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## Beta2-Adrenergic Receptor Gene Polymorphisms as Systemic Determinants of Healthy Aging in an Evolutionary Context

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### Abstract

The Gln<sup>27</sup>Glu polymorphism but not the Arg<sup>16</sup>Gly polymorphism of the beta2-adrenergic receptor (ADRB2) gene appears to be associated with a broad range of aging-associated phenotypes, including cancers at different sites, myocardial infarction (MI), intermittent claudication (IC), and overall/healthy longevity in the Framingham Heart Study Offspring cohort. The Gln<sup>27</sup>Gln genotype increases risks of cancer, MI and IC, whereas the Glu<sup>27</sup> allele or, equivalently, the Gly<sup>16</sup>Glu<sup>27</sup> haplotype tends to be protective against these diseases. Genetic associations with longevity are of opposite nature at young-old and oldest-old ages highlighting the phenomenon of antagonistic pleiotropy. The mechanism of antagonistic pleiotropy is associated with an evolutionary-driven advantage of carriers of a derived Gln<sup>27</sup> allele at younger ages and their survival disadvantage at older ages as a result of increased risks of cancer, MI and IC. The ADRB2 gene can play an important systemic role in healthy aging in evolutionary context that warrants exploration in other populations.

### Keywords

Healthy aging; ADRB2 polymorphisms; longevity; Framingham Heart Study

### 1. Introduction

The etiology of complex phenotypes entails epistatic and gene-environment interactions that makes relevant gene association studies a challenging task. Polymorphisms in the beta2-adrenergic receptor (ADRB2) gene have long attracted the attention of health researchers for their possible multiple physiological and health effects, particularly those involving vascular responses and airway function (Buscher et al., 1999; Contopoulos-Ioannidis et al., 2005; Hindorff et al., 2005; Snyder et al., 2005). ADRB2 is a member of the receptors family that mediates the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine. Adrenergic stimulation of the ADRB2 influences, for instance, cardiovascular function by regulating vasomotor tone (Guimaraes and Moura, 2001). This function makes ADRB2 an important target in cardiovascular disease therapy. The two most common functional single nucleotide polymorphisms of the ADRB2 gene are rs1042713 (46GA) and rs1042714 (79CG), which result in changes of amino acids at codon 16, Arg to Gly, and at

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codon 27, Gln to Glu, respectively (George, 2008). Various studies have shown that the role of these polymorphisms is of a broad nature because of their involvement not only in vascular responses but also in pulmonary, endocrine, and central nervous systems functioning (Brodde, 2008a; b). Specifically, the roles of Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu polymorphisms in the pathophysiology of diseases of the heart (Heckbert et al., 2003), hypertension (Puddu et al., 2007), obesity (Jalba et al., 2008), diabetes (Pinelli et al., 2006), asthma (Contopoulos-Ioannidis et al., 2005), Alzheimer disease (Yu et al., 2008), COPD (Matheson et al., 2006), and cancer (Huang et al., 2001) have been studied in different populations.

The pleiotropy of the effects of these genetic variants makes them promising candidates for studies of genetic predisposition to healthy aging (Melzer et al., 2007), which is often defined as life with preserved health and physical, social and mental wellness, independence, and quality of life (Peel et al., 2004). Although a common goal is to determine genetic pathway(s) which could directly modulate senescence (Johnson, 2005), a more realistic strategy is to understand mechanisms involved in the process of development of aging-associated disorders to improve the health of an increasing elderly population in contemporary societies (Hadley and Rossi, 2005; Melzer et al., 2007; Olshansky et al., 2007; Sierra et al., 2008).

Currently, a large number of genetic studies focus on aging per se in model organisms and humans (Vijg and Suh, 2005). Likewise, a large body of studies focus on a single particular disease phenotype (e.g., Brodde, 2008b). Integration of these diverse initiatives in a systematic way could greatly advance studies of the genetics of healthy aging. The problem is that an integrative approach requires rich data on health and longevity, which are typically collected in longitudinal studies. A majority of genetic studies of disease phenotypes address particular illnesses, as mandated by the current health care paradigm. These studies often use case-control designs, which prevent rigorous investigation of pleiotropic genetic effects because of the limited number of measured phenotypes. The other problem facing studies of pleiotropic effects is that they can be more susceptible to the effects of other genes (epistasis) or non-genetic factors (gene-environment interaction) – which can partly explain disagreements in replications of the results.

In this study, we focus on the effects of common polymorphisms of the ADRB2 gene, Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu, on the probability of staying free of cancer and cardiovascular diseases, as well as on association of these polymorphisms with overall and healthy longevity and survival. 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.

## 2. Data and Methods

### The FHSO phenotypic data

The FHSO cohort of respondents aged 5–70 years residing in Framingham, Massachusetts was launched in 1971–1975 with a focus on biological descendants (N=3514), their spouses (N=1576), and on adopted offspring (N=34) of the participants of the original Framingham Heart Study (FHS) cohort (Dawber, 1980; Gail and Johnson, 1989) resulting in a total sample of N=5,124 subjects; 52% women (Govindaraju et al., 2008; Kannel et al., 1979). The publicly-released limited-access FHSO data available for this study have phenotypic information assessed at six FHSO examinations performed in 1971–1975, 1979–1982, 1984–1987, 1987–1990, 1991–1995, and 1996–1997. The study participants have been followed for the occurrence of certain aging-associated diseases, with emphasis on cardiovascular diseases (CVDs) and cancer, and death through 2007. Onsets of CVDs and cancers were assessed at regular examinations at the FHS clinic and from medical records from outside clinics and hospitalization.

## Aging-associated phenotypes and risk factors

We selected CVDs and cancers as the most common aging-associated diseases which were studied on their associations with common variants of the ADRB2 gene. Skin cancers were disregarded in this study. An overall longevity phenotype was defined as reaching a certain old age at the end of the follow up period (i.e., in 2007) or at date of death. For the purpose of the study (see Section “Genetic effects on longevity”), cut-offs between long and short life were left flexible ranging from the youngest possible age (when too few individuals died before the cut-off age) to the oldest possible age (when there are too few individuals reaching the cut-off age). Individuals who survived cut-off ages were considered as long living (LL) individuals compared to those who died before or on the cut-off age; called as short lived (SL) individuals. A healthy longevity phenotype was considered if LL individuals did not develop cancer and/or CVDs.

## ADRB2 polymorphisms in the FHSO

DNA was collected for living participants of the FHSO in the late 1980s and through 1990s (Cupples et al., 2007). Genotyping of about 1,900 offspring (mainly unrelated) subjects was performed under the Cardio-Genomics program (<http://cardiogenomics.med.harvard.edu/projects/p5/assoc-results>) that focused on selected candidate genetic markers of cardiovascular development (a review of genotyped resources in the FHS and FHSO can be found in (Govindaraju et al., 2008; Levy et al., 2006)). Two common Arg<sup>16</sup>Gly and the Gln<sup>27</sup>Glu polymorphisms of the ADRB2 gene were selected for this study because of their documented *multiple* physiological and health effects (see Introduction) that warrants exploration of their systemic effect. The released data have information on the Arg<sup>16</sup>Gly and the Gln<sup>27</sup>Glu polymorphisms for 1565 (784 women) subjects. All genotyped members (but one) of the FHSO cohort participated in the 1<sup>st</sup> (1971–1974) and the 6<sup>th</sup> (1996–1997) examinations. There were 147 deaths (93 deaths occurred among men) in this sample that occurred after the 6<sup>th</sup> examination.

Table 1 shows mean age for samples of men and women stratified by the selected polymorphisms, as well as the number of individuals with a given compound genotype. Men with Glu<sup>27</sup>Glu genotype are somewhat younger (significantly with conventional tests and non-significantly with adjustment for multiple comparisons) than men with the Gln<sup>27</sup>Gln genotype. There are no other significant differences between mean ages. The Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu polymorphisms are in modest linkage disequilibrium (LD) with  $r^2=0.41$  (evaluated using Haploview, v. 4.1 (Barrett et al., 2005)). As a result, the Arg<sup>16</sup> allele is tightly linked to the Gln<sup>27</sup> allele (but not vice versa) and the Glu<sup>27</sup> allele is tightly linked to the Gly<sup>16</sup> allele (but again not vice versa) (Table 1). Both polymorphisms are in Hardy-Weinberg equilibrium with  $p=0.78$  (Arg<sup>16</sup>Gly) and  $p=0.86$  (Gln<sup>27</sup>Glu).

## Analyses

The Cox proportional hazards regression model was used to evaluate the relative risks (RRs) of death and incidence of the selected diseases as well as probability of staying free of a given disease (“survival patterns”) within the follow up period through 2007 for carriers of the Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu polymorphisms. Associations of these polymorphisms with longevity-related phenotypes were evaluated using the logistic regression.

The analyses were first performed with adjustment for sex (when necessary) and age. Then the models were adjusted for such potential effect-mediators as systolic (SBP) and diastolic (DBP) blood pressures (mm Hg), smoking (ever smoked), diabetes, body-mass index (BMI; kg/m<sup>2</sup>), total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol (mg/100 ml) (Jalba et al., 2008; Petrone et al., 2006; Pinelli et al., 2006; Puddu et al., 2007). The absolute values of SBP, DBP, TC, and HDL were used in the regression models with increments of 10 mm Hg

for SBP and DBP, and 10 mg/100 ml for TC and HDL cholesterol. BMI was categorized using the U.S. federal guidelines (Flegal et al., 2005) as underweight (<18.5 kg/m<sup>2</sup>), normal weight (18.5–24.9 kg/m<sup>2</sup>), overweight (25–29.9 kg/m<sup>2</sup>), and obesity (>30 kg/m<sup>2</sup>). Diabetes (present or absent) was defined if the study participant was under treatment with insulin/oral hypoglycemics or the level of fasting blood glucose  $\geq$  140 mg/dL (i.e., according to the WHO-1985 guidelines). Both types of analyses provided qualitatively similar results with slightly better estimates in the analyses with full adjustment.

Our analyses show that the Gln<sup>27</sup>Glu polymorphism is more relevant to diseases than the Arg<sup>16</sup>Gly polymorphism. Therefore, we present the results for the Gln<sup>27</sup>Glu polymorphism and show potential mediating/modulating role of the Arg<sup>16</sup>Gly polymorphism by evaluating the effect of six common compound genotypes of these two polymorphisms (i.e., excluding rare Arg<sup>16</sup>Gly/Glu<sup>27</sup>Glu and Arg<sup>16</sup>Arg/Gln<sup>27</sup>Glu genotypes; see Table 1).

Given prior evidences on the associations of the Gln<sup>27</sup>Glu and Arg<sup>16</sup>Gly polymorphisms with various aging-related phenotypes including those studied in this paper (see Introduction) and very limited number of independent tests, Type I sampling error is unlikely to be an issue for our analyses. Consequently, correction for multiple comparisons was not carried out. We note that robustness of our findings is further justified by the pleiotropic effect of the Gln<sup>27</sup>Glu polymorphism (revealed in this study) as well as by a hypothesized role of the ADRB2 polymorphisms in cardiovascular development (see Section “ADRB2 polymorphisms in the FHSO”) and by the candidate-gene-focused genotyping technique that offsets potential problems relevant to genome-wide technologies (Ziegler et al., 2008).

### 3. Results

#### The Gln<sup>27</sup>Glu polymorphism and health risks

Figure 1A shows the highly significant protective role of the Glu<sup>27</sup>Glu homozygous genotype against cancer (all sites but skin) for men and women combined. To ascertain that this effect is not an artifact of biased attrition of the FHSO cohort before the blood drawing (see “ADRB2 polymorphisms” section), we evaluated survival patterns and the relative risks focusing on the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> examinations which were performed on or after the blood drawing. In all three cases, the results resemble those in Figure 1A (e.g., the relative risk [RR] for the Gln<sup>27</sup>Gln homozygote was 2.03, 95% Confidence interval [CI]=1.29–3.21 compared to the Glu<sup>27</sup>Glu homozygote for the 5<sup>th</sup> FHSO examination). This protective effect holds for each sex, e.g., cancer risks are significantly higher for the Gln<sup>27</sup>Gln homozygote (RR=2.14; CI=1.23–3.72 for men and RR=2.02; CI=1.05–3.86 for women) compared to the Glu<sup>27</sup>Glu homozygote. Furthermore, the protective effect appears to be not specific to a cancer type; similar relationships were observed for breast and prostate cancers (Figure 1B) as well as for the remaining cancer sites (Figure 1C).

No effect of the Gln<sup>27</sup>Glu polymorphism on aggregate CVD phenotype was found (Figure 1D). Nevertheless, a significant effect was observed for more homogeneous phenotype of myocardial infarction (MI) (Figure 1E) that is in line with prior studies (Heckbert et al., 2003). We also hypothesized that MI could share common genetic background with intermittent claudication (IC) because both conditions could be linked to the development of systemic atherosclerosis. Indeed, IC is a symptom of peripheral arterial disease (PAD) and the latter is often a sign of widespread accumulation of fatty deposits in arteries, i.e., atherosclerosis. This condition may reduce blood flow not only to legs but also to heart and brain. Atherosclerosis may thus be a common risk factor for MI and PAD/IC (Murabito et al., 2002). As expected, the detrimental role of the Gln<sup>27</sup>Gln homozygote attains better significance for the compound phenotype of MI and IC, especially in men (Figure 1F).

Our analyses did not reveal significant association of the Gln<sup>27</sup>Glu polymorphism with mortality risk. For both sexes combined the RRs were RR=1.20 (CI=0.72–2.00) for the Gln<sup>27</sup>Gln homozygote and RR=1.25 (CI=0.78–2.03) for the Gln<sup>27</sup>Glu heterozygote compared to the Glu<sup>27</sup>Glu homozygote.

### The Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu compound genotypes and health risks

Table 1 shows that the Glu<sup>27</sup>Glu homozygote is tightly linked to the Gly<sup>16</sup>Gly homozygote. Consequently, the Gly<sup>16</sup>Gly/Glu<sup>27</sup>Glu compound genotype resembles the effect of the Glu<sup>27</sup>Glu genotype shown in Figure 1. Because the LD between the Gln<sup>27</sup>Glu and Arg<sup>16</sup>Gly polymorphisms is modest, carriers of the other two genotypes of the Gln<sup>27</sup>Glu polymorphism can carry different genotypes of the Arg<sup>16</sup>Gly polymorphism, e.g., the Gln<sup>27</sup>Glu genotype can be linked either to the Arg<sup>16</sup>Gly or Gly<sup>16</sup>Gly genotype (Table 1). Consequently, the effects of the Gln<sup>27</sup>Gln and Gln<sup>27</sup>Glu genotypes can theoretically be mediated or modulated by the Arg<sup>16</sup>Gly polymorphism.

Figure 2 shows that the effects on cancer of the Arg<sup>16</sup>Gly/Gln<sup>27</sup>Glu and Gly<sup>16</sup>Gly/Gln<sup>27</sup>Glu compound genotypes (i.e., carrying the Gln<sup>27</sup>Glu genotype) as well as the Arg<sup>16</sup>Arg/Gln<sup>27</sup>Gln, Arg<sup>16</sup>Gly/Gln<sup>27</sup>Gln, and Gly<sup>16</sup>Gly/Gln<sup>27</sup>Gln compound genotypes (i.e., carrying the Gln<sup>27</sup>Gln genotype) are the same (Figure 2A), i.e., the Arg<sup>16</sup>Gly polymorphism neither modulates nor mediates the effect of the Gln<sup>27</sup>Glu polymorphism on cancer. Although the effect of the Gln<sup>27</sup>Glu polymorphism on compound phenotype of MI and IC can be more sensitive to the Arg<sup>16</sup>Gly polymorphism than in the case of cancer, this sensitivity is not significant as well (Figure 2B). Therefore, these results provide no evidence on functional relationship between the Arg<sup>16</sup>Gly polymorphism and these aging-associated phenotypes. This result means that a protective effect is attributed to the Glu<sup>27</sup> allele (that is equivalent to the Gly<sup>16</sup>Glu<sup>27</sup> haplotype), whereas a detrimental effect is largely associated with the Gln<sup>27</sup> allele of the Gln<sup>27</sup>Glu polymorphism.

### Genetic effects on longevity

Because the Gln<sup>27</sup>Glu polymorphism is associated with risks of cancers and MI, and because these diseases are among the leading causes of death in developed societies, this polymorphism might be involved in characterization of overall and healthy longevity (see definition of the longevity phenotypes in section “Aging-associated phenotypes and risk factors”). Given that incidence rate of these diseases begin to notably increase in adulthood (at about 50 years), cut-off for longevity was left flexible conditional on the available sample size (see next paragraph). This technique helps also offset the chance nature of findings provided that the revealed associations exhibit consistent (but not stochastic) pattern across age.

Figure 3 shows the logarithm of odds ratios of reaching old ages for men and women carrying the Gln<sup>27</sup>Glu (Figure 3A) and Glu<sup>27</sup>Glu (Figure 3B) genotypes and the Glu<sup>27</sup> allele (Figure 3C). These analyses include a limited subset of the genotyped sample. This subset is maximal for cut-offs at young-old ages (e.g., N=773 for cut-off = 68 years). The number of SL individuals is, however, the smallest for these cut-offs due to a small number of deaths at those ages (e.g., 33 deaths occurred on or before the cut-off age = 68 years). When the cut-off age increases, the number of SL individuals gradually increases. However, the number of LL subjects (or, equivalently, the total number of individuals in the analysis) decreases because living individuals who are of cut-off ages or younger have to be censored out due to unknown life span. This results in a sample of N=135 SL subjects and N=30 LL subjects at cut-off = 86 years. These constraints limit flexibility of the cut-off ages.

Carriers of the Gln<sup>27</sup>Glu and Glu<sup>27</sup>Glu genotypes and the Glu<sup>27</sup> allele tend to *systematically* live shorter lives when longevity is defined as reaching young-old ages (e.g., cut-off = 72 years)

and longer lives when longevity is defined as reaching oldest-old ages (e.g., cut-off = 83 years) compared to carriers of the Gln<sup>27</sup>Gln genotype. These patterns are consistent and essentially the same for overall longevity and healthy life span (solid line in Figure 3 shows representative pattern for longevity without cancer). They also remain the same irrespective of adjustment for health-related risk factors. Estimates of the associations of compound genotypes with longevity are not reliable due to small sample sizes.

#### 4. Discussion and conclusions

In this work, we investigated associations of genetic variants of two common polymorphisms of the ADRB2 gene, Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu, with the risks of cancer, CVDs, mortality, and longevity. Our analyses show that the Gln<sup>27</sup>Glu polymorphism, but not the Arg<sup>16</sup>Gly polymorphism is associated with selected aging-associated phenotypes. The analyses suggest two insights on: (i) the systemic role of the ADRB2 gene in healthy aging and (ii) mechanisms linking systemic genetic health effects with overall and healthy longevity in evolutionary context.

##### Systemic role of the ADRB2 gene in healthy aging

Our results show that the ADRB2 Gln<sup>27</sup>Glu polymorphism might be involved in regulation of a broad range of complex aging-associated phenotypes including cancer, MI, IC, and longevity (but not survival). This finding suggests that the role of this polymorphism in healthy aging can be of a systemic origin that might provide a genetic rationale for accumulated epidemiological evidence on favorable healthy aging phenotypes of long-lived individuals who have smaller prevalence of cancer, CVD, and better physiological phenotypes (Barzilai et al., 2003; Willcox et al., 2008a; Willcox et al., 2008b). Importantly, the effect of the Gln<sup>27</sup>Glu polymorphism on the risk of cancer might be sex and cancer-site insensitive (see discussion of Figure 1)—which provides further support for its systemic role. Further evidence follows from the facts that: (i) the effect of the Gln<sup>27</sup>Glu polymorphism on these aging-associated diseases (Figure 1) and longevity (Figure 3) was not altered by common physiological and behavioral risk factors including blood pressure, total and high-density lipoprotein cholesterol, diabetes, BMI, and smoking and (ii) the effect of the Gln<sup>27</sup>Glu polymorphism holds for overall and healthy longevity (Figure 3).

The systemic role of the Gln<sup>27</sup>Glu polymorphism is further augmented by the consistently unfavorable role of the derived Gln<sup>27</sup> allele in etiology of cancer, MI and IC. The ancestral Glu<sup>27</sup> allele seems to be playing the protective role for these phenotypes. The mode of inheritance for these alleles is different for cancer, MI, and IC. Specifically, Figures 1A–C show the recessive effect of the Glu<sup>27</sup> allele on cancer phenotype whereas Figures 1E and 1F show its dominant effect on MI and on compound phenotype of MI and IC.

The ADRB2 receptor is expressed on cells of various tissues (e.g., immune, vascular, pulmonary, nervous (Brodde, 2008a; b)). Some studies suggest that a common ADRB2 signaling pathway (involving ADRB2 mediated catecholamine, e.g., epinephrine, induced activation of adenylate cyclase through the action of G proteins) interacts with multiple proteins from various cell molecular pathways (Benovic, 2002). This might provide a biological rationale for pleiotropic physiological effects of the ADRB2 gene (Brodde, 2008a; Leineweber et al., 2004).

In vascular tissue, for instance, the ADRB2 receptor regulates vasodilation and in healthy myocardium it regulates chronotropic and inotropic responses to endogenous and exogenous adrenergic agents (Brodde and Michel, 1999). The Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu polymorphisms are both associated with vasodilator responsiveness (Brodde, 2008b) and thus can be connected with cardiovascular events. Prior studies, however, are not in agreement on which specific

variants of these polymorphisms are associated with enhanced vasodilation (Brodde, 2008b). Our findings are in line with earlier epidemiologic studies that support the protective effect of the Glu<sup>27</sup> allele on risks of MI, as documented both in longitudinal population-based studies (e.g., the Cardiovascular Health Study (Heckbert et al., 2003)) and in case-control studies (e.g., Sala et al., 2001).

As for ADRB2 and cancer, ADRB2 has been shown to stimulate both fibroblast and endothelial cells proliferation (Iaccarino et al., 2005) and has been linked to inhibiting cellular immunity in cancer (Uotila, 1996). Recent studies also suggest a novel molecular pathway involving ADRB2 in the process of malignant transformation. This pathway is associated with repression of the ADRB2 gene expression by a Polycomb group protein (EZH2) that promotes prostate cells invasion (Thompson, 2007; Yu et al., 2007). Dysregulation of EZH2 has previously been linked to a number of cancers, including melanoma, lymphoma, breast and prostate cancers (Bracken et al., 2003; Varambally et al., 2005; Visser et al., 2001; Yu et al., 2007). Recent studies have also demonstrated a potential mechanism connecting behavioral stresses and certain types of cancer that includes ADRB2 mediated activation of the tumor cell cAMP-protein kinase A signaling pathway (Thaker et al., 2007). Epidemiological case-control studies have shown that carriers of the ADRB2 Glu<sup>27</sup> allele could be protected against breast cancer (Huang et al., 2001) and that the Gly<sup>16</sup> allele could be protective against lung cancer (Wang et al., 2006), which is broadly in line with our finding of favorable effect of the Gly<sup>16</sup>Glu<sup>27</sup> haplotype in relation to cancer.

### Mechanisms linking systemic genetic health effects with longevity in evolutionary context

The association patterns of the Gln<sup>27</sup>Glu polymorphism with longevity (Figure 3) exhibit consistent inverse relationships at younger and older ages supporting antagonistic pleiotropy theory on a change of the functional role of genetic variants with aging (Williams and Day, 2003). The effect of the Glu<sup>27</sup> allele tends to be of dominant nature in these associations. This allele, which is protective against the aging-associated diseases, appears to be unfavorable early in life but becomes favorable for longevity at older ages. Figure 4 helps understand why this role changes with aging.

Figure 4A shows that the Gln<sup>27</sup> allele is significantly more frequent than the Glu<sup>27</sup> allele in the young-old population (e.g., who reached ages 65 years, about 40% of the sample, at the end of follow up in 2007) resembling the pattern for the entire sample. This implies that evolutionary selection likely worked against ancestral Glu<sup>27</sup> allele and favored derived Gln<sup>27</sup> allele because mortality selection at young ages could not explain the observed proportions given small death rates of adults (e.g., <http://www.cdc.gov/nchs/deaths.htm>) and infants (Singh and Yu, 1995) in the contemporary U.S.

Figure 4B shows that frequencies of the Gln<sup>27</sup> and Glu<sup>27</sup> alleles are the same in the sample of individuals who died relatively early in life ( $LS \leq 72$  years) whereas the Gln<sup>27</sup> allele is significantly more frequent than the Glu<sup>27</sup> allele in the LL group (who are predominantly survivors;  $LS > 72$  years) resembling patterns in Figure 1A. Furthermore, the frequency of the Gln<sup>27</sup> allele in the sample of individuals who died later in life (Figure 4D,  $LS \leq 83$  years) becomes significantly larger than that of the Glu<sup>27</sup> allele, whereas frequencies of these alleles become the same in the LL group (Figure 4D,  $LS > 83$  years). This implies that the frequency of the Gln<sup>27</sup> allele increases in the group of deceased individuals and declines in the LL group who are predominantly survivors (equivalently, the frequency of the Glu<sup>27</sup> allele exhibits opposite behavior). This dynamics is explained by survival disadvantage of carriers of the Gln<sup>27</sup> allele attributable to increased risks of cancer, MI, and IC as shown in Figure 4C for the case of cancer (similar patterns hold when deaths among MI and IC patients are added). Note that because of the recessive effect of the Glu<sup>27</sup> allele on cancer phenotype (Figure 1A) this protective role is more pronounced for the Glu<sup>27</sup>Glu homozygote.

Thus, the derived Gln<sup>27</sup> allele was selected during evolution because of its favorable role for certain (unknown) conditions beneficial for the early life. However, this advantageous effect appears to be not relevant for the oldest-old ages—which follows from the change of the favorable role of the Gln<sup>27</sup> allele to an unfavorable one (Figure 3). This unfavorable role is the result of a survival disadvantage for carriers of the derived Gln<sup>27</sup> allele and, respectively, a survival advantage for carriers of the ancestral Glu<sup>27</sup> allele associated with the late-life health conditions. Given unknown forces driving the evolutionary advantage of the Gln<sup>27</sup> allele (that deserves further exploration), the entire biochemical mechanism relevant to the observed opposite genetic effects at different ages is unclear. It is clear, however, that in part it should be related to cancer- and CVD-specific pathways (discussed in the previous section). It is worth noting that these findings are in line with an apparently antagonistic effect of genetic variants of the ADRB2 gene on hypertension (Bao et al., 2005) and cognition (Bochdanovits et al., 2009) in early and late life with some indications on a putative role of the white matter integrity in the splenium (Penke et al., 2010).

Our results also add to the discussion on replication of genetic associations in independent samples. Replication is sometimes viewed as a “gold standard” in the era of genome-wide association studies given relevant statistical and technological issues (McCarthy et al., 2008; Ziegler et al., 2008). Only a few candidate longevity-associated genes, among many others, have been replicated thus far in two or more human studies, including APOE (see, e.g., Christensen et al., 2006; Salvioli et al., 2006), CETP (Barzilai et al., 2003; Koropatnick et al., 2008), and FOXO3A (Flachsbart et al., 2009; Willcox et al., 2008a). The question is if lack of replication is a definitive signature of a false positive or not?

Replication in independent samples implies that the effects of the respective genetic variants can: (i) have smaller chances of being an artifact of a particular procedure (e.g., technology, genotyping, sampling, etc.), (ii) be weakly sensitive to population heterogeneity and, consequently, have a stronger systemic component, and (iii) have strong but the same-type sensitivity to population heterogeneity in all studied samples. Therefore, lack of replication might be informative, because this could indicate differences in sensitivity to population heterogeneity in different samples, i.e., provide clues on factors affecting possible discordance.

Our study provides arguments that lack of replication can be evolutionary-driven. For instance, because evolution has worked differently in whites, blacks, and Asians, the frequency of the derived Gln<sup>27</sup> allele appears to be markedly different in these ethnic groups, i.e., 0.54–0.65 in whites, 0.73–0.80 in blacks, and 0.80–0.9 in Asians (Brodde, 2008a). Therefore, an unfavorable role of this allele for the late-life phenotypes (that implies the case of antagonistic pleiotropy) revealed in the FHSO sample of whites might not necessarily be replicated in a sample of blacks or Asians because these late-life phenotypes may not have driven evolutionary selection of the Gln<sup>27</sup> allele. Lack of replication, however, might reveal race-specific factors contributing to the late-life phenotypes. This is in line with increasingly recognized sensitivity of the effect of common polymorphisms to local environment or/and genetic heterogeneity (Zintzaras et al., 2008). The latter factors can even selectively alter the effect of specific polymorphisms not affecting the others even if they are in the same gene and are in modestly-strong LD (Kulminski et al., 2010).

Broadly, evolutionary selection did not directly work against genetic variants which could be harmful for inherently non-Mendelian late-life phenotypes that was suggested in prior works (Medawar, 1952) and evidently seen in our study. The human genome was established to provide better robustness under an adverse environment, which is markedly different from modern conditions, especially in developed societies (Kuningas et al., 2008). Evolutionary forces (e.g., genetic drift) can be responsible for entirely stochastic change in allele frequencies (Zimmer, 2001). These arguments along with others suggest that even a truly positive effect



can be not necessarily replicated in other populations because of the inherent complexity of a net of causation of aging-associated phenotypes, which is not a result of a global genetic program similar to, for instance, the program of development (Thomas and Clayton, 2004; Vijg and Suh, 2005). As a consequence, replication studies should be accompanied by strategies for revealing potential effect modulators.

Our analyses and current knowledge about molecular-biological mechanisms of ADRB2 mediating health effects support a systemic role of the ADRB2 gene in healthy aging. We also show that the same allele of the Gln<sup>27</sup>Glu polymorphism of this gene could be detrimental early in life but its role may change to a favorable one in late life implying the phenomenon of antagonistic pleiotropy. These important findings warrant further exploration in other populations.

Major limitation of our study is a small sample of the oldest-old individuals that prevents generalization of the case of antagonistic pleiotropy to the very old ages. Given concerns on definitive validity of strategy on replication of genetic associations in different setting (see above), the best strategy would be to replicate the results on antagonistic pleiotropy in the same cohort but followed for longer time period. This is a reasonable strategy given the ongoing nature of the FHS.

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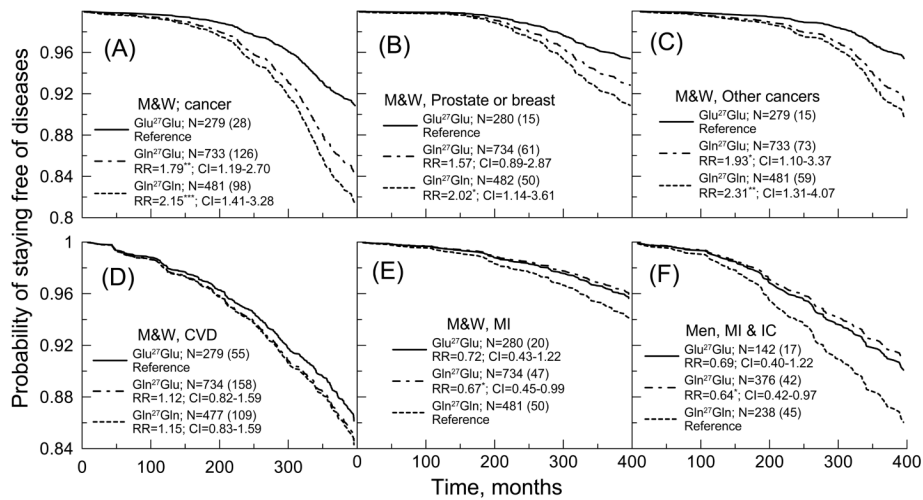
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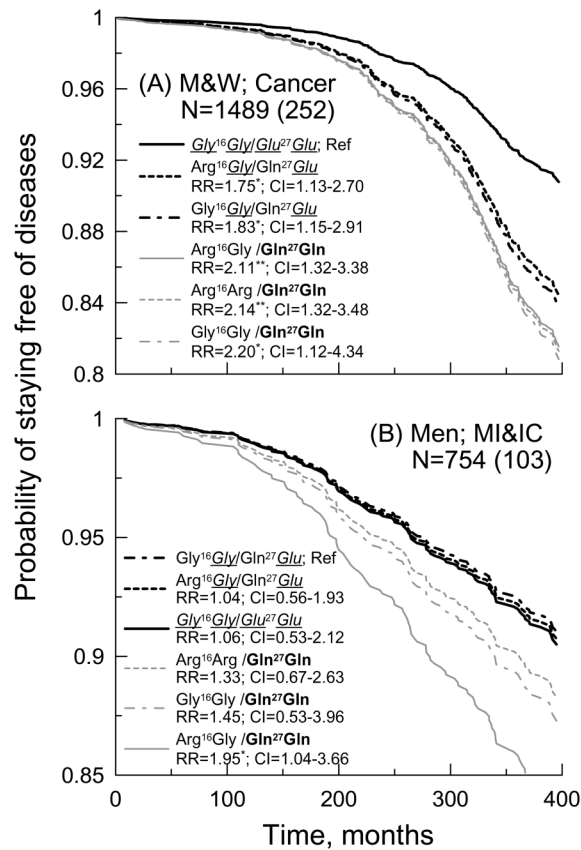
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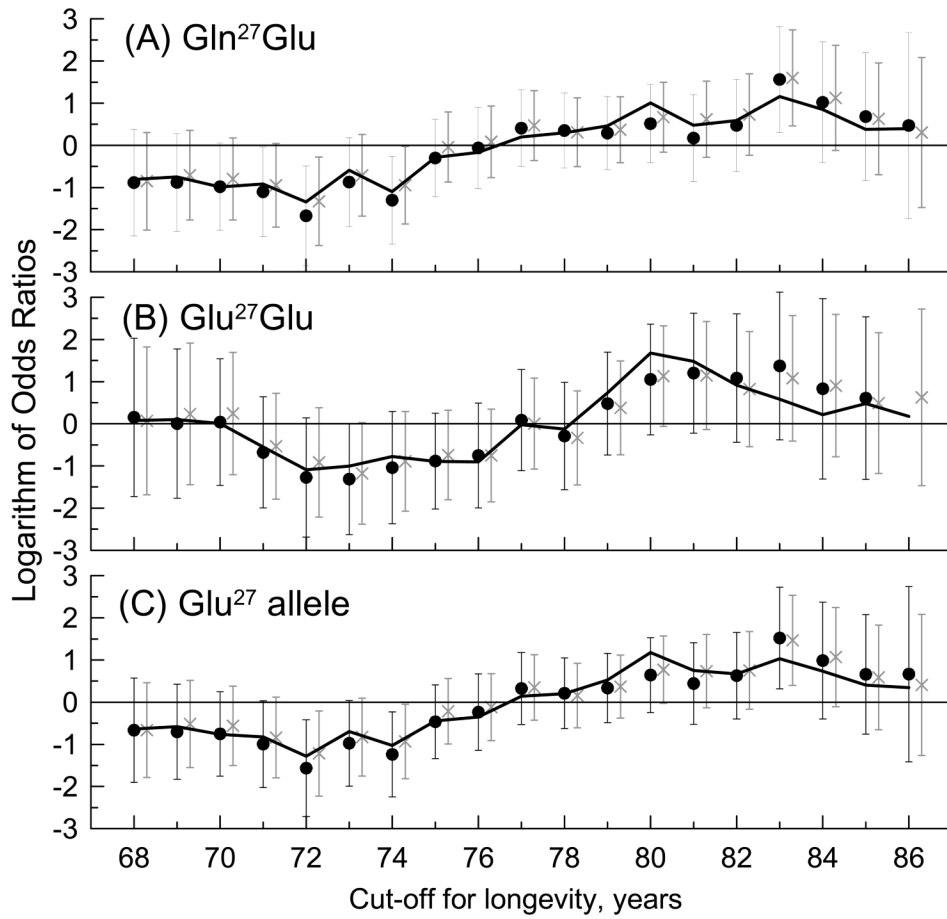


**Figure 1.** Probability of staying free of selected geriatric diseases for carriers of different genotypes of the ADRB2 Gln<sup>27</sup>Glu polymorphism in the FHSO sample (see insets). M&W denotes combined sample of men and women. MI=myocardial infarction. IC=intermittent claudication. The letter “N” in the inset denotes sample size for a given genotype with non-missing information on all covariates. The numbers in parentheses are counts of individuals with onsets of respective condition within the follow-up period (months). The RR (CI) denotes relative risks (confidence intervals) calculated using the Cox regression model adjusted for age, sex (when necessary), systolic and diastolic blood pressures, total and high density lipoprotein cholesterol, body mass index, smoking, and diabetes. \*0.01<p<=0.05; \*\*0.0005<p<=0.01; \*\*\*p<=0.0005.



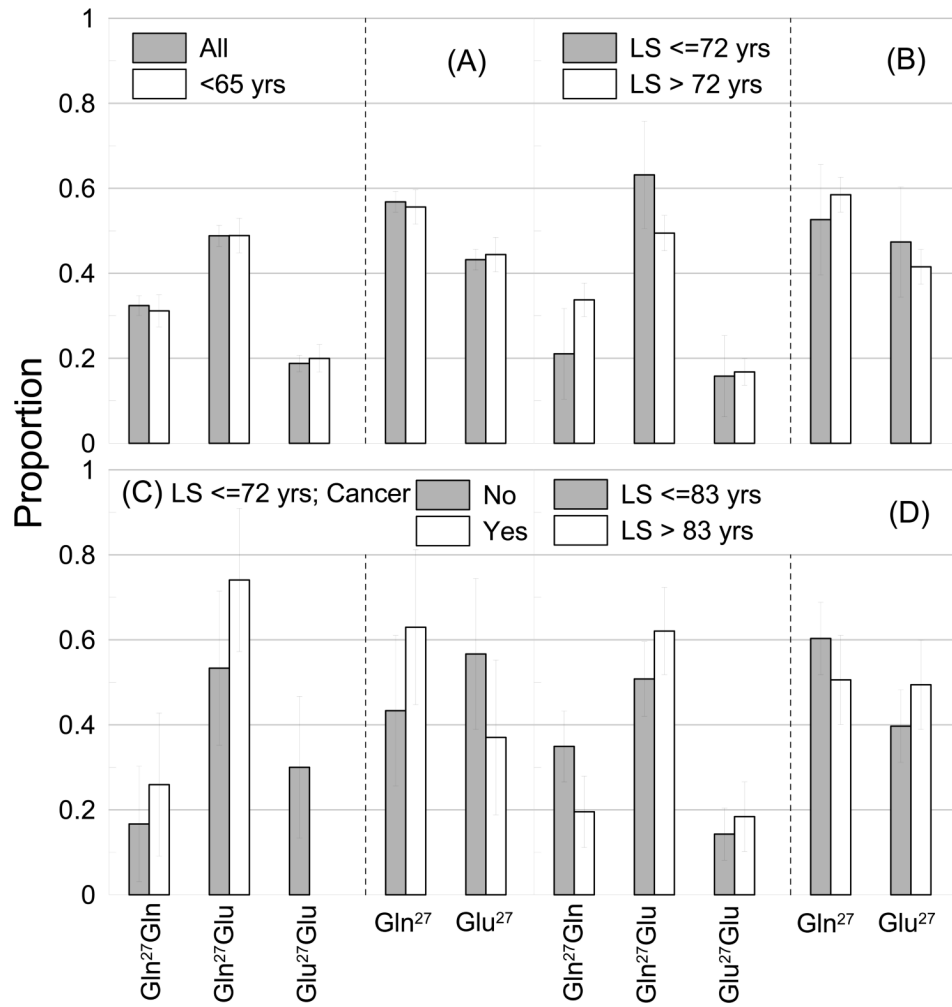
**Figure 2.**

Probability of staying free of (A) cancer and (B) compound phenotype of myocardial infarction (MI) and intermittent claudication (IC) for (A) men and women and (B) men carrying compound genotypes of the ADRB2 Arg<sup>16</sup>Gly and Gln<sup>27</sup>Glu polymorphisms (patterns of probability are ordered according to increasing relative risks; see insets). Protective Gly<sup>16</sup>Glu<sup>27</sup> haplotype is italicized and underlined (given that the Arg<sup>16</sup>Glu<sup>27</sup> haplotype is virtually lacking, see Table 1, haplotypes are uniquely determined from the respective genotypes). The letter “N” in the inset denotes sample size with non-missing information on all covariates. The numbers in parentheses are counts of individuals with onsets of respective condition within the follow-up period (months). The RR (CI) denotes relative risks (confidence intervals) calculated using the Cox regression model adjusted for age, sex (when necessary), systolic and diastolic blood pressures, total and high density lipoprotein cholesterol, body mass index, smoking, and diabetes. \*0.01<p<=0.05; \*\*p<=0.01.



**Figure 3.**

Patterns of chances to live long life for carriers of the (A) Gln<sup>27</sup>Glu and (B) Glu<sup>27</sup>Glu genotypes and (C) Glu<sup>27</sup> allele compared to the Gln<sup>27</sup>Gln genotype (horizontal line). The x-axis shows varying cut-off for longevity (see Section “Aging-associated phenotypes and risk factors”). The logarithm of odds ratios is shown for models with adjustment for (i) age and sex (crosses); (ii) age, sex, systolic and diastolic blood pressures, total and high density lipoprotein cholesterol, body mass index, smoking, and diabetes (filled dots), and (iii) life span without cancer (solid line). Bars show 95% confidence interval.



**Figure 4.** Proportions of carriers of the Gln<sup>27</sup>Gln, Gln<sup>27</sup>Glu, and Glu<sup>27</sup>Glu genotypes in different samples. (A) The entire sample (All) and a subsample of young-old individuals (aged <65 years at the end of follow up in the year 2007). (B) Samples of short-lived ( $LS \leq 72$  years) and long-living ( $LS > 72$  years) individuals. LS denotes life span. (C) Samples of short-lived individuals as in (B) but with history of cancer (Yes) and without it (No). (D) The same as in (B) but for cut-off at 83 years. Bars show 95% confidence interval.



**Table 1**

Mean age (MA) and standard deviation (SD) for carriers of each genotype of the ADRB2 Gln<sup>27</sup>Glu or Arg<sup>16</sup>Gly polymorphism at first FHSO examination, and number of individuals carrying given compound genotypes of the Gln<sup>27</sup>Glu and Arg<sup>16</sup>Gly polymorphisms in the sample of the genotyped FHSO participants.

	Men				Women			
	MA yrs	SD yrs	Gln <sup>27</sup> Gln	Gln <sup>27</sup> Glu	MA yrs	SD yrs	Gln <sup>27</sup> Gln	Gln <sup>27</sup> Glu
MA, yrs	37.2		36.4	35.0	35.5		36.3	36.4
SD, yrs	9.3		10.2	9.5	8.9		9.7	10.1
Arg <sup>16</sup> Arg	36.9	8.9	0	0	36.5	8.7	2	0
Arg <sup>16</sup> Gly	36.8	10.1	235	2	35.3	9.4	125	236
Gly <sup>16</sup> Gly	35.7	9.8	33	144	36.6	9.9	32	148