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

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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 ($r^2 = 0.77$) *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/and 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,

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however, largely failed to confirm this association.[14,15] Because I/D is an intronic polymorphism, other variant(s) can be associated with MI and be involved in regulation of the ACE expression. For instance, the rs4363 SNP of the *ACE* gene, which is relevant for our work, was studied for its association with MI,[16,17] blood pressure,[18] renal disease,[19] and obesity.[16] It has been found to be involved in regulation of ACE concentration.[20,21] The other relevant SNP, rs12449782, was found to be associated with diabetic nephropathy.[22]

The *ADRB2* and *ACE* genes are both attractive targets in therapy for CVDs and other diseases (e.g., asthma). For instance, beta-blockers and ACE inhibitors are major lines of drugs in the treatment of hypertension and other CVDs. They also improve the survival of CVD patients.[23–25] Recent meta-analysis suggests, however, that these associations should not necessarily be linked to the commonly studied ACE I/D polymorphism because it does not consistently explain variation in treatment of CVDs.[26] Nevertheless, connections of these two genes with risks of such common aging-associated phenotypes as MI make them plausible candidates for studies of genetic determinants of healthy aging.

In this study, we use the approaches of multimarker analyses and analyses of compound genotypes of two common SNPs in the *ADRB2* gene (rs1042713 and rs1042714) and 11 SNPs of the *ACE* gene to elucidate systemic effect of these markers on risk of a complex (non-Mendelian) aging-associated phenotype. As an example, we consider commonly studied connections of these genes to risks of MI. Phenotypic and genotypic information are assessed for participants of the longitudinal Framingham Heart Study Offspring (FHSO) cohort that has been followed up for about 36 years.

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[27,28] 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.[29,30] 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.[31] 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 through 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 Haploview[33]), 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, they were disregarded. Statistical characteristics of the remaining 11 SNPs of the ACE gene are shown in Table 1.

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

SNP	*Position*	*HW* p *value*	*% Genotyped*	*MAF*	*Minor allele*
ACE gene					
rs4305	58911961	0.53	93.9	47.6	A
rs4309	58913655	0.83	98.2	40.5	T
rs4311	58914495	0.19	93.5	49.0	T
rs4316	58916041	0.09	87.4	42.9	T
rs4329	58917190	0.97	98.5	44.0	G
ID_polymorphism	58919632	0.87	56.7	44.2	II
rs4363	58928224	0.95	98.0	44.7	A
rs9896208	58929841	0.36	97.8	40.3	T
rs12449782	58929981	1.00	96.9	45.6	A
rs4968653	58932830	0.63	96.8	49.5	T
rs7221678	58933344	0.76	99.1	48.0	T
rs4459610	58938452	0.62	92.4	40.7	A
ADRB2 gene					
rs1042713	148186633	0.78	93.0	36.0	A
rs1042714	148186666	0.86	95.2	43.0	G

SNP, Single-nucleotide polymorphism; HW, Hardy–Weinberg; MAF, minor allele frequency; Position, chromosome position.

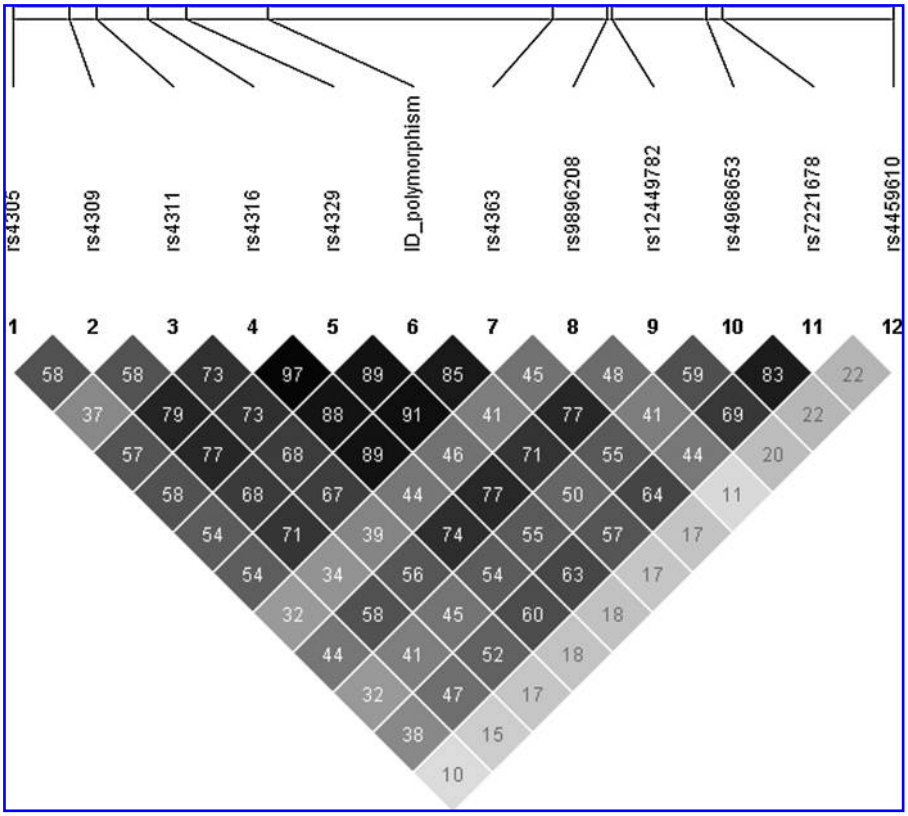

FIG. 1. Linkage disequilibrium (r^2) between single-nucleotide polymorphisms (SNPs) and an insertion/deletion (I/D) polymorphism of the *ACE* gene.

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/m^2), 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[35] as underweight ($<18.5\,kg/m^2$), normal weight ($18.5–24.9\,kg/m^2$), overweight ($25–29.9\,kg/m^2$), and obesity ($>30\,kg/m^2$). 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

Table 2. Baseline Characteristics of Groups of Genotyped FHSO Participants Who Contracted Myocardial Infarction Within 36 Years of Follow Up and Those Who Did Not

Risk factors	*No MI group,* n = *1382*		*MI group,* n = *118*	
Quantitative traits	*Mean*	*SE*	*Mean*	*SE*
Age, years	35.7	0.26	41.6	0.76
SBP, mmHg	120.1	0.38	126.8	1.36
DBP, mmHg	77.6	0.26	82.3	0.92
TC, mg/100 mL	192.5	1.00	216.7	3.29
HDL-C, mg/100 mL	51.5	0.40	44.8	1.20
Categorical traits	n	%	n	%
Smoking, no	539	39.0%	30	25.4%
Smoking, yes	843	61.0%	88	74.6%
BG, <110 mg/dL	1080	81.4%	67	58.8%
BG, 110–125 mg/dL	224	16.9%	42	36.8%
BG, ≥126 mg/dL	23	1.7%	5	4.4%
BMI, <18.5 kg/m^2	26	1.9%	0	0%
BMI, 18.5–24.9 kg/m^2	747	54.1%	40	33.9%
BMI, 25–29.9 kg/m^2	460	33.3%	54	45.8%
BMI, >30 kg/m^2	149	10.8%	24	20.3%

FHSO, Framingham Heart Study Offspring; MI, myocardial infarction; SE, standard error; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; BG, blood glucose; BMI, body mass index.

individuals who contracted MI and for those who did not contract this disease.

Multimarker analyses with all SNPs of interest included in the model are feasible because the number of SNPs is less than the number of subjects. This strategy, however, leads to sample size reduction (about 20%) because of missing information on the SNPs (Table 1). Therefore, it is reasonable to preselect prospective genetic determinants of the MI risks to maximize the sample size.

Preselection combined traditional univariate and multivariate analyses. We considered conditions at the first examination in these analyses. Specifically, we analyzed the effects of individual SNPs on the risks of MI using Cox regression models adjusted as defined above. Univariate analysis of individual ACE SNPs revealed six SNPs with a significance level of $p < 0.1$ (rs4305, rs4309, rs4363, rs9896208, rs12449782, rs7221678). This analysis, however, ignored LD between SNPs. Given the data (Fig. 1), this issue is of importance because such univariate analyses can generate false associations just because of correlation between SNPs. This issue can be offset by performing simultaneous adjustment of the effects of six SNPs, i.e., by performing multivariate analysis. Such analyses reveal only two significant signals for the rs4363 ($p = 0.009$ for genotypic model with two degrees of freedom) and rs12449782 ($p = 0.01$) SNPs in the Cox regression models with adjustment for age and sex. The same SNPs were selected using the model with full adjustment. When all eight *ACE* SNPs selected on the basis of LD are tested in the same model using iterative shrinking procedures (e.g., a backward elimination and likelihood ratio tests), the result remains qualitatively the same, i.e., only two the same SNPs (rs4363 and rs12449782) are selected in the best-predicting model.

Although the revealed associations did not reach significance level adjusted for Bonferroni correction (i.e., adjusted significance level should be $0.05/8 = 0.00625$), it can be argued that Bonferroni correction in this case is overly conservative given the level of LD in each loci.[36] Furthermore, 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.

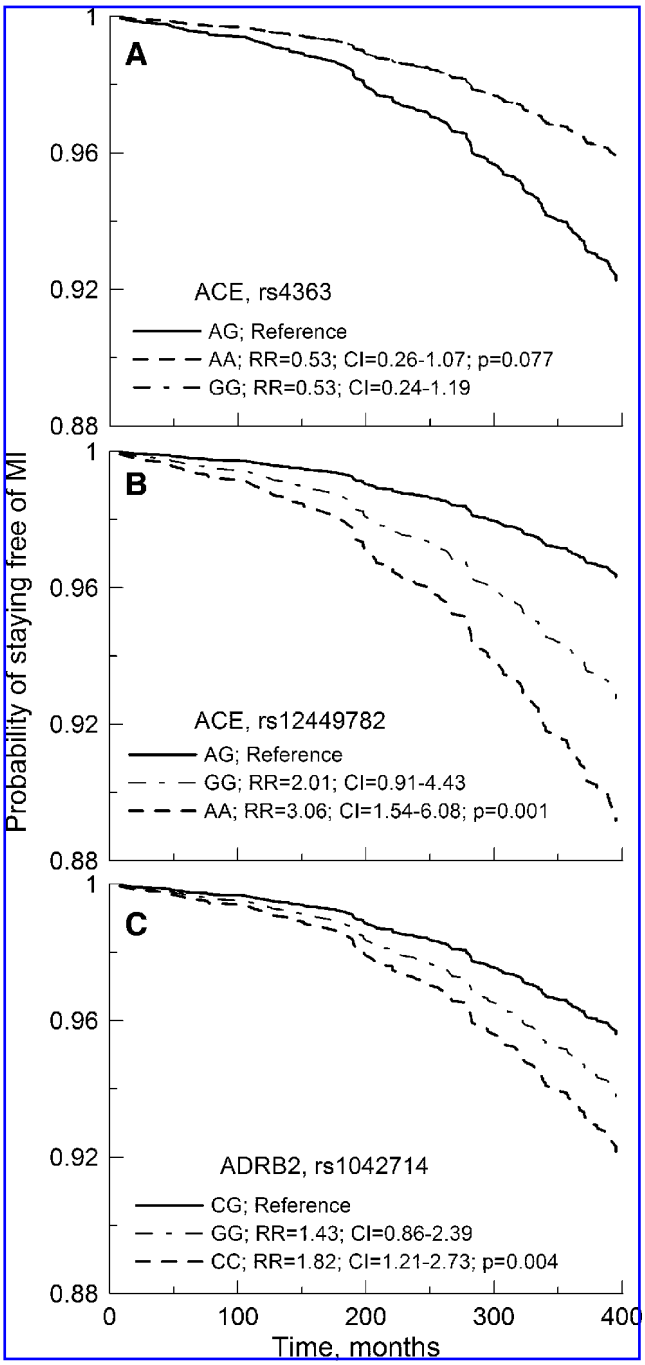

FIG. 2. Probability of staying free of myocardial infarction (MI) for $n = 1495$ Framingham Heart Study Offspring (FHSO) participants with nonmissing information on myocardial infarction (MI) status and *ACE* and *ADRB2* polymorphisms. rs4363 (A→G) (**A**) and rs12449782 (A→G) (**B**) are for the *ACE* 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.

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 do (compare survival curves for the most and the least favorable genotypes in Fig. 3A,B). 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). Similarly, individuals carrying the AA/AA_CG compound genotype have about two times smaller risks ($p = 0.092$) of MI compared to the AA/AA_CC genotype. The rs1042714 polymorphism shows no effect (the best p value is $p = 0.56$) on the heterozygous AG/AG genotype of the *ACE* gene.

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,

Table 3. Relative Risks of Incidence of Myocardial Infarction in the FHSO Sample of Genotyped Subjects

			rs12449782		*rs4363*		*rs1042714*	
Ex	*N (MI)*	*MA (SD)*	*AA*	*GG*	*AA*	*GG*	*CC*	*GG*
1	1434 (113)	36.2 (9.6)	$n=265$, MI = 36	$n=402$, MI = 33	$n=261$, MI = 30	$n=411$, MI = 30	$n=417$, MI = 50	$n=254$, MI = 20
			3.03 (1.47–6.27)	2.14 (0.95–4.78)	0.54 (0.26–1.14)	**0.43 (0.19–0.98)**	**1.68 (1.12–2.54)**	1.28 (0.75–2.19)
4	1373 (86)	51.7 (9.5)	**3.31 (1.58–6.93)**	2.40 (0.96–6.01)	**0.37 (0.17–0.82)**	**0.34 (0.13–0.88)**	**1.87 (1.15–3.02)**	1.42 (0.78–2.59)
5	1383 (71)	55.3 (9.6)	**5.19 (2.22–12.1)**	**3.87 (1.50–9.96)**	**0.36 (0.15–0.87)**	**0.32 (0.12–0.84)**	**1.80 (1.06–3.06)**	1.46 (0.75–2.83)
6	1426 (57)	59.2 (9.5)	**3.92 (1.44–10.7)**	**3.92 (1.42–10.8)**	0.41 (0.15–1.16)	0.37 (0.13–1.02)	**1.93 (1.05–3.54)**	1.93 (0.96–3.87)

95% Confidence intervals are given in parentheses.

The reference in all analyses was the heterozygous genotype. Bold font denotes significant estimates.

Relative risks were evaluated using the Cox regression model adjusted for age, sex, systolic and diastolic blood pressures, total and high density lipoprotein cholesterol, body mass index, blood glucose, and smoking.

FHSO, Framingham Heart Study Offspring; Ex, examination; MA, mean age; SD, standard deviation; MI, myocardial infarction; N(MI), total number of subjects in the analysis (the number of incident MI cases).

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[37] and epistatic interactions,[38] 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,[39] 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)

Table 4. The Number of Individuals with Compound Genotypes of the rs4363 and rs12449782 SNPs of the *ACE* Gene and the rs1042714 SNP of the *ADRB2* Gene in a Sample of $N = 1500$ Genotyped *FHSO* Participants

			rs12449782			
rs4363			*AA*	*AG*	*GG*	*Total*
AA	rs1042714	CC	86	7	0	93
		CG	136	17	0	153
		GG	49	10	1	60
	Total		271	34	1	306
AG	rs1042714	CC	14	218	18	250
		CG	21	319	13	353
		GG	10	116	10	136
	Total		45	653	41	739
GG	rs1042714	CC	1	14	132	147
		CG	0	22	194	216
		GG	0	11	81	92
	Total		1	47	407	455

FHSO, Framingham Heart Study Offspring.

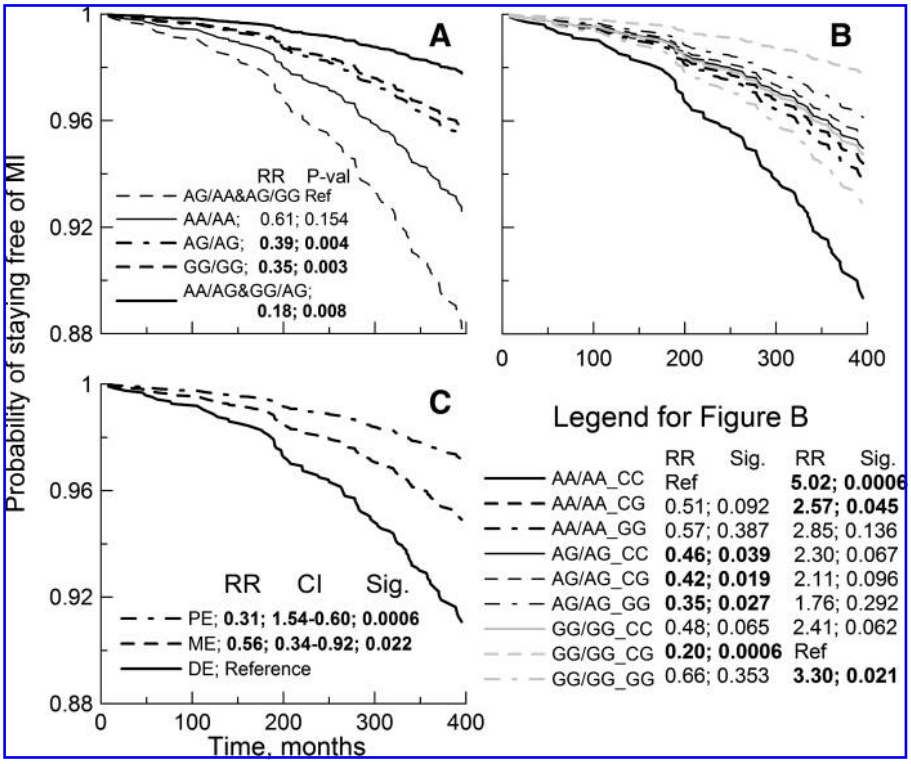

FIG. 3. Probability of staying free of myocardial infarction (MI) for individual (**A,B**) and aggregated (**C**) compound genotypes constructed using rs4363 (A→G) and rs12449782 (A→G) SNPs of the *ACE* gene (**A**) and rs4363 and rs12449782 SNPs of the *ACE* gene and rs1042714 (C→G) SNP of the *ADRB2* gene (**B,C**). Alleles in compound genotypes follow the order of the SNPs, i.e., rs4363, rs12449782, and rs1042714. (**C**) The protective-effect (PE) group includes the GG/GG_CG and AG/AG_GG compound genotypes; the deleterious-effect (DE) group includes AA/AA_CC and GG/GG_GG compound genotypes; all other genotypes seen in ***B*** constitute the moderate-effect (ME) group. (**A**) Analyses involved $n = 1432$ individuals including 113 incident MI cases. (**B** and **C**) Analyses involved $n = 1272$ individuals including 98 incident MI cases. Probability patterns were evaluated using the Cox regression model adjusted for age, sex, systolic and diastolic blood pressures, total and high-density lipoprotein cholesterol, body mass index, blood glucose, and smoking. RR, Relative risks; CI, 95% confidence interval. Bold font in the insets denotes significant estimates.

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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