex 34-cycle PCR amplification using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Two probes were designed for each biallelic site to detect and distinguish between the variant sequences. Probes were conjugated at their 5′ ends to bovine serum albumin and then applied in a linear array to sheets of backed nylon membrane using a linear striper and Multispense 2000 controller (IVEK, N. Springfield, VT, USA). Base-denatured PCR products were added to the typing tray wells containing the strip and hybridization solution. After 15 min at 55°C in a rotating water bath, the hybridization solution was replaced with fresh buffer containing SA-HRP and hybridized for another 5min at 55°C. A stringent wash was carried out for 12min at 55°C followed by color development. Independent genotype interpretations were made by two investigators (DB and SJC) who were blind to pedigree structure and to the clinical status of the family members; discrepant results were reviewed and assays repeated if necessary.

Statistical techniques

Briefly, in the first step of MDR, the data are divided into a training set and an independent testing set for cross-validation.[33,34] As is traditional with MDR, 10-fold cross-validation is used, so that 9/10 of the data is used for training and 1/10 of the data is used for testing.

Second, a set of *n* SNP polymorphisms are selected. These polymorphism combinations are divided in *n*-dimensional space. Then the ratio of cases to controls is calculated for each combination. Each combination is then labeled 'high risk' or 'low risk' (using a ratio of 1.0 as the defining point) based on the ratio calculated, therefore reducing the *n*-dimensional space to one dimension with two levels (high risk/low risk). The combination of risk cells composes the MDR model. For each possible model size (one locus, two locus, etc.), a single MDR model is chosen that has the lowest number of misclassified individuals. To assess the predictive ability of the model, PE is calculated using 10-fold cross-validation. The result is a set of models, one for each model size considered. From these models, a final model is chosen based on minimization of PE and maximization of cross-validation consistency (number of times a particular set of factors is identified across the cross-validation subsets). The statistical significance of the final best model is determined through permutation testing. Permutation testing involves creating 1000 permuted data sets by randomizing the disease status labels. The entire MDR procedure is repeated for each, generating a distribution of PEs and cross-validation consistencies that could be expected by chance alone. The significance of the final model is determined by comparing the PE and cross-validation consistency of the final model to the distribution. A P-value is extracted for the model by its theoretical location in the distribution.

Acknowledgments

We are grateful to the MS patients and their families for participating in this study. We thank R.R. Lincoln, C. DeLoa, R. Harrison, W. Chin, H. Mousavi and R. Guerrero for recruitment of patients to the study, database management, and expert technical help. This work was funded by Grant RG3060 from the National Multiple Sclerosis Society and by National Institutes of Health Grants NS046297, GM31304, AG20135, and in part by HL65962, the Pharmacogenomics of Arrhythmia Therapy U01 site of the Pharmacogenetics Research Network.

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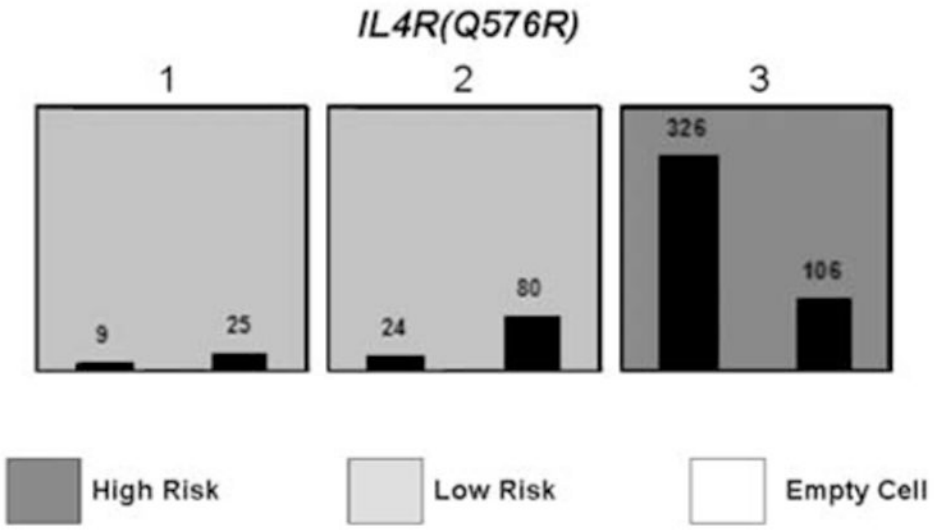

Figure 1.

Best single-locus multifactor dimensionality reduction (MDR) model. Light gray cells are low risk, while dark gray cells are high risk. The number of cases is shown in the histogram on the left in each cell, whereas controls are shown by the histogram on the right for each genotype combination.

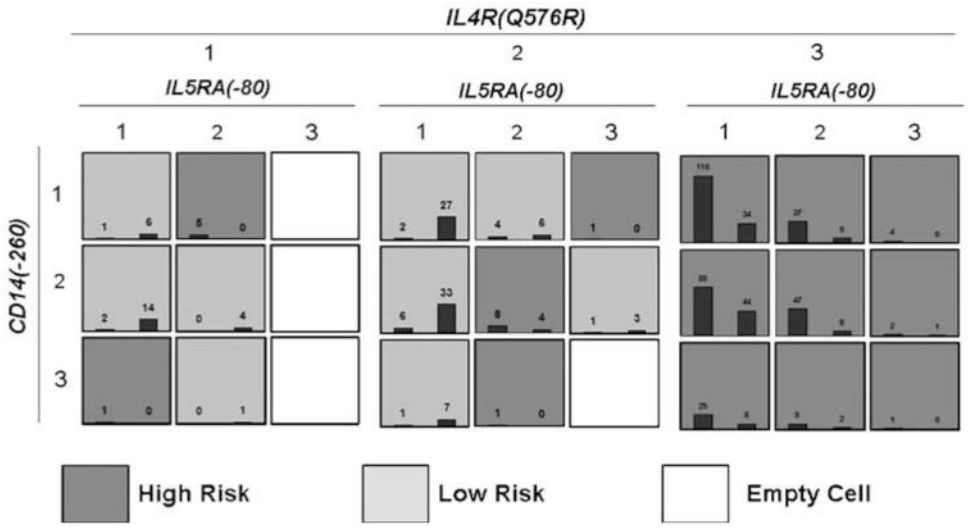

Figure 2.

Best three-locus multifactor dimensionality reduction (MDR) model. Light gray cells are low risk, while dark gray cells are high risk. The number of cases is shown in the histogram on the left in each cell, while controls are shown by the histogram on the right for each genotype combination.

Table 1

List of candidate genes

Gene	Gene symbol/SNP	NCBI locus ID	Genomic location	NCBI SNP ID	Alleles
Interleukin 4	*IL4* (−589)	3565	5q31.1	rs22433250	C/T
Interleukin 4 receptor (CD142)	*IL4R* (I50V)	3566	16p11.2–p12.1	rs 1805010	A/G
Interleukin 4 receptor	*IL4R* (S478P)	3566	16p11.2–p12.1	rs1805015	T/C
Interleukin 4 receptor	*IL4R* (Q576R)	3566	16p11.2–p12.1	rs1801275	A/G
Interleukin 13	*IL13* (intron3)	3546	5q31	rs1295686	C/T
Adrenergic beta-2 receptor	*ADRB2* (R16G)	154	5q31–q32	rs1042713	G/A
Adrenergic beta-2 receptor	*ADRB2* (Q27E)	154	5q31–q32	rs1042714	C/G
Adrenergic beta-2 receptor	*ADRB2* (T164I)	154	5q31–q32	rs 1800888	C/T
Intercellular adhesion molecule I (CD54)	*ICAM1* (K56M)	3383	19p13.3–p13.2	rs5491	A/T
Intercellular adhesion molecule I	*ICAM1* (G241R)	3383	19p13.3–p13.2	rs1799969	G/A
Vascular cell adhesion molecule I	*VCAM1 (−1594)*	4412	1p32–p31	rs1041163	G/A
Selectin E (Endothelial adhesion molecule 1), ELAM 1	*SELE* (S149R)	6401	1q22–q25	rs5361	A/C
Selectin P (granule membrane protein 140kD, CD62)	*SELP* (S330N)	6403	1q22–q25	rs6131	G/A
Selectin P	*SELP* (L640V)	6403	1q22–q25	rs6133	T/G
Fc fragment IgE, high affinity I R	*FCER1B* (E237G)	2206	11q13	rs569108	A/G
CD14 55 kDa antigen	*CD14* (−260)	929	5q31.1	rs2569190	C/T
Uteroglobulin (Clara-cell)	*UGB* (+38)	7356	11q12.3–q13.1	rs3741240	G/A
Transforming growth factor beta 1	*TGFB1* (−509)	7040	19q13.2	rs1800469	C/T
Small inducible cytokine subfamily A, eotaxin	*SCYA11* (A23T)	6356	17q21.1–q21.2	rs3744508	G/A
Chemokine receptor 2	*CCR2* (U62I)	1231	3p21	rs1799864	G/A
Chemokine receptor 3	*CCR3 (P39L)*	1232	3p21	rs5742906	C/T
Chemokine receptor 5	*CCR5* (−2454)	1234	3p21	rs333	G/A
Chemokine receptor 5	*CCR5 (Δ580–611)*	1234	3p21	rs333	deletion
T cell transcription factor	*TCF7* (P19T)	6932	5q31.1	rs5742913	C/A
T cell transcription factor	*TCF7* (UAS)	6932	5q31.1		A/T
Interleukin 9	*IL9* (T113M)	3578	5q31.1	rs2069885	C/T
Interleukin 1alpha	*IL1A* (−889)	3552	2q14	rs1800587	C/T
Interleukin 1beta	*IL1B* (−1418)	3553	2q14	rs16944	C/T

Gene	Gene symbol/SNP	NCBI locus ID	Genomic location	NCBI SNP ID	Alleles
Interleukin 1beta	*IL1B* (F105F)	3553	2q14	rs1143634	C/T
Interleukin 5 receptor alpha	*IL5RA* (−80)	3568	3p26–p24	rs2290608	G/A
Interleukin 6	*IL6* (−572)	3569	7p21	rs1800796	G/C
Interleukin 6	*IL6* (−174)	3569	7p21	rs1800795	G/C
Interleukin 10	*IL10* (−571)	3586	1q31–q32	rs1800872	C/A
Complement component 3	*C3* (R102G)	718	19p13.3–p13.2	rs2230199	C/G
Complement component 5	*C5* (I802V)	727	9q32–q34	rs17611	C/A
Colony stimulating factor 2	*CSF2* (I117T)	1437	5q31.1	rs25882	T/C
Cytotoxic T lymphocyte-associated protein 4	*CTLA4* (−318)	1493	2q33	rs5742909	C/T
Cytotoxic T lymphocyte-associated protein 4	*CTLA4* (T17A)	1493	2q33	rs231775	A/G
Leukotriene C4 synthase	*LTC4S* (−444)	4056	5q35	rs730012	A/C
Nitric oxide synthase (inducible)	*NOS2A* (D346D)	4843	17q11.2–q12	rs1137933	T/C
Nitric oxide synthase 3 (endothelial cell)	*NOS3* (−948)	4846	7q36	rs1800779	A/G
Nitric oxide synthase 3	*NOS3* (E298D)	4846	7q36	rs1799983	G/T
Small inducible cytokine A	*SCYAII* (−1328)	6356	17q21.1–q21.2	rs4795895	G/A
Stromal cell-derived factor 1	*SDF1* (800)	6387	10q11	rs1801157	G/A
Lymphotoxin alpha	*LTA* (252)	4049	6p21.3	rs909253	G/A
Tumor Necrosis Factor	*TNF* (252)	7124	6p21.3	rs1800629	G/A
Tumor Necrosis Factor	*TNF* (−308)	7124	6p21.3	rs361525	G/A
Vitamin D Receptor	*VDR* (M1T)	7421	12q13.11	rs2228570	C/T
Vitamin D Receptor	*VDR* (Bsm1)	7421	12q13.11	rs1544410	G/A
Vitamin D binding protein	*GC*	2638	4q12–q13	rs7041/rs4588	GA/GC/TA

Table 2
Results of MDR analysis in case–control sample

Number of loci	Polymorphism in model	Cross validation consistency	Prediction error
1	*IL4R* (*Q576R*)	10	24.39*
2	*IL4R (Q576R), ICAM1 (G241R)*	5	25.09
3	*IL4R (Q576R), CD14 (−260), IL5RA (−80)*	5	23.9*
4	*IL4R (Q576R), CD14 (−260), IL5RA (−80), GC(2416D)*	4	24.73

* $P \leq 0.01$.