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RISK FACTOR INTERACTIONS AND GENETIC EFFECTS ASSOCIATED WITH POST-OPERATIVE ATRIAL FIBRILLATION

ALISON A. MOTSINGER[1], BRIAN S. DONAHUE[2], NANCY J. BROWN[3], DAN M. RODEN[3], MARYLYN D. RITCHIE[1]

[1]*Department of Molecular Physiology & Biophysics, Center for Human Genetics Research*
[2]*Department of Anesthesiology*
[3]*Departments of Medicine and Pharmacology*
Vanderbilt University, Nashville, TN 37232, USA

Postoperative Atrial Fibrillation (PoAF) is the most common arrhythmia after heart surgery, and continues to be a major cause of morbidity. Due to the complexity of this condition, many genes and/or environmental factors may play a role in susceptibility. Previous findings have shown several clinical and genetic risk factors for the development of PoAF. The goal of this study was to determine whether interactions among candidate genes and a variety of clinical factors are associated with PoAF. We applied the Multifactor Dimensionality Reduction (MDR) method to detect interactions in a sample of 940 adult subjects undergoing elective procedures of the heart or great vessels, requiring general anesthesia and sternotomy or thoracotomy, where 255 developed PoAF. We took a random sample of controls matched to the 255 AF cases for a total sample size of 510 individuals. MDR is a powerful statistical approach used to detect gene-gene or gene-environment interactions in the presence or absence of statistically detectable main effects in pharmacogenomics studies. We chose polymorphisms in three (IL-6, ACE, and ApoE) candidate genes, all previously implicated in PoAF risk, and a variety of environmental factors for analysis. We detected a single locus effect of IL-6 which is able to correctly predict disease status with 58.8% ($p<0.001$) accuracy. We also detected an interaction between history of AF and length of hospital stay that predicted disease status with 68.34% ($p<0.001$) accuracy. These findings demonstrate the utility of novel computational approaches for the detection of disease susceptibility genes. While each of these results looks interesting, they only explain part of PoAF susceptibility. It will be important to collect a larger set of candidate genes and environmental factors to better characterize the development of PoAF. Applying this approach, we were able to elucidate potential associations with postoperative atrial fibrillation.

1. Introduction

Atrial fibrillation (AF) is the most frequent complication after cardiac surgery, occurring in 25-40% of patients[1-3]. It is an abnormal irregular heart rhythm whereby electrical signals are generated apparently randomly throughout the upper chambers (atria) of the heart. Its onset leads to a significantly higher risk for stroke compared with patients in sinus rhythm and other adverse events[4]. In

addition, patients who develop PoAF are more likely to have other postoperative complications such as peri-operative myocardial infarction, congestive heart failure and respiratory failure[5]. Post-op AF (PoAF) has been associated with increased frequency of inotropic and mechanical circulatory support, ventilation time[4], and increased length of hospital stay. Management strategies have focused on reducing PoAF mainly through antiarrhythmic drugs such as beta-blockers, sotalol, and amiodarone. These drugs have had some success in reducing risk, but are far from universally effective[6].

The mechanism of PoAF is complex and not fully understood, but almost certainly multifactorial, involving susceptibility and triggering factors. Genomic approaches offer one way of analyzing risk, but a significant challenge involves identifying sequence variations associated with increased risk. In the case of rare, Mendelian single-gene disorders such as sickle-cell anemia or cystic fibrosis, the genotype to phenotype relationship is often apparent, as disease phenotypes can be explicitly attributed to a mutant genotype. In the case of common complex diseases and pharmacological responses, such relationships are more difficult to characterize since the phenotype is likely the result of many genetic and environmental factors. In addition, epistasis, or gene-gene interaction, is increasingly assumed to play a crucial role in the genetic architecture of common diseases[7-9] and pharmacological responses[10].

There has been strong evidence for both genetic and environmental risk factors contributing to the development of AF[11]. The most frequently identified risk factors include increased age, valvular heart disease, atrial enlargement, preoperative atrial arrhythmias and chronic lung disease[12-14]. AF was first reported in a familial form in 1943[15]. More recent studies have indicated a genetic susceptibility to disease shown by the fact that parental AF increases the risk of AF in their offspring[16]. Linkage analysis has indicated a number of genetic loci in kindreds with a familial form of AF. Mutations in three potassium channel genes have been identified, each in a single kindred[17,18,19]. Other loci have been implicated, but no disease gene within these regions has yet been identified (10q22[20]; 6q14-16[21]; 5p13[22]). Though family studies have been successful in demonstrating a genetic component to AF, the familial form is uncommon. It is possible that this is largely a genetic disorder with highly variable penetrance. Association studies of acquired forms of AF have identified several candidate genes, but without much replication of results. One report form Japan identified a polymorphism in the ACE gene that confers disease risk[23]. Also, recently, a small study (110 patients) implicated the –174C/G polymorphism in the interleukin-6 (IL-6) gene as a risk factor for PoAF[24]. Earlier reports from our group confirm the association of the IL6 promoter polymorphism with PoAF[25].

The goal of this study was to determine whether interactions among candidate genes and a variety of clinical factors are associated in PoAF risk. We selected polymorphisms in six candidate genes, all chosen because of previous work implicating them in PoAF risk. We also chose a variety of recognized environmental factors to analyze.

For this study, we used Multifactor Dimensionality Reduction (MDR), a method for analyzing interactions designed to address many of the limitations of traditional methods. A key problem in traditional parametric methods is that the dimensionality involved in the evaluation of combinations of many genetic and environmental variables quickly diminishes their usefulness. Referred to as the curse of dimensionality[26], as the number of genetic or environmental factors increases and the number of possible interactions increases exponentially, many contingency table cells will be left with very few, if any, data points. This can result in increased type I errors and parameter estimates with very large standard errors[27]. Traditional approaches using logistic regression modeling are limited in their ability to deal with many factors and simultaneously fail to characterize epistasis models in the absence of main effects due to the hierarchical model building process[28]. This leads to an increase in type II errors and decreased power[29]. This is particularly a problem with relatively small sample sizes.

MDR reduces the dimensionality of multilocus data to improve the ability to detect genetic combinations that confer disease risk. MDR pools genotypes into "high-risk" and "low-risk" or "response" and "non-response" groups in order to reduce multidimensional data into only one dimension. It is a nonparametric method, so no hypothesis concerning the value of any statistical parameter is made. It is also a model free method, so no inheritance model is assumed [30].

MDR has been used to identify higher order interactions in the absence of any significant main effects in simulated data. In addition, MDR has demonstrated gene-gene interactions in a variety of different clinical datasets, including sporadic breast cancer[30], essential hypertension[28], type II diabetes[31], atrial fibrillation[32], amyloid polyneuropathy[33], and coronary artery calcification[34]. Studies with simulated data (of 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 datasets (including missing data and genotyping error), while errors such as heterogeneity (genetic or locus), and phenocopy diminish the power of MDR [35]. Additionally, theoretical mathematical approaches strongly support the idea that MDR is an optimal method to discriminate between clinical endpoints using multi-locus genotype data more efficiently than any other method[36].

Using MDR, we identified both a genetic effect and an environmental interaction that confers increased risk of PoAF. These findings demonstrate the utility of novel computational approaches for the detection of disease susceptibility genes and risk factors.

2. Methods

2.1. *Sample Population*

Since 1999, our group has been enrolling elective cardiac surgery patients into a genetic registry to study genetic variables that impact clinical outcomes. Following IRB approval and informed consent, we evaluated 940 adult cardiac surgery patients in the registry, and determined the following polymorphisms: -174G/C of IL-6, angiotensin converting enzyme (ACE) intron 16 insertion/deletion (I/D) polymorphism, and the apolipoprotein E alleles 2, 3, and 4. These loci have been previously identified as genetic risk factors for cardiovascular disease[24,37-40]. PoAF was defined as having occurred if present on either a postoperative ECG or rhythm strip, or documented by at least two of the following: progress notes, nursing notes, discharge summary, consultation, or change in medication. Other clinical variables were determined by chart review, and are listed in Table 2. These include clinical variables which have previously been associated with PoAF[41]. Prophylactic beta blockade was defined as receiving beta blockers after surgery but before discharge or onset of atrial fibrillation, whichever happened first. Of the 940 subjects enrolled, 255 developed PoAF. We took a random sample of 255 controls along with the 255 AF cases for a total sample size of 510 individuals.

Table 1: Genetic Parameters Measured in PoAF sample

Number of IL-6 -174G alleles (0,1,2)
Number of ACE D alleles
Number of ApoE2 alleles
Number of ApoE3 alleles
Number of ApoE4 alleles

2.2. *Laboratory Techniques*

Genomic DNA was isolated from blood sampled at the time of surgery. DNA processing was performed by the Vanderbilt Center for Human Genetics DNA Core Laboratory using Puregene (Gentra Systems). ACE insertion/deletion polymorphism was determined by amplification of intron 16 and agarose gel fragment size determination, similar to that of Perticone et al[42]. The ABI Prism 7900HT Sequence Detection System (Applied Biosystems) was used for

genotyping the IL-6 -174G/C and Apolipoprotein E (ApoE) alleles. This system utilized the 5' nuclease allelic discrimination *Taq*man assay in a 384-well format, a fluorescent method similar to that of MacLeod et al[43].

Table 2: Clinical Parameters:

Patient demographics			
Gender	Age	Ethnicity	
Surgical procedure			
Valve operation	Coronary bypass operation	Non-coronary, non-valve operation	Open chamber procedures
Duration of cardiopulmonary bypass	Offpump procedures	Repeat sternotomy	
Preoperative medications			
Aspirin	Corticosteroids	Nonsteroidal anti-inflammatory drugs	Alpha-2 antagonists
Beta-blockers	ACE inhibitors	Antilipid drugs	Calcium antagonists
Diuretics	Inotropes		
Medical history			
Hypertension	Diabetes	Preoperative tobacco history	Left ventricular ejection fraction
Preoperative use of intra-aortic balloon pump	History of congestive heart failure	Atrial fibrillation at time of surgery	History of atrial fibrillation
Postoperative events			
Reoperation for bleeding	Death during hospitalization	Use of intra-aortic balloon pump	New neurologic deficit
Use of prophylactic beta blockers	Blood loss during first 24 hours after surgery	Units of blood products transfused after surgery	Length of hospital stay

2.3. *Statistical Techniques*

To explore potential multifactor interactions, we applied the Multifactor Dimensionality Reduction (MDR) method. The details of the MDR algorithm have previously been described[30,35,44]. A diagram explaining the steps of the MDR algorithm is shown in Figure 1.

In the first step of MDR, the dataset is divided into multiple partitions for cross-validation. MDR can be performed without cross-validation; however, this is rarely done due to the potential for over-fitting[45]. Cross-validation[46] is an important part of the MDR method, as it tries to find a model that not only fits the given data, but can also predict on future, unseen data. Since attainment of a second dataset for testing is time-consuming and often cost-prohibitive, cross-validation produces a testing set from the given data to evaluate the predictive ability of the model produced. The training set is comprised of 9/10 of the data while the testing set is comprised of the remaining 1/10 of the data.

Second, a set of *n* genetic and/or environmental factors is selected for analysis, and a list of all possible combinations of factors is created. In the third step these *n* factors are arranged in contingency tables in *n*-dimensional space with all possible multifactorial combinations as individual cells in the table. The cases and controls for each locus combination are counted and in the fourth step the ratio of cases to controls within each cell is calculated. Each multilocus genotype combination is then labeled as "high risk" or "low risk" based on a threshold set at 1: if the ratio within a multifactor combination is >1, it is labeled as "high risk" for disease and if it is <1, it is labeled as "low risk" for disease. This step compresses multidimensional data into one dimension with two classes. For pharmacogenomic endpoints, each genotype combination could be labeled "response" or "non-response" based on the ratio of responders to non-responders.

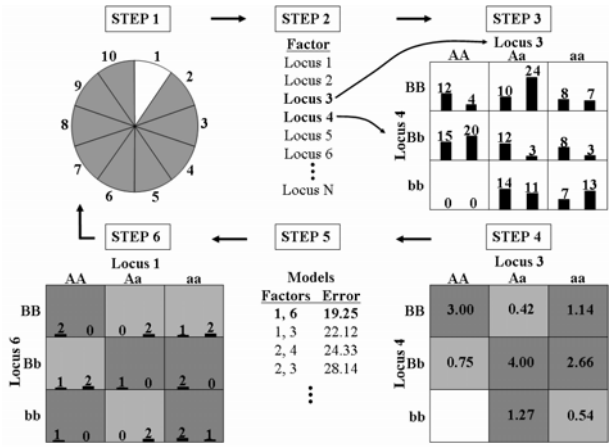

Figure 1. Summary of the general steps to implement the MDR method (adapted from [30]).

The disease risk classifications from each of the multifactorial combinations represent the MDR models for a particular combination of multilocus genotypes. The classification error for each model is calculated based on the number of individuals within the model that are actually cases in genotype combinations classified as "low risk" and the number of individuals that are actually controls in the genotype combination classified as "high risk." The best *n* locus model is selected and the model is evaluated against the testing group and prediction error is calculated. Prediction error is based on the number of misclassified individuals in the testing set, based on the model developed in the training set. This is repeated for each training set and the average classification error and prediction error are calculated. Among all of the models created, the one model with the lowest prediction error is chosen. This process is completed

for each number of loci combinations that is computationally feasible. For this analysis, single-locus through four-way interactions were evaluated. A model is chosen for each number of loci considered; so a one-locus model, two-locus model, three-locus model, etc will each comprise a set.

Once this set of models is completed, a final model is chosen. The final model is selected based on minimization of prediction error and maximization of cross-validation consistency. Prediction error is how well the model predicts risk/disease status in independent testing sets - generated through cross-validation. The error for the model is calculated by taking the average of the prediction errors in each of the ten testing sets. Cross-validation consistency is the number of times a model is identified across the cross-validation sets. Therefore, for ten-fold cross-validation, the consistency can range from one to ten. The higher the cross-validation consistency is, the stronger the support for the model. When prediction error and cross-validation indicate different models, the rule of parsimony, or the simpler model, is used to choose between them.

Once a best/final model is chosen, permutation testing is used to test the significance of the hypothesis generated. Permutation testing involves creating multiple permuted datasets by randomizing the disease status labels. One thousand randomized datasets are generated. The entire MDR procedure is repeated for each randomized dataset. The best model is extracted for each random data set as described above which generates a distribution of one thousand prediction errors and cross-validation consistencies that could be expected by chance alone. The significance of the final model is determined by comparing the prediction error of the final model to the distribution. A p-value is extracted for the model by its location in this empirical distribution. A p-value < 0.05 is considered statistically significant.

3. Results

Table 3 shows the results of the MDR analysis of the genotype data. We detected a single locus effect of IL-6 which is able to correctly predict disease status with 58.8% accuracy (Figure 2). This model was significant at the $p<0.001$ level.

Table 3. Results of MDR Analysis in Genotype Sample

Number of Loci	Polymorphism in Model	Cross Validation Consistency	Prediction Error
1	*IL6*	10	41.2*
2	*IL6, APOE4*	5	46.8
3	*IL6, ACE, APOE3*	9	46.01
4	*IL6, ACE, APOE2, APOE3*	10	42.77

* p=<0.001

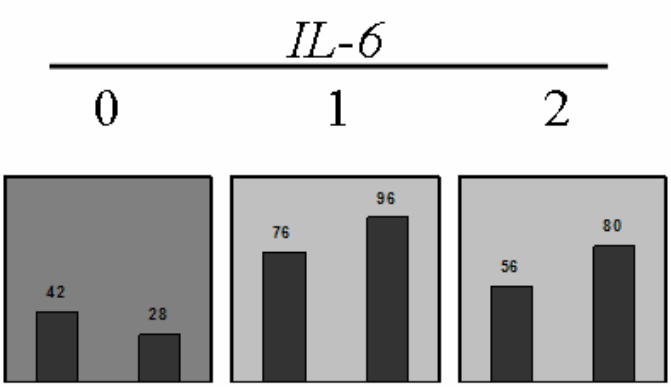

Figure 2. Single-locus MDR Model demonstrating effect of IL-6 which is able to correctly predict disease status with 58.8% accuracy. Light grey cells are low-risk, while dark grey cells are high risk. The number of cases is shown in the histogram on the left in each cell, while controls are shown by the histogram on the right. The genotype labels indicate number of IL-6 promoter -174 G alleles.

Table 4 shows the results of the clinical risk factor analysis. All four MDR models were found to be statistically significant at the $p<0.001$ level, but we focus on the two-locus interaction model because of the low prediction error and high cross-validation consistency. We detected an interaction between history of AF and length of hospital stay that predicted disease status with 68.34% accuracy (Figure 3).

Table 4. Results of MDR Analysis in Clinical Risk Factor Sample

Number of Loci	Variable in Model	Cross Validation Consistency	Prediction Error
1	*Length of stay*	10	33.06*
2	*History of AF, length of stay*	8	31.46*
3	*AF at time of surgery, age, length of stay*	3	38.64*
4	*AF at time of surgery, age, coronary bypass operation, length of stay*	10	33.4*

* p=<0.001

4. Discussion

Post-operative atrial fibrillation is likely the result of multiple genetic and environmental factors. In this study, we investigated potential associations between both candidate genes and risk of PoAF, and clinical risk variables and PoAF. A case-control study design was used, with a large population size and randomly selected controls. We detected an interesting single locus effect of IL-6 which is able to correctly predict disease status with 58.8% accuracy. PoAF is known to prolong length of hospital stay, and preoperative history of AF is a risk factor for postoperative AF[41,47]. Consistent with these findings, we also detected an interaction between history of AF and length of hospital stay that predicted disease status with 68.34% accuracy. These findings demonstrate the

utility of novel computational approaches for the detection of disease susceptibility genes.

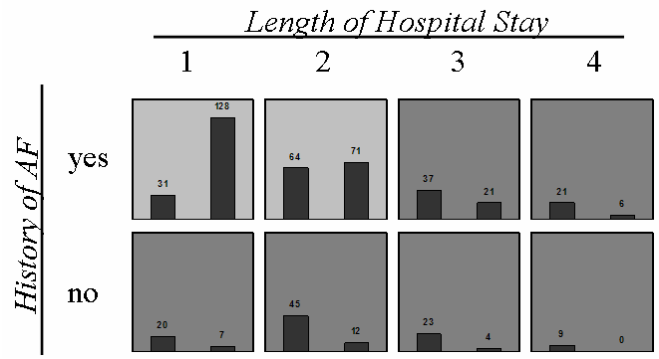

Figure 3. MDR model demonstrating an interaction between history of AF and length of hospital stay that predicted disease status with 68.34% ($p<0.001$) accuracy. Light grey cells are low-risk, while dark grey cells are high risk. The number of cases are shown in the histogram on the left in each cell, while controls are shown by the histogram on the right. Length of stay was defined as the date difference between surgery date and discharge date. It is coded as follows: 1=0-4 days, 2=5-7 days, 3= 8-13 days, 4=14 or more days. These cutoffs correspond to the 40th, 80th and 95th percentile cutoffs.

Part of the challenge in exploring epistatic interactions in pharmacogenomics or genetic epidemiology is the interpretation of results. Two interesting associations were found – both a main effect and an interactive effect. That IL-6 was shown to have an association with disease risk replicates the findings of[24] providing support for a postulated role for activation of inflammatory pathways in this[48] and perhaps other forms of AF[49]. This underscores a possible role for anti-inflammatory approaches for the prevention of this common complication.

The interaction model demonstrates the importance of genetic and environmental interactions, and represents a possible approach for detection of at-risk subgroups. The occurrence of multiple significant models also demonstrates the extreme complexity of the phenotype and could imply the importance of complicating issues such as heterogeneity and phenocopy.

Future studies may focus on a larger set of candidate genes and environmental factors to better characterize the development of post-operative AF. In addition, cases and controls could be matched on a number of clinical factors to control for confounding. This study demonstrated the importance of looking for both main effects and interactive effects, as well as demonstrating the utility of MDR in analyzing multiple gene-gene and gene-environment interactions.

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