Genomics of human health and aging

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Abstract Despite notable progress of the candidate-gene and genome-wide association studies (GWAS), understanding the role of genes contributing to human health and lifespan is still very limited. We use the Framingham Heart Study to elucidate if recognizing the role of evolution and systemic processes in an aging organism could advance such studies. We combine throughput methods of GWAS with more detail methods typical for candidate-gene analyses and show that both lifespan and ages at onset of CVD and cancer can be controlled by the same allelic variants. The risk allele carriers are at highly significant risk of premature death (e.g., $RR=2.9$, $p=5.0 \times 10^{-66}$), onset of CVD (e.g., $RR=1.6$, $p=4.6 \times 10^{-17}$), and onset of cancer (e.g., $RR=1.6$, $p=1.5 \times 10^{-6}$). The mechanism mediating the revealed genetic associations is likely associated with biological aging. These aging-related phenotypes are associated with a complex network which includes, in this study, 62 correlated SNPs even so these SNPs can be on non-homologous chromosomes. A striking result is three-fold, highly significant ($p=3.6 \times 10^{-10}$) enrichment of non-synonymous

SNPs ($N=27$) in this network compared to the entire qualified set of the studied SNPs. Functional significance of this network is strengthened by involvement of genes for these SNPs in fundamental biological processes related to aging (e.g., response to stimuli, protein degradation, apoptosis) and by connections of these genes with neurological (20 genes) and cardiovascular (nine genes) processes and tumorigenesis (10 genes). These results document challenging role of gene networks in regulating human health and aging and call for broadening focus on genomics of such phenotypes.

Keywords Aging · Lifespan · Survival · Healthy aging · Gene networks

Introduction

Aging of populations worldwide becomes a matter of governmental and public concern requiring effective strategies to extend healthy lifespan (Sierra et al. 2008; Olshansky et al. 2007). An effective strategy could be yielding insights into genetic predisposition to health traits and longevity. Candidate-gene and genome-wide association studies (GWAS) focus basically on two approaches to address this problem in humans. The most developed is the approach focusing on genetic predisposition to specific geriatric diseases and related traits (Martin et al. 2007; Depp et al. 2007; Franco et al. 2009). The other approach focuses on genetics of

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individuals with exceptionally long life (Bergman et al. 2007; Melzer et al. 2007; De Benedictis et al. 2001; Willcox et al. 2008a). Despite notable progress of these approaches (Salvioli et al. 2006; Christensen et al. 2006; Teslovich et al. 2010; Barzilai et al. 2003; Koropatnick et al. 2008; Willcox et al. 2008a; Flachsbarth et al. 2009; Sebastiani et al. 2010; Ku et al. 2010; Bloss et al. 2011), unraveling the role of genes in regulating health and lifespan and, consequently, potential of these factors for effective interventional strategies is still very limited (Manolio et al. 2008; Cutler and Mattson 2006; McClellan and King 2010; Gibson 2009; Goldstein 2009; Plomin et al. 2009; Gorlov et al. 2008; Lunetta et al. 2007; Newman et al. 2010; Beekman et al. 2010) explaining only a small fraction of genetic susceptibility (Frazer et al. 2009; Kraja et al. 2011). For instance, combined associations of 13 SNPs with blood pressure (BP) revealed by the CHARGE (Levy et al. 2009) and Global BPgen (Newton-Cheh et al. 2009) consortia explained less than 2% of the BP variance (Kraja et al. 2011).

Even more disappointing result is that some genes predisposing to geriatric diseases discovered by GWAS appear to be not correlated with human longevity (Beekman et al. 2010; Deelen et al. 2011). This result questions whether findings obtained from GWAS may provide insights into the bio-genetic mechanisms underlying a healthy lifespan. In fact, this finding is very surprising because (1) genetic studies of non-human species have discovered numerous genes predisposing to aging-related processes (Cutler and Mattson 2006; Vijg and Suh 2005; Kenyon 2005; Johnson 2006; Greer and Brunet 2008), (2) non-genetic association studies show that the long-living individuals are typically in better health compared to the short-living individuals (Barzilai et al. 2003; Willcox et al. 2008b; Willcox et al. 2008a; Evert et al. 2003), and (3) candidate-gene studies (but not GWAS) document that the same genes can affect diseases and lifespan (Koropatnick et al. 2008; Kulminski et al. 2011).

This is an apparent paradox which has to be carefully examined. A prominent geneticist and evolutionary biologist T. G. Dobzhansky asserts that “nothing in biology makes sense except in the light of evolution.” Evolution primarily maximizes fitness of individuals of reproductive age. The classical evolutionary biological theory of aging claims that aging occurs because of decline in the force of natural selection with age (Kirkwood and Austad 2000). Then, according to that

theory, aging-related (senescent) phenotypes with post-reproductive manifestation are non-adaptive and subject to stochastic variation. Therefore, at a first glance evolution should not be relevant to senescent phenotypes (apart so-called grandmother hypothesis; Hawkes et al. 1998). Such phenotypes, however, can be caused by reproductive-age-related risk factors making, thus, evolution to be relevant to them (Vijg and Suh 2005; Di Rienzo and Hudson 2005; Drenos and Kirkwood 2010).

The problem, however, is that the risk factors for senescent phenotypes and the factors maximizing fitness are not necessarily the same. Therefore, while the reproductive-age-related factors can well be relevant to fitness, they can (1) be not relevant to post-reproductive-age diseases at all, (2) predispose to them, (3) be protective of them, or (4) predispose to some diseases but be protective of the others. Accordingly, different modalities of gene action which can modulate the process of aging and the senescent phenotypes are known (Martin 2007) [two notable examples are antagonistic pleiotropy (Martin 2007; Williams and Day 2003; Kulminski et al. 2010; Summers and Crespi 2010; Alexander et al. 2007) and genetic trade-offs (Finch 2010; Charlesworth 1996; Martin 1999; Kulminski et al. 2011)].

Therefore, in general, there are stochastic and non-stochastic (i.e., evolutionary driven) components in genetic predisposition to the senescent phenotypes. The non-stochastic component is largely determined by correlation among the fitness-related and disease-related factors, i.e., mode (1) above implies no correlation and entirely stochastic variation whereas modes (2)–(4) imply some correlation and, accordingly, some deterministic variation. The problem is that we know little about the fitness-related factors; we know, however, that they do exist (Vijg and Suh 2005). Accordingly, there are chances to discover non-stochastic, evolutionary-driven genetic predisposition to the senescent phenotypes. These chances are better if we consider phenotypes which can be caused by multiple risk factors (i.e., highly heterogeneous) assuming that some of them can correlate with the fitness-related factors.

Surprisingly, the role of evolution in many GWAS appears to be underestimated. This is, perhaps, a reason why traditional strategy in GWAS is to make a complex post-reproductive trait of interest to be less heterogeneous (Arking and Chakravarti 2009). Although this might be entirely compelling strategy for studies of

conventional (non-genetic) risk factors, it definitely decreases chances of discovering evolutionary-driven genetic determinants of the senescent phenotypes.

On the other hand, the same evolutionary-motivated strategy suggesting to focus on more heterogeneous phenotypes (as opposite to more homogenous) can be highly beneficial for unraveling genetic predisposition to fundamental mechanisms of intrinsic biological aging and, consequently, to geriatric diseases. Indeed, aging is associated with systemic remodeling of an organism's functioning which increases chances of virtually all geriatric disorders (Franco et al. 2009; Franceschi et al. 2000; Martin et al. 2007; Cutler and Mattson 2006). Experiments with laboratory animals (Johnson 2006) and heritability estimates in humans (Christensen et al. 2006; Iachine et al. 1998) show that aging can be genetically regulated (Finch and Tanzi 1997; Martin et al. 2007; Vaupel 2010). Accordingly, yielding insights in genetic predisposition to aging-related processes in an organism could be a major breakthrough in preventing and/or ameliorating not one geriatric trait, but perhaps a major subset of such traits (Martin et al. 2007) that can greatly advance progress in solving the problem of extending healthy lifespan in humans.

Recognizing the role of evolution and systemic aspects of the aging-related changes in an organism suggests a promising strategy for unraveling genes predisposing to health and lifespan. This strategy combines throughput methods of GWAS for screening large arrays of SNPs for potentially useful associations with more detail analyses of mechanisms driving these associations which are typical for candidate gene studies. In this study, we follow the proposed strategy to elucidate if it can essentially advance our understanding of human health and lifespan. We focus on the Framingham Heart Study (FHS) participants followed for up to about 60 years.

Materials and methods

Study design and population

The FHS data are available from the NIH SHARe through dbGaP. In this study, we focus on participants of the FHS (launched in 1948, 5,209 respondents aged 28–62 years) and the FHS Offspring (FHSO, launched in 1971–1975, 5,124 offspring and their spouses aged

5–70 years) cohorts for whom survival information is available. Phenotypic and genotyping data have been previously described (Govindaraju et al. 2008; Splansky et al. 2007; Cupples et al. 2009). We have used SNP data from the custom Affymetrix 50K Human Gene Focused chip available for 5,036 participants of these cohorts. After quality control test [excluding if HWE p values $<10^{-2}$, missingness $>10\%$, Mendel errors $>2\%$, and MAF (minor allele frequency) $<2\%$] and exclusion of sex chromosomes, about 38K SNPs were left for the analyses. Given random missing information on SNPs and phenotypes, specific number of the study participants is detailed for each analysis.

Analysis

A two-stage strategy is motivated by systemic processes in an aging organism and the role of evolution in developing traits with post-reproductive manifestation (as discussed in the “Introduction”). This strategy is implemented through combining methods of GWAS (first stage) with those which are typical for candidate gene (second stage) studies. GWAS is used for tentative pre-selection of candidate SNPs on the basis of their potential associations with complex, aging-related traits. More detail analysis of action of the pre-selected SNPs is performed at the second stage.

If the same loci are associated with multiple senescent traits, this likely implies that these associations are mediated by basic biology of aging (Goh et al. 2007; Martin et al. 2007). Accordingly, to increase chances of revealing genes that can affect this biology we use several aging-related phenotypes (called endophenotypes, EPs) for SNP pre-selection at the first stage.

First stage

Representatively, four highly heterogeneous unadjusted aging-related traits were selected as EPs. They include prevalence of cardiovascular disease (CVD; 1,669 cases) and prevalence of cancer (1,060 cases) (measured during the entire follow-up period through 2007) as well as total cholesterol (TC, mg/100 ml) and systolic blood pressure (SBP, mmHg). CVD was categorized as having being diagnosed with any disease of heart or stroke vs. no those diagnoses. Cancer was characterized as having being diagnosed with any cancer except skin. Quantitative EPs were representatively assessed at the baseline

examinations (mean age is 36.6 years, SD=9.4 years). The SBP was measured in all genotyped subjects ($N=5,036$) whereas TC was measured in 4,472 subjects.

Screening of all SNPs for their potential association with each of the selected EPs was performed using genome-wide univariate technique. Specifically, each qualified SNP from the 38 K set was tested for its association with each selected EP using unadjusted logistic/linear regression model, as appropriate, and additive genetic model using *plink* v. 1.07 (Purcell et al. 2007). The result of these analyses is a list of SNP–EP associations. Each SNP can be associated with one to four EPs. Significance of the associations was used for pre-selection of the reasonable number of the candidate SNPs. SNPs were pre-selected given conservative genome-wide significance level for 38 K tests, i.e., $p < 10^{-6}$ for at least one of the four EPs.

Second stage

At this stage, we focus only on the pre-selected SNPs and use traditional candidate-gene-like techniques and models to ascertain mechanisms of SNP actions. Adjusted linear and logistic regression models were used to more rigorously ascertain the associations among the pre-selected SNPs and quantitative or prevalence-type EPs. Associations of the pre-selected SNPs with time-to-event phenotypes were characterized by empirical Kaplan–Meier estimates of the age at onsets of CVD (diseases of heart and stroke) and cancer (all sites but skin) and the age at death. The FHS and FHSO cohorts have been followed for the onset of CVD and cancer, and death through regular examinations at the FHS clinic, surveillance of hospital admissions, and death registries (Govindaraju et al. 2008; Splansky et al. 2007) for up to about 60 years currently through 2007. Kaplan–Meier empiric was complemented by evaluation of the relative risks of death using the proportional hazard Cox regression model. Chronological age, e.g., age at baseline plus time elapsed since the baseline examination through 2007, was used as a time variable in these analyses. The models were adjusted as discussed in the text. Relatedness for the Cox regression model was categorized as singleton and one individual per extended family (“an extended family is made up of any related nuclear families”; Splansky et al. 2007) vs. other family members. These analyses were performed using SPSS software (release 17.0, Chicago, IL, USA).

Functional annotation

Statistical evidences were integrated with functional genomics annotation using Gene Ontology (GO) for Functional Analysis (GOFFA) tool (Sun et al. 2006). p values for gene enrichment in biological processes and molecular functions were calculated for heuristic proposes only using right-sided Fisher's exact test implemented in GOFFA. The analyses were limited to biological processes and molecular functions with two and more genes.

Results

The EP-based pre-selection

Following our strategy (see “Materials and methods”), we selected four representative EPs (prevalence of CVD and cancer, total cholesterol, and systolic blood pressure) and screened each of them for associations with each of 38 K SNPs passed quality control tests (see “Materials and methods”). Adopting genome-wide significance level ($p < 10^{-6}$), 63 candidate SNPs were pre-selected (Online Resource 1). Of them, 12, 19, 17, and 15 SNPs were associated with one, two, three, and four EPs, respectively (Online Resource 1). These multiple associations can be the result of (1) correlations among SNPs, (2) correlations among EPs, (3) trivial factors (e.g., stochasticity), and (4) underlying biology. Further analyses explore factors which can drive those associations.

Linkage disequilibrium (LD)

First, we evaluated LD among the pre-selected SNPs by calculating pair-wise r^2 statistics for founders only (Wigginton et al. 2005) using *plink* v. 1.07 (Purcell et al. 2007) in order to reduce the number of independent SNPs. Figure 1 shows that virtually all SNPs are correlated. Important result is that these SNPs show not only intra-chromosomal LD but also inter-chromosomal LD. Correlation among SNPs implies coherent clustering of alleles of different SNPs in the same individuals [e.g., minor allele of one SNP sticks together with minor allele of another SNP(s)]. Accordingly, SNPs in strong LD are statistically indistinguishable and any of them can be used as a proxy of an entire set of such SNPs. We selected three

Fig. 1 Map of intra- and inter-chromosomal linkage disequilibrium among the pre-selected SNPs. SNPs are ordered along the x- and y-axes by chromosome and base pair number. Pixels report pair-wise linkage disequilibrium level (r^2) using colored scale shown in the inset. SNP IDs correspond to those in Online Resource 1

representative SNPs for further analyses. The rs9330200 (Fig. 1, SNP ID #35) and rs2292664 (SNP ID #41) SNPs were selected to represent two SNP sets exhibiting largely non-overlapping LD patterns. The rs5491 (SNP ID #54) represents SNPs with overlapping LD patterns. These three SNPs absorb statistical effects of the other SNPs in LD (Online Resource 2) and, thus, they can be considered as proxies for all SNPs in LD.

Candidate-gene-like analyses

Adjustment of the SNP–EP associations revealed at the pre-selection stage by sex makes no difference in the effects (Online Resource 3). Contrarily, adjustment by age considerably attenuates those associations making certain of them to be above genome-wide significance level (Online Resource 3). Relatedness of the FHS participants plays at most minor role in the revealed associations (Online Resource 3) and, thus, it can be disregarded (some analyses below, however, were adjusted for relatedness to ensure that it makes no difference either in the results or conclusions).

Next we elucidate mechanisms which could attenuate the revealed associations. For instance, sensitivity to age might be due to biased selection of the risk and

non-risk allele carriers or due to unbalanced aging of carriers of these alleles, e.g., when the risk allele carriers die prematurely. In the latter case, such SNPs (and genes for them) can be compelling candidates to characterize the aging-related processes.

The nature of a mechanism driving sensitivity to age can be conveniently tested by evaluating empirical life expectancy (LE) and survival patterns of age at death for the risk and non-risk allele carriers. Kaplan–Meier estimates show that the minor (risk) allele carriers of the proxy SNPs do indeed live significantly shorter lives than their major allele homozygous age-peers (Fig. 2; LE estimates). For instance, the rs5491 explains 8.7 year difference in the LE for them. The minor allele carriers are at highly significant risk of premature death (Fig. 2, log-rank estimates). Importantly, Fig. 2 shows that empirical survival curves for the risk allele carriers resemble those for the non-risk allele homozygotes except virtually parallel shift to the left. This pattern implies that the minor allele carriers are at high risk of death at any age, i.e., that they can age faster than their major allele homozygous age-peers.

The revealed associations with lifespan were further tested in the Cox regression models (Table 1). First we show that the model estimates of significance of the relative risks of dying prematurely for the minor allele carriers (i.e., at younger ages compared to those for the major allele homozygotes) resemble the results of the empirical estimates in Fig. 2. Adjustment for sex did not change the estimates (not shown). Further adjustment for age improves the unadjusted estimates. As expected (see Online Resource 3), adjustment for relatedness (defined in the “Materials and methods” section) does not make any difference.

Further insights on the relevance of the correlated SNPs to the process of aging can be gained from the analyses of age at onset of major aging-related diseases available in the FHS, i.e., CVD and cancer. Empirical estimates show that the minor-allele carriers typically contract CVD and cancer earlier in life than the major-allele homozygotes (Online Resource 4). The difference in healthy LE (defined as life without CVD or cancer; see Online Resource 4) for them can be about 7 years for CVD (rs5491) and cancer (rs2292664). Significance of these associations was slightly improved by adjustments for age and sex in the Cox regression models (Table 2). Similarly to survival, relatedness did not affect these estimates.

Fig. 2 Empirical Kaplan–Meier survival age patterns. Survival curves show the age at death and the age at the end of follow-up in 2007 for deceased (living) individuals. Survival curves are plotted for the minor-allele carriers and major-allele homozygotes of **a** rs9330200, **b** rs2292664, and **c** rs5491. Letter “n” denotes the number of total/died individuals. *LE* life expectancy, *CI* confidence interval

Table 1 Relative risks of dying at younger ages for the minor allele carries for each of the three proxy SNPs compared to the major allele homozygotes in the Cox regression model

Adjustment	rs9330200		rs2292664		rs5491	
	RR	<i>p</i> value	RR	<i>p</i> value	RR	<i>p</i> value
No	2.31	9.5E–42	2.23	2.5E–33	2.58	2.1E–58
Sex and age	2.76	2.0E–52	2.40	1.7E–37	2.89	5.1E–66
Sex, age, and relatedness	2.79	6.5E–53	2.40	1.4E–37	2.89	5.0E–66

The sample size is shown in Fig. 2

RR relative risk

Adjustment of the Cox models for the ages at onset of CVD by prevalence of cancer virtually did not change the estimates (Table 2). Adjustment of the models for cancer (Table 2) by prevalence of CVD shows modest improvement of the relative risk and significance estimates for the rs9330200 making this association to be close to the originally pre-determined genome-wide level ($p=10^{-6}$). Weak sensitivity of the models for CVD to adjustment for cancer and vice versa implies that the associations of the proxy SNPs with CVD and cancer are virtually independent.

The latter result is intuitively clear because CVD and cancer are diseases with distinct etiologies that results in the lack of noticeable correlation among them (e.g., Pearson two-tailed correlation coefficient for prevalence of CVD and cancer is $r=0.054$). Therefore, probability of a sampling error (due to multiple testing) when the same SNP shows pleiotropic

associations with uncorrelated phenotypes is a product of probabilities for each phenotype. This means that the threshold for genome-wide significance for the associations with CVD and cancer can be set at $p < 10^{-3}$ (because $10^{-3} \times 10^{-3} = 10^{-6}$) strengthening significance of the estimates.

Table 2 shows that unlike survival (Table 1), the associations with ages at onset of CVD and cancer are modest and less significant implying that differences in survival for the minor-allele carriers and major-allele homozygotes can be unlikely explained solely by the CVD- and cancer-related deaths. Table 3 shows that this is indeed the case. Specifically, adjustment of the Cox survival models by either prevalence of CVD or cancer explains minor reduction of the relative risks of premature death for the minor allele carriers. Adjustment for both CVD and cancer shows that they modulate the relative risks of premature deaths additively.

Table 2 Relative risks of having onset of CVD or cancer at younger ages for the minor allele carries for each of the three proxy SNPs compared to the major allele homozygotes in the Cox regression model

Model	Adjustment	rs9330200		rs2292664		rs5491	
		RR	<i>p</i> value	RR	<i>p</i> value	RR	<i>p</i> value
CVD	No	1.51	2.1E–08	1.63	3.0E–10	1.72	4.2E–16
	Sex and age	1.60	4.9E–10	1.65	1.7E–10	1.76	1.1E–16
	Sex, age, and relatedness	1.60	6.5E–10	1.65	1.7E–10	1.76	1.3E–16
	Sex, age, relatedness, and cancer	1.63	1.8E–10	1.68	6.0E–11	1.55	4.6E–17
Cancer	No	1.21	4.6E–02	1.48	6.2E–05	1.32	1.7E–03
	Sex and age	1.35	2.4E–03	1.55	1.0E–05	1.41	1.2E–04
	Sex, age, and relatedness	1.37	1.1E–03	1.55	9.7E–06	1.43	7.3E–05
	Sex, age, relatedness, and CVD	1.47	8.2E–05	1.69	1.7E–07	1.55	1.5E–06

The sample size is shown in Online Resource 4

CVD cardiovascular disease, RR relative risk

Table 3 Relative risks of premature death for the minor allele carries for each of the three proxy SNPs in the Cox regression model adjusted for prevalence of cancer or/and CVD

Model	Covariate	rs9330200		rs2292664		rs5491	
		RR	<i>p</i> value	RR	<i>p</i> value	RR	<i>p</i> value
Base ^a	SNP	2.79	6.5E–53	2.40	1.4E–37	2.89	5.0E–66
Base and cancer	SNP	2.68	5.8E–49	2.37	2.3E–36	2.86	1.0E–64
	Cancer	1.44	3.9E–11	1.48	6.7E–13	1.49	2.6E–13
Base and CVD	SNP	2.65	5.8E–50	2.31	6.6E–34	2.81	1.1E–61
	CVD	1.26	2.5E–05	1.25	5.6E–05	1.24	9.6E–05
Base, cancer, and CVD	SNP	2.62	1.9E–46	2.27	1.0E–32	2.78	2.9E–60
	Cancer	1.45	1.2E–11	1.49	2.7E–13	1.50	1.2E–13
	CVD	1.28	8.1E–06	1.27	2.3E–05	1.25	4.5E–05

The sample size is shown in Fig. 2

RR relative risk

^a Base is a model from Table 1 adjusted for sex, age, and relatedness; it is given for convenience

Biological role

The results of the survival analyses and analyses of morbidity show that the risk alleles of the revealed SNPs can be involved in the aging process. Because virtually all these SNPs are in LD (Fig. 1), risk alleles of these SNPs cluster in the same individuals. This implies that the correlated SNPs should be working in some kind of biological network to influence complex, aging-related phenotypes. Do these SNPs really pinpoint genes with important biological role?

Annotation of the 62 pre-selected SNPs in LD (one SNP, rs12512353, did not show LD to other SNPs and, thus, it was disregarded in further analyses) reveals a striking result that 27 of 62 SNPs, i.e., 43.5%, are non-synonymous coding polymorphisms (Online Resource 5). Because these SNPs can alter amino acid sequence of proteins, they are biologically important. This high proportion is contracted by modest proportion of about 15% of such SNPs in the entire qualified set of about 38 K SNPs (from the Affymetrix 50 K array; see “Materials and methods”). The three-fold enrichment of non-synonymous coding SNPs in the pre-selected set is highly significant with a two-sided *p* value for the difference in these two proportions $p=3.6 \times 10^{-10}$. This result implies that probability of stochastic clustering of these SNPs in the pre-selected set is immensely small compared to that expected by chance and it is far below the 5% (i.e., $p=0.05$) cut-off for significance in this analysis.

Further inspection of a set of these 62 SNPs shows that 48 of them are within regions of protein coding genes (Online Resource 5). The remaining 14 SNPs are within regions of non-coding genes (five) and are intergenic variants. To characterize biological role of genes for these 14 SNPs, we identified the nearest protein coding gene or locus (includes two to three genes) within 60-kb region (for rs4335625 the nearest protein coding gene was about 75 kb apart) for these 14 SNPs. This annotation results in 76 genes (Online Resource 5).

Analysis of published research shows that functional significance of the revealed genes and their connections with diseases has been extensively studied (Online Resource 5).

Analysis of GO biological processes using GOFFA (see “Materials and methods”) reveals 50 (of 76) genes with known GO terms (Online Resource 7). These genes cluster in the GO biological processes playing a fundamental role in functioning of an aging organism related to cell adhesion, cell communication, response to stimuli, multi-organism interaction, transport, metabolism, regulation, development, cellular organization, and neurological processes (Online Resource 6). Significant over-representation of genes is observed in several specific processes related to leukocyte adhesion, neuropeptide signaling, photoreceptor cell maintenance, cholesterol transport, leukocyte migration, digestion, maintenance of organ identity, and cell differentiation (Table 4). However, because statistical inferences on

Table 4 Selected results of the analysis of 50 genes for the revealed SNPs on enrichment in Gene Ontology (GO) biological processes

GO categories and subcategories	Count	<i>p</i> value	Genes
Cell adhesion	6	0.025	<i>AZGP1, CD36, GPR98, ICAM1, ITGB2, PCDN9</i>
Cell–cell adhesion	4	0.012	<i>GPR98, ICAM1, ITGB2, PCDN9</i>
Leukocyte adhesion	2	2.7×10^{-3}	<i>ICAM1, ITGB2</i>
Signal transduction	13		
Small GTPase-mediated signal transduction	3	0.049	<i>RGL3, TTN, USP6</i>
Neuropeptide signaling pathway	3	2.4×10^{-3}	<i>GPR133, GPR98, HCRT2</i>
Second-messenger-mediated signaling	3	0.043	<i>CD36, HCRT2, PI4KA</i>
Neurological system process	6		
Synaptic transmission	4	0.029	<i>DBH, HCRT2, KCNQ2, PI4KA</i>
Photoreceptor cell maintenance	2	1.3×10^{-3}	<i>GPR98, USH2A</i>
Response to stimulus	20		
Leukocyte-mediated immunity	3	0.015	<i>DBH, ICAM1, TUBB2C</i>
Response to inorganic substance	3	0.020	<i>EEF1A2, NEDD4L, TTN</i>
Localization	13		
Lipid transport	3	0.012	<i>ABCA7, CD36, SOAT2</i>
Cholesterol transport	2	6.8×10^{-3}	<i>CD36, SOAT2</i>
Endocytosis	3	0.040	<i>ABCA7, CD36, NEDD4L</i>
Leukocyte migration	3	1.8×10^{-3}	<i>DBH, ICAM1, ITGB2</i>
Regulation of biological quality	10	0.057	<i>CD36, DBH, FGGY, ITGB2, MUC6, NEDD4L, SOAT2, SERPIND1, SOAT2, TPO</i>
Anatomical structure homeostasis	2	0.030	<i>FGGY, MUC6</i>
Digestive system process	2	6.8×10^{-3}	<i>MUC6, SOAT2</i>
Regulation of molecular function	7	0.022	<i>HCRT2, ICAM1, MAP4K1, PSMB1, TTN, USP6, WRN</i>
Developmental process	14		
Maintenance of organ identity	2	3.5×10^{-5}	<i>GPR98, USH2A</i>
Foam cell differentiation	2	2.2×10^{-3}	<i>CD36, SOAT2</i>

p value was evaluated using Fisher's exact test. Genes were not shown if *p* > 0.1 (complete information is given in Online Resource 6)

enrichment of relatively small number of genes are not highly reliable (Online Resource 6), these *p* values should be considered as heuristic measures.

Analysis of biological role of 26 genes not annotated by GOFFA for GO processes (see non-bolded gene symbols in Online Resource 5) shows that they likely complement the GO biological processes presented in Table 4. For example, the *CNBD1*, *DENND3*, *DOCK8*, *RAI14*, *RIMBP2*, and *SAMD7* genes likely extend the set of genes involved in signaling events and the *CSMD2*, *CSMD3*, *DCDC2C*, *FLNC*, *RAI1*, *SAMD7*, and *SAMD11* genes likely extend the set of genes involved in the developmental processes.

Analysis of the GO terms reveals 57 genes with the GO molecular functions related to binding, catalytic

activity, and enzyme regulator activity (Online Resource 7). Genes related to fatty acid binding ($p = 6.8 \times 10^{-3}$), purine ribonucleotide binding ($p = 1.5 \times 10^{-3}$), cytoskeletal protein binding ($p = 4.3 \times 10^{-3}$), nucleoside-triphosphatase activity ($p = 6.7 \times 10^{-3}$), and GTPase regulator activity ($p = 7.6 \times 10^{-3}$) were highly over represented (Online Resource 7).

Discussion

Common practice in GWAS of complex, aging-related phenotypes is to focus on unraveling associations of SNPs with residual variations in such phenotypes after adjustment for potentially confounding non-genetic

factors (see, e.g., Kathiresan et al. 2009). Insights from the evolutionary aging theories and biology of aging (see “Introduction”) suggest that such strategy might be not entirely compelling. This might be one of the explanations of minor progress of GWAS in discovering new genes with a potential to regulate lifespan (Newman et al. 2010; Lunetta et al. 2007) as well as senescent traits and lifespan (Beekman et al. 2010; Deelen et al. 2011). This is an important problem because GWAS is thought should advance the progress on revealing genetic determinants of healthy life. Our study suggests that this situation can be improved by employing strategies which can deal with complexity of the senescent phenotypes. One such a strategy, implemented in this study, combines GWAS, used for tentative pre-selection of potentially important SNPs, with candidate-gene-like analyses of the pre-selected SNPs in order to better map mechanisms of the SNP-phenotype associations.

First, most important results of this study is that following our strategy (called here as systemic) we show that, unlike prior GWAS-based studies, both lifespan and ages at onset of CVD and cancer can be controlled by the same allelic variants. For instance, these alleles can explain the 8.7-year difference in the lifespan of carriers of the risk and non-risk alleles. The risk allele carriers are at highly significant risk of premature death (e.g., $RR=2.9$, $p=5.0\times 10^{-66}$), onset of CVD (e.g., $RR=1.6$, $p=4.6\times 10^{-17}$), and onset of cancer (e.g., $RR=1.6$, $p=1.5\times 10^{-6}$).

We further show that empirical survival age patterns for the risk and non-risk allele carriers are virtually parallel with the age pattern for the risk allele carriers shifted to the left (Fig. 2). This finding, along with premature development of CVD and cancer in these individuals (Table 2) are epidemiological signatures of premature aging of the risk allele carriers. The observation that the same allelic variants favor premature onset of CVD and cancer, i.e., diseases having distinct etiologies, and influence lifespan implies that the revealed genetic associations have to be mediated by a common mechanism. Given these epidemiological evidences, this mechanism should be relevant to the aging process. This conclusion is further strengthened by weak sensitivity of the associations of the revealed allelic variants with the risks of premature death to prevalence of CVD and cancer (Table 3).

Aging is an extremely complex process associated with interplay of genetic, biochemical, and metabolic

factors in an organism in a given environment. Although genetic studies of various animal models suggest that even a single-gene mutation can remarkably extend lifespan (Kenyon 2005; Johnson 2006) and, thus, modulate aging, no such genes are revealed in humans so far. Given that a human organism is a much more complex system than a model organism (Christensen et al. 2006), it is evident that genetic effects on the aging process should be mediated via coordinate action of a large number of inter-related processes (Kirkwood 2011). Coordinated function is rather relevant to complex biological (Soltow et al. 2010; Slagboom et al. 2011) and genetic (Bloss et al. 2011) networks than to individual genes.

In agreement with these insights, our study documents that indeed lifespan and ages at onset of CVD and cancer can be regulated by a complex network which includes, in this study, 62 correlated SNPs even so these SNPs can be on non-homologous chromosomes. This is an important result implying that genes for these SNPs can work together in a coordinated fashion. This result is in line with recent findings in the mouse (Graber et al. 2006; Petkov et al. 2005, 2007) and it deserves separate detail analyses (Kulminski 2011).

Second most important result is three-fold highly significant ($p=3.6\times 10^{-10}$) enrichment of 27 SNPs which can alter amino acid sequence of proteins in the network of 62 correlated SNPs compared to the entire qualified set of 38 K studied SNPs. The importance of this result is two-fold. First, the observation that 27 of 62 SNPs are non-synonymous coding polymorphisms is contrasted by typical situation in GWAS that most of the discovered SNPs are located either in intron, intergenic or gene desert regions and that only a small number of the risk alleles are non-synonymous SNPs (Ku et al. 2010). Second, highly significant enrichment of 27 non-synonymous SNPs in the pre-selected set and highly significant associations of SNPs from this network with survival and ages at onset of CVD and cancer provide compelling evidences that our findings are real in the FHS data by showing immensely small probability ($p=3.6\times 10^{-10}$) that this network has been generated by a stochastic process of a genetic or non-genetic origin (e.g., genotyping errors, sampling errors, etc.).

Functional significance of the revealed network is further strengthened by biological role of the revealed genes. Specifically, such genes cover GO biological processes related to cell adhesion, cell communication,

response to stimulus, metabolic processes including macromolecular processes such as DNA repair and replication, homeostasis, developmental processes, and regulatory processes among other biological processes in an aging organism. Importantly, a large group of genes play a role in complex cellular processes (e.g., metabolism and cell communication) thereby implying that the same gene can be involved in different processes. These genes are also involved in certain important GO molecular functions (Online Resource 7).

Analysis of prior research (Online Resource 5) shows that the revealed genes can be explicitly involved in other key biological processes in an organism whose role is known to be changing with aging. Specifically, ten genes (*BAZ2B*, *HMGB4*, *NOC2L*, *RAI1*, *SIK1*, *SMARCA2*, *SPZ1*, *TBP*, *TRIP13*, and *ZKSCAN1*) regulate transcription which is believed to be disrupted when an organism is getting older (Roy et al. 2002). The *DBH*, *TPO*, and *LSS* genes are involved in synthesis of catecholamine, thyroid, and vitamin D hormones, respectively. The *GPER* binds estrogen and *HCRT2* binds orexin-A and orexin-B neuropeptide hormones. Hormonal deregulation with aging is considered to be one of the major components of senescent processes in an organism (Barzilai and Gabriely 2010). Five genes (*ATG2A*, *NEDD4L*, *PSMB1*, *UBXN4*, and *USP6*) are involved in degradation of proteins through ubiquitin–proteasome and the lysosomal/autophagic system. Dysfunction of this system leads to accumulation of damaged proteins in an organism that is associated with aging (Koga et al. 2011). Protein degradation through ubiquitin-mediated proteolysis plays an important role in cell-cycle regulation (Reed 2003). The *PSMB1*, *SIK1*, *TRIP13*, and *TTN* genes in the revealed set coordinate cell cycle. Cell cycle is linked with the aging-related processes in humans through a gradual increase in cell division errors in all tissues in an organism (Ly et al. 2000). Five genes (*EEF1A2*, *DBH*, *ITGB2*, *TUBB2C*, and *WRN*) take part in regulation of apoptosis which plays an important role in the aging process and tumorigenesis (Salvioli et al. 2008). Seven genes (*ABCA7*, *AZGP1*, *CD36*, *DEGS2*, *LSS*, *PI4KA*, and *SOAT2*) are involved in lipid metabolism which plays one of the key roles in human longevity and healthy aging (Barzilai et al. 2003).

Involvement of genes in a wide range of fundamental biological processes suggests also a broad role of these genes in regulating the aging-related phenotypes.

Analysis of published research reveals (Online Resource 5) that 20 genes (*CSMD2*, *CSMD3*, *DBH*, *DCDC2C*, *DOCK8*, *FGGY*, *GPR98*, *HCRT2*, *KANK1*, *KCNQ2*, *KLHL17*, *PCDH9*, *RAI1*, *RIMBP2*, *SMARCA2*, *TBP*, *TRAPPC9*, *UBXN4*, *USH2A*, and *WRN*) can play a role in the nervous system development and in the development of neurological traits. Recent studies suggest that the nervous system may act as a central regulator of aging by coordinating the physiology of extraneural tissues (Bishop et al. 2010). Nine genes (*CD36*, *DOCK8*, *GPR133*, *MAP4K1*, *NEDD4L*, *SERPIND1*, *SIK1*, *TNNI3K*, and *TTN*) can coordinate functioning of the cardiovascular system. Importantly, recent studies show that neurological disorders can be causally linked with CVD and that these traits can share the same mechanisms (Manev 2009; Samuels 2007; Finch 2005). Ten genes (*AZGP1*, *CSMD2*, *DOCK8*, *ICAM1*, *KANK1*, *LSS*, *MAP4K1*, *MUC6*, *TRIP13*, and *USP6*) were shown to be associated with various cancers.

Currently prevailing studies of genetic and biological origin of human health and longevity follow largely two approaches which focus on the aging-related diseases and on individuals with exceptionally long lives (Martin et al. 2007). This study provides de facto the rationale for a new approach. Specifically, Fig. 2 suggests that a promising strategy could be to focus on individuals who died prematurely. Studies of genetic profiles of short-lived subjects compared to those who aged more successfully (i.e., those who lived longer and perhaps healthier lives) can be a core of this strategy. Importantly, this strategy can be naturally implemented in longitudinal studies of aging and longevity by focusing on individuals who died first.

Thus, this work provides compelling evidences that senescent phenotypes and lifespan can be efficiently controlled by complex genome-wide networks of coherently (i.e., in the same individuals) working genes which can be involved in regulation of important biological processes in an aging organism. According to this result, in addition to the investigation of the effect of single genes on aging-related phenotypes, association studies should more widely focus on genomics of such phenotypes. As a consequence, traditional GWAS approach might be not sufficient to gain more insights into genomics of healthy life and more rigorous methods are needed.

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