Polymorphisms in the ACE and ADRB2 Genes and Risks of Aging-Associated Phenotypes: The Case of Myocardial Infarction

Alexander M. Kulminski, Irina V. Culminskaya, Svetlana V. Ukrainseva, Konstantin G. Arbeev, Igor Akushevich, Kenneth C. Land, and Anatoli I. Yashin

Abstract

Multiple functions of the beta2-adrenergic receptor (ADRB2) and angiotensin-converting enzyme (ACE) genes warrant studies of their associations with aging-related phenotypes. We focus on multimarker analyses and analyses of the effects of compound genotypes of two polymorphisms in the ADRB2 gene, rs1042713 and rs1042714, and 11 polymorphisms of the ACE gene, on the risk of such an aging-associated phenotype as myocardial infarction (MI). We used the data from a genotyped sample of the Framingham Heart Study Offspring (FHSO) cohort (n = 1500) followed for about 36 years with six examinations. The ADRB2 rs1042714 (C → G) polymorphism and two moderately correlated (r² = 0.77) ACE polymorphisms, rs4363 (A → G) and rs12449782 (A → G), were significantly associated with risks of MI in this aging cohort in multimarker models. Predominantly linked ACE genotypes exhibited opposite effects on MI risks, e.g., the AA (rs12449782) genotype had a detrimental effect, whereas the predominantly linked AA (rs4363) genotype exhibited a protective effect. This trade-off occurs as a result of the opposite effects of rare compound genotypes of the ACE polymorphisms with a single dose of the AG heterozygote. This genetic trade-off is further augmented by the selective modulating effect of the rs1042714 ADRB2 polymorphism. The associations were not altered by adjustment for common MI risk factors. The results suggest that effects of single specific genetic variants of the ADRB2 and ACE genes on MI can be readily altered by gene–gene or gene–environmental interactions, especially in large heterogeneous samples. Multimarker genetic analyses should benefit studies of complex aging-associated phenotypes.

Introduction

The aging of populations is becoming a challenging issue worldwide, making research on healthy aging a matter of major priority.1 Because genetics along with the environment are major components of pathways to healthy aging, gaining insight into the genetic component could greatly benefit these studies.2 Healthy aging phenotypes, however, are inherently complex and are not typically associated with a single genetic variant.3 Therefore, studies of such complex phenotypes should include multiple markers/loci. Such multimarker/multilocus approaches appear to be beneficial not only for studies of the aging-associated phenotypes but also for studies of drug efficacy, given that drug response could be better controlled when the predictive value of polygenic profiles is known.4

Polymorphisms in the beta2-adrenergic receptor (ADRB2) and angiotensin-converting enzyme (ACE) genes have long attracted the attention of health researchers for their possible connections with cardiovascular diseases (CVDs), the leading causes of deaths worldwide.5,6 For instance, two common single-nucleotide polymorphisms (SNPs) of the ADRB2 gene, rs1042713 (A → G, Gly16Arg) and rs1042714 (C → G, Gln27Glu), have been associated with myocardial infarction (MI) in a number of studies, including cohort (e.g., ref. 7), case–control (e.g., ref. 8), and trial (e.g., ref. 9) studies. Some studies, however, did not reveal such associations.10

Studies of the effects of the ACE gene on MI were largely focused on a common insertion/deletion (I/D) polymorphism within intron 16 of this gene, which has shown association with serum ACE levels linked to blood pressure regulation.11,12 Association with risk of MI was typically reported for the D allele predominantly in small-scale studies.5 Importantly, recent large-scale meta-analysis of 118 studies confirmed the positive effect of an I/D polymorphic variant with coronary artery disease.13 Large-scale studies,
The FHSO cohort represents biological descendants of residents of Framingham, Massachusetts, aged 5–70 years at the entry (in 1971–1975). The FHSO phenotypic data were assessed for participants of the sixth examination through 2007. Virtually all genotyped subjects participated in the first (1971–1974) and the sixth (1996–1997) examinations. There were 147 (54 women) deaths in this sample occurring after the sixth examination occurring after 2007.

The FHSO cohort contains residents of Framingham, Massachusetts, aged 5–70 years at the entry (in 1971–1975). The FHSO cohort represents biological descendants (n = 3514), their spouses (n = 1576), and adopted offspring (n = 34) of the participants of the original Framingham Heart Study (FHS) cohort. The FHSO respondents were followed longitudinally for the occurrence of certain aging-associated diseases, with an emphasis on CVDs and deaths through 2007. The health status of the study participants was examined during extensive physical and laboratory tests. Health assessments at six FHSO examinations performed in 1971–1975, 1979–1982, 1984–1987, 1987–1990, 1991–1995, and 1996–1997 were available for the present study. The FHSO assessed onsets of diseases (e.g., CVDs) at regular examinations at the FHS clinic and from medical records from outside clinics and hospitalization. Incidence of CVDs and death were followed through 2007.

ACE and ADRB2 polymorphisms in the FHSO

For this work, we used data from the Cardio-Genomics project on genotyping of 1888 mainly unrelated FHSO participants. DNA was collected for living participants of the FHSO in the late 1980s and through 1990s. The Cardio-Genomics project focused on candidate genetic markers of cardiovascular development (a review of genotyped resources in the FHS and FHSO can be found in refs. 28 and 32). Virtually all genotyped subjects participated in the first (1971–1974) and the sixth (1996–1997) examinations. There were 147 (54 women) deaths in this sample occurring after the sixth examination occurring after 2007.

Because of missing information in the available data, the selection of rs1042713 (A→G) and rs1042714 (C→G) SNPs of the ADRB2 gene limits the sample size to 1565 (784 women) individuals. These SNPs are in relatively weak linkage disequilibrium (LD), $r^2 = 0.41$ (LD was evaluated using version 4.1 of Haplovew33), with the A (rs1042713) allele linked to the C (rs1042714) allele (but not vice versa) and the G (rs1042714) allele linked to the G (rs1042713) allele (but again not vice versa).

Of 16 SNPs of the ACE gene available for the analyses, five SNPs have less than 1.5% minor allele frequency (MAF) and, thus, were disregarded. Statistical characteristics of the remaining 11 SNPs of the ACE gene are shown in Table 1.

### Data and Methods

#### The FHSO phenotypic data

The FHSO cohort includes residents of Framingham, Massachusetts, aged 5–70 years at the entry (in 1971–1975). The FHSO cohort represents biological descendants (n = 3514), their spouses (n = 1576), and adopted offspring (n = 34) of the participants of the original Framingham Heart Study (FHS) cohort. The FHSO respondents were followed longitudinally for the occurrence of certain aging-associated diseases, with an emphasis on CVDs and deaths through 2007. The health status of the study participants was examined during extensive physical and laboratory tests. Health assessments at six FHSO examinations performed in 1971–1975, 1979–1982, 1984–1987, 1987–1990, 1991–1995, and 1996–1997 were available for the present study. The FHSO assessed onsets of diseases (e.g., CVDs) at regular examinations at the FHS clinic and from medical records from outside clinics and hospitalization. Incidence of CVDs and death were followed through 2007.

**Table 1. Statistical Characteristics of the ACE and ADRB2 Gene SNPs**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>HW p value</th>
<th>% Genotyped</th>
<th>MAF</th>
<th>Minor allele</th>
</tr>
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<tbody>
<tr>
<td><strong>ACE gene</strong></td>
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</tr>
<tr>
<td>rs4305</td>
<td>58911961</td>
<td>0.53</td>
<td>93.9</td>
<td>47.6</td>
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<td>rs4309</td>
<td>58913655</td>
<td>0.83</td>
<td>98.2</td>
<td>40.5</td>
<td>T</td>
</tr>
<tr>
<td>rs4311</td>
<td>58914495</td>
<td>0.19</td>
<td>93.5</td>
<td>49.0</td>
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<tr>
<td>rs4316</td>
<td>58916041</td>
<td>0.09</td>
<td>87.4</td>
<td>42.9</td>
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<tr>
<td>rs4329</td>
<td>58917190</td>
<td>0.97</td>
<td>98.5</td>
<td>44.0</td>
<td>G</td>
</tr>
<tr>
<td>ID polymorphism</td>
<td>58919632</td>
<td>0.87</td>
<td>56.7</td>
<td>44.2</td>
<td>II</td>
</tr>
<tr>
<td>rs4363</td>
<td>58928224</td>
<td>0.95</td>
<td>98.0</td>
<td>44.7</td>
<td>A</td>
</tr>
<tr>
<td>rs9896208</td>
<td>58929841</td>
<td>0.36</td>
<td>97.8</td>
<td>40.3</td>
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<td>rs12499782</td>
<td>58929981</td>
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<tr>
<td>rs4968653</td>
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<td>96.8</td>
<td>49.5</td>
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<tr>
<td>rs7221678</td>
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<td>rs4459610</td>
<td>58938452</td>
<td>0.62</td>
<td>92.4</td>
<td>40.7</td>
<td>A</td>
</tr>
<tr>
<td><strong>ADRB2 gene</strong></td>
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<tr>
<td>rs1042713</td>
<td>14818663</td>
<td>0.78</td>
<td>93.0</td>
<td>36.0</td>
<td>A</td>
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<tr>
<td>rs1042714</td>
<td>14818666</td>
<td>0.86</td>
<td>95.2</td>
<td>43.0</td>
<td>G</td>
</tr>
</tbody>
</table>

SNP, Single-nucleotide polymorphism; HW, Hardy–Weinberg; MAF, minor allele frequency; Position, chromosome position.
Table 1 also shows statistical characteristics of two SNPs of the ADRB2 gene and the I/D ACE polymorphism. The ACE I/D polymorphism was not included into the analyses because of small genotyping frequency (56.7%). Analysis of LD, however, shows that the I/D polymorphism is in relatively strong LD with three other SNPs (Fig. 1 and Table 1). Consequently, we selected one SNP (rs4363) from the group of the highly correlated SNPs (rs4316, rs4329, I/D polymorphism, and rs4363) as a proxy for this group.34 Similarly, rs4968653 was dropped from further analyses because of redundancy. Thus, eight SNPs (rs4305, rs4309, rs4311, rs4363, rs9896208, rs12449782, rs7221678, and rs4459610) were selected for further analysis.

Analyses

Because of the focus of this work on the aggregated (or systemic) effect of the ACE and ADRB2 gene polymorphisms on the risk of such complex (non-Mendelian) aging-associated phenotypes as MI, relying only on traditional single marker (univariate) methods would be of limited help. Therefore, in this work we basically use multimarker approaches. In addition, the proposed multimarker analyses help better delineate false-positive associations compared to single-marker approaches (e.g., arising due to LD between SNPs). The relative risks and “survival patterns” (i.e., probability of staying free of MI) were evaluated using the Cox proportional hazard regression model (SPSS 17.0, Chicago, IL) with follow-up time as a time-to-incidence variable. The individuals were censored: (1) If they were diagnosed with MI on or before an examination considered as a baseline in a model, (2) if they died within the follow-up period, and (3) at the end of follow up in 2007. Basic analyses were performed considering health conditions at the baseline (first) examination. Because DNA was collected at different time points on or after the fourth examination, we provided also the results for the latest examinations (considering age, health status, and behavioral factors at the respective examination as appropriate) to ensure that attrition of cohorts did not bias the results.

The analyses were performed using two types of adjustments: (1) Age and sex, and (2) age, sex, and CVD-related risk factors consistently measured in all six FHSO examinations, i.e., systolic (SBP) and diastolic (DBP) blood pressures (mmHg), smoking (ever smoked), fasting serum glucose, body-mass index (BMI; kg/m²), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) (mg/100 mL). The absolute values of SBP, DBP, TC, and HDL were used in the analyses. These covariates were evaluated in the regression models with increments of 10 mmHg for SBP and DBP, and 10 mg/100 mL for TC and HDL-C. BMI was categorized using the U.S. federal guidelines as underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²), and obesity (>30 kg/m²). Grades of obesity for our analyses were disregarded. Fasting glucose was categorized according to the WHO-1999 and ADA-1997 guidelines, i.e., normal (<110 mg/dL), impaired (110–125 mg/dL), and diabetic (≥126 mg/dL) levels. Table 2 shows patterns of mean values of quantitative risk factors and frequencies of categorical risks factors that are typical for
When all eight SNPs were selected using the model with full adjustment. The same SNPs selected on the basis of LD are maximized because the rs4363 is linked to the commonly studied I/D polymorphism (Fig. 1) and rs12449782 was studied in relation to other diseases (see Introduction), the effect of these two SNPs could be not due to sampling error. Given this typical situation in candidate gene studies, we further investigated the additive systemic action of these two potentially interesting markers of the ACE gene as well as two (rs1042713 and rs1042714) SNPs of the ADRB2 gene on the risk of a complex aging-associated phenotype, the MI. This focus limits the overall sample size to \( n = 1500 \) (752 women) with 118 MI cases (31 among women).

Finally, we used Cox regression models with both types of adjustment to evaluate survival chances of individuals carrying different genotypes of the preselected SNPs (i.e., rs4363, rs12449782, rs1042713, and rs1042714). A total of 140 individuals died within the follow-up period (i.e., from the first examination to the end of follow up in 2007). The analyses reveal no effect of the SNPs of interest on mortality (the best \( p \) value was \( p = 0.38 \) for rs1042714 SNP in the model with full adjustment).

### Results

Figure 2 shows that the effects of the rs12449782 (ACE) and rs1042714 (ADRB2) SNPs on risks of incident MI are significant in the multimarker models with the rs4363, rs12449782, rs1042713, and rs1042714 SNPs included and adjusted for age and sex even after Bonferroni correction (i.e., \( p \) values are less than the power level of 0.005 for 10 tests). The effect of the rs4363 SNP attains marginal unadjusted significance and the rs1042713 SNP did not show significant effect.
Adjustments for potential risk factors did not qualitatively change the estimates for the rs12449782 and rs1042714 SNPs, but the effect of the rs4363 GG genotype attained unadjusted significance compared to the heterozygous (AG) genotype (Table 3; Ex 1). Because these results can be biased by attrition of the FHSO cohort until DNA collection, we evaluated the relative risks (RRs) at the latest examinations which were performed on or after the time of DNA collection (Table 3). The results remain consistent despite aging of this cohort (Table 3, column MA). Significance of the estimates at the last (sixth) examination, however, decreases due to decreasing number of incident MI cases.

Frequency distributions of genotypes (Table 4) show that due to modest LD between rs4363 and rs12449782 SNPs of the ACE gene the minor allele (A) of one SNP is predominantly linked to the minor allele (A) of the other SNP (consequently, the major alleles G are also predominantly linked as well). Nevertheless, the effects of these SNPs on the risk of MI are of an opposite nature, e.g., the rs12449782 AG heterozygote is the most protective against MI, whereas the rs4363 AG heterozygote is the least protective (Fig. 2 and Table 3). This means that the protective and detrimental effects are basically attributed to rare compound genotypes of the rs4363 and rs12449782 SNPs, i.e., the AA/AG (n = 34) and GG/AG (n = 47) genotypes (the allele pairs follows the ascending order of rs4363 and rs12449782 SNP coordinates on chromosome) are protective against MI whereas the AG/AA (n = 45) and AG/GG (n = 41) genotypes show detrimental effect (Fig. 3A).

Of three common compound genotypes (i.e., AA/AA, AG/AG, and GG/GG), the AA/AA genotype is the least protective, i.e., the risk of MI for carriers of the AG/AG genotype is 0.64 times smaller [95% confidence interval (CI) = 0.39–1.03, p = 0.064] and for carriers of the GG/GG genotype is 0.58 times smaller (CI = 0.34–0.97, p = 0.038) than the risks for the AA/AA genotype.

Figure 3B shows the effects of the compound genotypes composed of the common genotypes of the rs4363 and rs12449782 SNPs of the ACE gene (i.e., AA/AA, AG/AG, and GG/GG) and genotypes of the rs1042714 SNP of the ADRB2 gene. Two observations are of importance. First, the ADRB2 gene modulates the effect of common genotypes of the ACE gene to the same extent as rare genotypes of the ACE gene (Fig. 3A). Second, only homozygous genotypes of the ACE gene (AA/AA and GG/GG) are significantly sensitive to the rs1042714 polymorphism of the ADRB2 gene. Specifically, the GG genotype of the rs1042714 ADRB2 SNP increases risks of MI for carriers of the GG/GG (rs4363 and rs12449782) homozygotes by 3.3 times (p = 0.021) compared to the most favorable GG/GG.CG genotype (Fig. 3B).

Although Fig. 3B provides insights into systemic effect of compound genotypes composed of SNPs of two different genes (i.e., rs4363, rs12449782, and rs1042714) on such complex phenotype as MI, the effects in polygenic framework can be more robustly estimated for compound genotypes aggregated into the same-effect groups. For instance,

FIG. 2. Probability of staying free of myocardial infarction (MI) for n = 1495 Framingham Heart Study Offspring (FHSO) participants with nonmissing information on myocardial infarction (MI) status and ACE and ADRB2 polymorphisms. rs4363 (A→G) (A) and rs12449782 (A→G) (B) are for the ACE gene and rs1042714 (C→G) (C) is for the ADRB2 gene. Mean age for this sample at the first examination performed in 1971–1975 was 36.1 years (standard deviation was 9.7 years). A total of 117 incident MI cases were diagnosed after the first examination. Probability patterns were evaluated using the Cox regression model adjusted for age and sex. RR, Relative risks; CI, 95% confidence interval.
Fig. 3C shows the result of aggregation based on statistical significance of the relative risks as contrasted by the most protective GG/GG_CG genotype and by the least protective AA_AA_CC genotype (see legend for Fig. 3B). That is, compound genotypes are aggregated into a protective-effect (PE) group if the RR for a given compound genotype has a large p value (p > 0.1) as contrasted by the GG/GG_CG genotype (i.e., they have no difference in the risks). The PE group in this case includes the GG/GG_CG and AG/AG_GG compound genotypes. Similarly, the deleterious-effect (DE) group is a result of aggregation of the least protective compound genotypes, i.e., AA/AA_CC and GG/GG_GG. All other genotypes including the AA/AA_GG, which shows no effect compared to either the GG/GG_CG or AA/AA_CC genotypes, are included into moderate-effect (ME) group.

**Discussion and Conclusions**

Phenotypes of aging-associated disorders are multifactorial and unlikely caused by a mutation in a single locus. Nevertheless, major strategies in genetic analyses highlight the effect of a single genetic variant. There is growing understanding, however, that studies of complex phenotypes should involve systemic approaches including, for instance, analyses of genetic pathways and epistatic interactions, which do involve multiple markers. Better understanding of genetic predisposition to complex phenotypes can be gained from comparative analyses of different methods of genetic analyses, especially in a systemic context. In this study, we focused on multimarker analyses and analyses of compound genotypes to investigate aggregated (or systemic) effects of two common SNPs, rs1042713 and rs1042714, of the ADRB2 gene and 11 SNPs of the ACE gene on the probability of staying free of such a complex aging-associated phenotype as MI in participants of the FHSO cohort, who were followed for about 36 years. Our analyses reveal significant associations for two SNPs, rs4363 and rs12449782, of the ACE gene and one SNP, rs1042714, of the ADRB2 gene. Associations for the ADRB2 rs1042714 (nonsynonymous coding) and the ACE rs12449782 (intronic region) SNPs were significant after adjustment for multiple comparisons. The effect of the ACE rs4363 (splice site) SNP was marginally significant in some analyses (Fig. 2B), but attained unadjusted significance in the other analyses (Table 3).

A major result of our systemic analyses is the finding of opposite effects of predominantly linked ACE genotypes on the risks of MI. For instance, the protective AA (rs4363) genotype is predominantly linked to the detrimental AA (rs12449782)
genotype (see Fig. 2A,B and Tables 3 and 4). This result can be better understood if we consider compound genotypes of these two ACE SNPs instead of individual genotypes. For instance, 271 individuals (Table 4) carry the AA=AA compound genotype. Therefore, the effect of rs4363 and rs12449782 SNPs for these individuals is indistinguishable. Consequently, the detrimental and protective effects are attributed to selectively opposite effects of rare compound genotypes with a single dose of the AG heterozygote (Fig. 3A), which characterize deviations from perfect LD between rs4363 and rs12449782 SNPs (Table 4). For instance, the AG rs4363 genotype selectively increases chances of MI only for carriers of the homozygous AA and GG genotypes of the rs12449782 SNP. The homozygous AA and GG genotypes of the rs4363 SNP selectively decrease chances of MI only for carriers of the heterozygous AG rs12449782 genotype. The net effect of such selective modulation, for instance, is a significant difference in the risks of MI for the AG and AA genotypes of the rs12449782 SNP (Fig. 2B). This result means that the effect on such complex phenotypes as MI of even predominantly linked polymorphisms of the ACE gene can be readily altered either by other polymorphism(s) or/and by environment.

This genetic trade-off in the ACE gene is further augmented by a significant selective modulating effect of the rs1042714 SNP of the ADRB2 gene, which does not belong to the same genetic pathway. For instance, modulation is significant for the GG/GG ACE compound genotype, but not for the AG/AG ACE compound genotype. This result explicitly shows the presence of different pathways to healthy aging. One is when the net protective effect of a single genetic variant (e.g., the GG=GG genotype) on an aging-associated phenotype (MI) is a result of superposition of opposite effects of more specific genetic variants (e.g., GG=GG_CG and GG=GG_GG compound genotypes). This pathway reflects high sensitivity to gene–gene and gene–environment interactions. The other is when such a protective effect can potentially be attributed to the effect of a given genetic variant (e.g., AG/AG) itself, which is less sensitive to other markers or the environment (e.g., because the effects of extended compound genotypes are the same as in the case for AG/AG_CG, AG/AG_CC, and AG/AG_GG). However, even if the genetic effect of a given variant holds in the polygenic profile studied, this does not guarantee yet that the effect of this variant is not mediated or modulated by other genetic variants because the entire polygenic profile of complex phenotypes is typically not known.

Sensitivity of the effects of the ACE polymorphisms to gene–gene and gene–environment interactions is an example...
of inherent difficulties in determining effects of a single specific genetic variant on a complex phenotype especially in large heterogeneous samples. This, particularly, might explain the remarkable concordance of the results on associations of the ACE SNPs with MI in small-scale studies and the lack of such an association in large-scale studies.5,14,15 This situation calls for extending single-marker analyses to analyses of the effects of polygenic profiles on complex phenotypes in large (and heterogeneous) samples.

Thus, our results suggest that role of single specific genetic variants of the ADRB2 and ACE genes in the etiology of complex aging-associated phenotypes such as MI can be altered by gene–gene or and gene–environmental interactions, especially in large heterogeneous samples. Multimarker genetic analyses should benefit studies of complex aging-associated phenotypes.

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Author Disclosure Statement

No competing financial interests exist.

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