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A genome-wide association study of aging

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Abstract

Human longevity and healthy aging show moderate heritability (20%–50%). We conducted a meta-analysis of genome-wide association studies from 9 studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium for 2 outcomes: (1) all-cause mortality, and (2) survival free of major disease or death. No single nucleotide polymorphism (SNP) was a genome-wide significant predictor of either outcome ($p < 5 \times 10^{-8}$). We found 14 independent SNPs that predicted risk of death, and 8 SNPs that predicted event-free survival ($p < 10^{-5}$). These SNPs are in or near genes that are highly expressed in the brain (*HECW2*, *HIP1*, *BIN2*, *GRIA1*), genes involved in neural development and function (*KCNQ4*, *LMO4*, *GRIA1*, *NETO1*) and autophagy (*ATG4C*), and genes that are associated with risk of various diseases including cancer and Alzheimer's disease. In addition to considerable overlap between the traits, pathway and network analysis corroborated these findings. These findings indicate that variation in genes involved in neurological processes may be an important factor in regulating aging free of major disease and achieving longevity.

Keywords: Genome-wide association analysis; Mortality; Disease-free survival; Longevity; Aging; Brain aging

1. Introduction

The recent, remarkable extension of life expectancy is largely attributed to the postponement of mortality at old age (Vaupel, 1997, 2010). The years of life gained in the older population residing in developed nations are a success story of public health measures and improved health care. In addition to such external factors, longevity and healthy aging consistently show a modest heritability between 20% and 50% and aging-associated genetic research may provide further insights into the mechanisms of aging (Herskind et al., 1996; McGue et al., 1993; Reed and Dick, 2003). It has been postulated that genes involved in pathways associated with aging identified in animal models, such as insulin-like growth factor (IGF)-insulin signaling, regulation of lipoprotein metabolism, the mTOR pathway, and the oxidative stress response may also influence survival to old or even exceptionally old age in humans (Christensen et al., 2006; Kenyon, 2010; Vellai et al., 2003). However, in humans, common variants within genes involved in these pathways have not been consistently associated with lifespan (Christensen et al., 2006; Kenyon, 2010; Kuningas et al., 2008; Vijg and Suh, 2005).

The lack of success in the identification of genes related to aging in humans may be due to the complexity of the phenotype. One approach to investigate aging and longevity is to compare frequencies of genetic variants between nonagenarians or centenarians and the general population. This approach led to the discovery of an association between *APOE* (Deelen et al., 2011; Ewbank, 2007; Gerdes et al., 2000) and more recently *FOXO3A* (Anselmi et al., 2009; Flachsbart et al., 2009; Li et al., 2009a; Pawlikowska et al., 2009; Willcox et al., 2008) and human aging and longevity. However, a recent genome-wide association study (GWAS) of individuals reaching the age of 90 or older failed to identify genome-wide significant variants (Newman et al., 2010).

Prospective follow-up studies with a continuous outcome such as time to death are more powerful than case-control analyses. A study of time to death simultaneously addresses the effects of genetic variation related to life span, the progression toward death, and disease-specific mortality. This design has been successfully applied in animal models (Finch and Ruvkun, 2001; Kenyon, 2010) and also in human genetics research of blood pressure (Levy et al., 2009; Newton-Cheh et al., 2009; van Rijn et al., 2007), a trait with heritability similar to longevity, where examination of a continuous outcome has been more successful in identifying genetic loci than studies that have solely used hypertension

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as a dichotomous trait. Frailty and survival free of disease have been suggested as more promising phenotypes for studies of aging because mortality is a very heterogeneous outcome caused by multiple chronic conditions (Vijg and Suh, 2005).

This study addresses the genetics of aging in a broad, sequential way using data from cohort studies participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. First, we aimed to identify single nucleotide polymorphism (SNPs) associated with all cause mortality (time to death) in a hypothesis-free GWAS in approximately 25,000 unselected persons of European ancestry. Second, we performed GWAS of time to event, defined by major incident events (myocardial infarction, heart failure, stroke, dementia, hip fracture, or cancer) or death, as an alternative phenotype for healthy aging. Last, we analyzed the SNPs along with their respective most likely associated genes identified in the GWAS meta-analyses to identify pathways and networks associated with aging and longevity.

2. Methods

2.1. Participants

The participants are of recent European ancestry and stem from cohorts of the CHARGE Consortium (Psaty et al., 2009). All cohorts are follow-up studies periodically assessing the health and vital status of their participants. Although some of the cohorts included multiple ethnic groups, only data from self-reported Caucasians were used. In addition, population structure was assessed using principal components in each CHARGE study and outliers were removed. Any remaining within-study structure was adjusted for using appropriate methods (Price et al., 2006). All participants included in this analysis were at least 55 years of age at the time of blood draw for DNA and provided written informed consent. A brief description of each population is given in the Supplementary Information.

2.2. Phenotype

We conducted a survival analysis, adjusted for age at baseline and sex, to model continuous time to death or end of follow-up in 25,007 participants (deceased "cases" = 8444; mean follow-up time = 10.6 [SD 5.4] years) that were older than 55 years at baseline. As research demonstrated that the likelihood of incident disease is genetically determined, we defined a second phenotype: survival free of major disease or mortality (Atzmon et al., 2004; Lunetta et al., 2007; Vijg and Suh, 2005). The outcome was defined as time to the first of the following adjudicated events: myocardial infarction, heart failure, stroke, dementia, hip fracture, cancer, or death. For this analysis, participants at baseline were older than 55 years of age and free of any of the aforementioned conditions. Inclusion in the study required complete follow-up information on mortality and at least 4 out of 6 of the health conditions. Genome-wide information on polymorphisms was available for 16,995 participants free of disease at the beginning of the study. These participants were followed for 8.8 (SD 5.7) years and we registered 7314 major events.

2.3. Genotyping and imputation

As different genotyping platforms were used across studies, we imputed to 2.5 million SNPs using the HapMap 22 CEU (Build 36) genotyped samples as a reference. For details on the study-specific quality control procedures for genotyping and imputation please consult Supplementary Table S1.

2.4. Statistical analysis

We used the semiparametric Cox proportional hazard to model time to event for both phenotypes in each study. Follow-up time since baseline was used as time scale. An additive genetic model was used in this analysis. We subsequently combined the individual study results in a meta-analysis using a fixed effects model that combined the study-specific regression parameters and standard errors using inverse variance weighting. We included SNPs that had a minor allele frequency (MAF) of at least 1% and an imputation quality ratio (de Bakker et al., 2008) (equivalent to the MaCH r^2 statistic; Li et al., 2009b) of at least 0.3. The study-specific inflation factors (λ_{GC}) were computed using the set of chi-square statistics used for the meta-analysis for each study. The inflation factor is computed as the median of all chi-square statistics divided by the expected median of the statistics (approximately 0.456) for a chi-square distribution with 1 degree of freedom. SNP associations at $p < 5 \times 10^{-8}$ were considered to be genome-wide significant. SNPs with $p < 5 \times 10^{-5}$ were considered suggestive associations. The combined meta-analysis hazard ratio (HR) can be interpreted as the increase in the risk of dying or having a major event during follow-up per additional copy of the coded allele. Power analysis revealed 80% statistical power to detect SNPs with a minor allele frequency of 5% and relative risk of 1.10 using a nominal significance level of 0.05 (Supplementary Table S2).

In addition, we incorporated gene annotation information, a technique that has successfully been applied in the field of aging research (de Magalhaes et al., 2009a, 2010). Protein ANalysis THrough Evolutionary Relationships (PANTHER; Mi et al., 2007; Thomas et al., 2003) and Ingenuity Pathway Analysis (IPA) (www.ingenuity.com) were used for identification and classification of networks, pathways, biological processes, and molecular functions of the genes identified in this study. For both phenotypes we generated lists of candidate genes. These genes were the closest reference genes to the SNPs associated with the outcome at $p < 1 \times 10^{-3}$. PANTHER compares these gene lists to the reference list using the binomial test for each molecular function, biological process, or pathway term.

IPA builds networks by searching the Ingenuity Pathways Knowledge Base for interactions between the identified genes and all other gene objects stored in the knowledge base.

3. Results

We conducted a meta-analysis of GWAS on time to death adjusted for baseline age and sex in participants of European origin, 55 years of age or older from 9 longitudinal cohort studies participating in the CHARGE Consortium (Psaty et al., 2009). In total, we observed 8444 deaths (mean age at death: 81.1, SD 8.4) in 25,007 participants (55% female) after an average follow-up of 10.6 (SD 5.4) years. Descriptive characteristics of participants and Manhattan plots showing genome wide *p*-values for association are displayed in the Supplementary data (Supplementary Fig. S1, and Supplementary Tables S3 and S4). The quantile-quantile plot (Q-Q plot) of observed versus expected *p*-values showed only a small deviation from the null hypothesis, indicating no significant population stratification (Fig. 1a, $\lambda_{GC} = 1.066$). Although there were no genome-wide significant findings ($p < 5 \times 10^{-8}$), 14 independent SNPs were associated with time to death at a suggestive threshold of $p < 1 \times 10^{-5}$ (Table 1). Among these SNPs, rs4936894 (chromosome 11, near the von Willebrand factor A domain containing 5A gene [*VWA5A*]) had the strongest association with time to death ($p = 3.4 \times 10^{-7}$). We sought replication for 5 of the 14 top SNPs with the strongest association with time to death in 4 independent samples ($n = 10{,}411$, deaths = 1295) of the same ancestry. None of the SNPs were consistently associated with time to death at a nominally significant level of $p < 0.05$ across all replication samples (Supplementary Tables S5–S8). In the combined meta-analysis of the discovery and replication studies only rs1425609 in the vicinity of otolin-1 (*OTOL1*) showed a stronger association (1.61×10^{-6}).

Likewise, no genome-wide significant findings were identified in the time to event analysis following 16,995 participants free of disease at baseline and registering 7314 events over an average of 8.8 (SD 5.7) years of follow-up (Table 2). Events included incident myocardial infarction, heart failure, stroke, dementia, hip fracture, and cancer or death. The Q-Q plot (Fig. 1a, $\lambda_{GC} = 1.019$) showed no evidence of inflation of type I error. In total, there were 8 independent SNPs associated with event-free survival at $p < 10^{-5}$. The SNP with the strongest association was rs10412199 (chromosome 19, $p = 3.02 \times 10^{-6}$), which is in close proximity to ataxia, cerebellar, Cayman type (*ATCAY*). Additional descriptive information including definitions of each event and association results with $p < 10^{-4}$ are provided in Supplementary Figure S2, and Supplementary Tables S9–S12.

As both phenotypes may provide different but complimentary information about the aging process, we evaluated the overlap between their association results (Table 3). Interpretation of the overlap between the phenotypes requires caution as both phenotypes are correlated, nevertheless it helps to focus on specific loci and put them into the context of aging. From the 14 loci passing the prespecified, suggestive threshold of $p < 1 \times 10^{-5}$ in the time to death analysis, 5 had corresponding SNPs within 500 kilo base pairs distance, in linkage disequilibrium (LD; $r^2 > 0.1$) associated with $p < 1 \times 10^{-4}$ and the same overall direction of the effect in the time to event analysis. These 5 regions were in the vicinity of the following genes: *OTOL1* (3q26.1), bridging integrator 2 (*BIN2*, 12q13), ATG4 autophagy related 4 homolog C (*ATG4C*, 1p31.3), origin recognition complex,

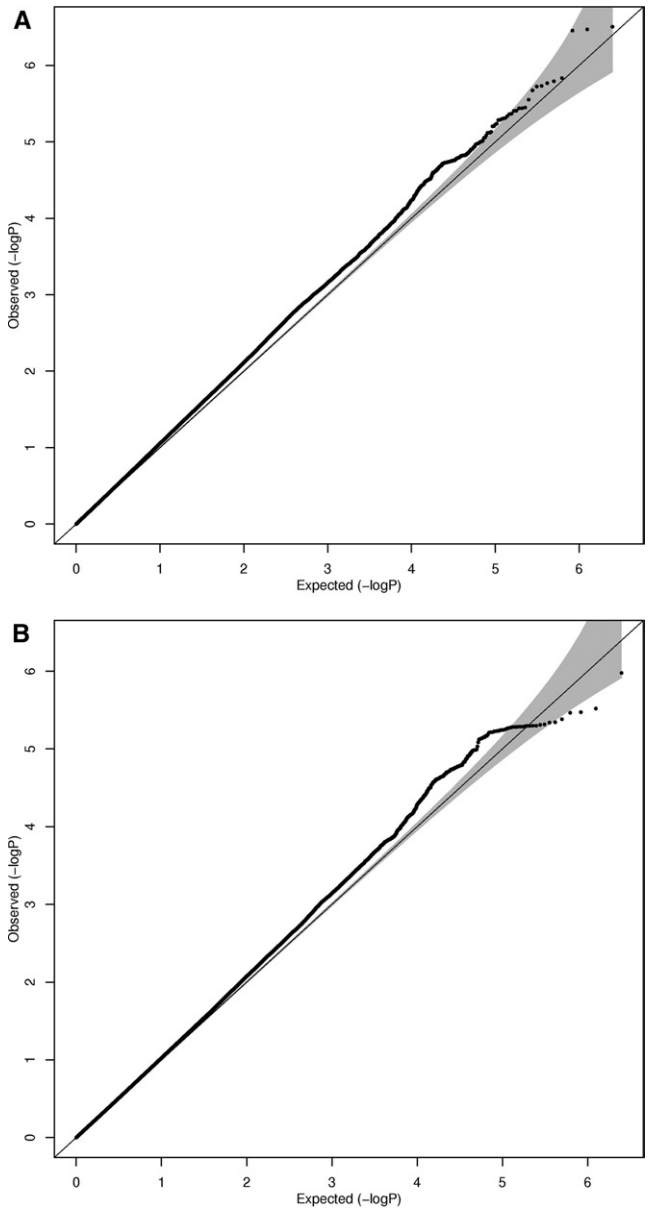

Fig. 1. (a) Quantile-quantile (Q-Q) plot after meta-analysis for time to death. (b) Quantile-quantile (Q-Q) plot after meta-analysis for time to event.

Table 1
Top 14 SNPs (p-value $< 10^{-5}$) for time to death ranked by p-value, from meta-analysis of 9 cohorts[a]

Number	SNP	Chr	Position	Closest reference gene	Distance from closest gene	Coded allele	Noncoded allele	Frequency coded allele	HR	p-value	Study effect direction [b]	Number of supporting SNPs
1	rs4936894	11	123522703	*VWA5A*	123	A	G	0.226	1.11	3.38E-07	++++-++-+	224
2	rs1425609	3	164164689	*OTOL1*	1,460,265	A	G	0.381	0.92	1.46E-06	—-------	399
3	rs766903	12	49990101	*BIN2*	14,104	A	G	0.834	0.90	1.61E-06	———————+----	7
4	rs12042640	1	63139384	*ATG4C*	36,747	T	C	0.284	1.09	1.71E-06	++++-+-+-	19
5	rs17149227	7	75073485	*HIP1*	72,141	T	G	0.959	0.79	3.56E-06	−??———————+-?	0
6	rs3128591	9	136741940	*COL5A1*	68,468	A	G	0.754	0.92	3.64E-06	—-------	20
7	rs11582903	1	87618642	*LMO4*	34,804	A	C	0.150	1.12	3.94E-06	++-+++++	38
8	rs4850695	2	196861504	*HECW2*	89,283	A	G	0.766	1.09	4.62E-06	+++++++++	95
9	rs10259086	7	103680248	*ORC5L*	44,549	T	G	0.686	1.08	5.16E-06	++++++-++	72
10	rs2769255	1	41017941	*KCNQ4*	4329	T	C	0.374	1.08	5.17E-06	++++++-++	95
11	rs17291546	6	2660681	*LOC340156*	35,472	A	G	0.957	0.82	7.65E-06	−?-------	8
12	rs12606100	18	69102967	*NETO1*	417,177	T	C	0.202	1.11	8.72E-06	+??-++++-	4
13	rs1274214	11	122979741	*GRAMD1B*	18,987	T	C	0.500	0.93	8.87E-06	—-------	42
14	rs10811679	9	2224701	*SMARCA2*	41,080	T	C	0.330	1.08	9.53E-06	+++++++++	37

n = 25,007 participants with 8444 deaths, only SNPs with MAF > 3% are presented. p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36. HRs are for each additional coded allele. Number of supporting SNPs is the number of SNPs within 500 kilo base pairs of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 ($r^2 >= 0.10$) and have association p-value < 0.05.

Key: Chr, chromosome; LD, linkage disequilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

[a] For information on all SNP associations with p-value $< 10^{-4}$ see Supplementary Table S2.

[b] Study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI, SHIP; "+" = coded allele increases risk of mortality, "-" = coded allele decreases risk of mortality, "?" = not tested.

Table 2
Top 8 SNP (p-value $< 10^{-5}$) associations from meta-analysis of 8 cohorts for time to event, ranked by p-value (n = 16,995 with 7314 events)

Number	SNP	Chr	Position	Closest reference Gene	Distance (bp) from closest gene	Coded allele	Noncoded allele	Frequency coded allele	HR	p-value	Study effect direction [a]	Number of supporting SNPs
1	rs10412199	19	3878771	ATCAY	307	A	G	0.33	0.91	3.02E-06	–?-++--	6
2	rs16852912	3	170169370	MECOM	114610	T	C	0.08	1.18	3.37E-06	++++-+-+	72
3	rs8001976	13	47285723	SUCLA2	129069	T	C	0.44	1.09	3.43E-06	+++-+++--	173
4	rs11162963	1	80507169	ELTD1	1262086	T	C	0.63	1.09	4.15E-06	+++++++++	40
5	rs4764043	12	14006749	GRIN2B	17570	T	C	0.08	1.17	6.10E-06	+++++++++	2
6	rs3112530	5	152619870	GRIA1	230628	A	G	0.08	0.85	6.79E-06	----------+---	130
7	rs10202497	2	237935633	COL6A3	38233	A	C	0.14	0.89	8.22E-06	----------	36
8	rs2367725	1	43988415	ST3GAL3	42611	T	C	0.42	1.08	9.31E-06	+++++++-	119

p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36. HRs are for each additional coded allele. Number of supporting SNPs is the number of SNPs within 500 kilo base pair of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 ($r^2 \geq 0.10$) and have association p-value < 0.05. For information on all SNP associations with p-value $< 10^{-4}$ see Supplementary Table S12.
Key: bp, base pair; Chr, chromosome; HR, hazard ratio; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.
[a] Study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI; "+" = coded allele increases risk of event; "-" = coded allele decreases risk of event; "?" = not tested.

subunit 5-like (*ORC5L*, 7q22.1), and potassium voltage-gated channel, KQT-like subfamily, member 4 (*KCNQ4*, 1p34). Similarly, in the time to event analysis 3 of the 8 top SNPs showed considerable overlap and the same direction of effect in the time to death analysis. These SNPs were close to the following genes: MDS1 and EVI1 complex locus (*MECOM*, 3q24-q28), succinate-CoA ligase, ADP-forming, beta subunit (*SUCLA2*, 13q12.2-q13.3), and ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (*ST3GAL3*, 1p34.1).

Finally, we evaluated candidate genes for aging by identification and classification of networks, pathways, biological processes, and molecular functions. The candidate genes were derived from the meta-analyses of GWAS and included the reference genes closest to the SNPs associated with $p < 1 \times 10^{-3}$ (time to death: 862 genes, time to event: 704 genes). We used PANTHER (Mi et al., 2007; Thomas et al., 2003, 2006) and IPA software (www.ingenuity.com) for these analyses. PANTHER compares these gene lists to the reference list using the binomial test for each molecular function, biological process, or pathway term. IPA builds networks by searching the Ingenuity Pathways Knowledge Base for interactions between the identified genes and all other gene objects stored in the knowledge base.

For the analysis of time to death, the relevant biological processes overrepresented in the PANTHER analysis were developmental processes, neuronal activities, signal transduction, neurogenesis, ectoderm development, and cell adhesion. For the analysis of time to incident event, developmental processes and neuronal activities were overrepresented among other biological process (Table 4). The analyses also highlighted the Wnt signaling pathway. The Wnt signaling pathway is ubiquitous and known to be involved in cancer but also plays an important role in the early stages of the development of the central nervous system, in synaptic formation by axon guidance, and in modulating fibrosis during muscle repair scored high in both traits under study (Brack et al., 2007; Inestrosa and Arenas, 2010; Keeble et al., 2006; Ulloa and Martí, 2010). For extended tables see Supplementary Tables S13 and S14. In addition, Ingenuity identified 1 network with $p = 10^{-31}$ containing 26 genes involved in processes related to nervous system development and function for the analysis of time to death (Fig. 2) and 1 network with $p = 10^{-40}$ containing 28 genes involved in cellular function and development for time to event (Supplementary Fig. S3).

IPA analysis highlighted the following genes associated with the time to death trait: *NTRK2* (neurotrophic tyrosine kinase, receptor, type 2)—a member of the neurotrophic tyrosine receptor kinase family. This kinase is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and members of the mitogen-activated protein kinase (MAPK) pathway. Signaling through this kinase leads to cell differentiation. Second in line were *NCAM1* (neural cell adhesion molecule 1)—a cytoskeletal binding

Table 3
Overlap between the associations of time to death and time to event[a]

Top hit	SNP	Chr	Closest reference gene	Time to death		Time to event		Top SNPs from time to death (time to event) analysis associated with different *p*-values in time to event (time to death) analysis					
				p	Effect	*p*	Effect	Total	$p \leq 0.05$	$p < 0.05$	$p < 0.01$	$p < 0.001$	$p < 0.0001$
Time to death													
1	rs1425609	3	*OTOL1*	1.46E-06	−	0.005704	−	1119	693	235	132	37	22
2	rs766903	12	*BIN2*	1.61E-06	−	0.01315	−	37	27	4	5	0	1
3	rs12042640	1	*ATG4C*	1.71E-06	+	0.03701	+	93	60	19	4	0	10
4	rs11582903	1	*LMO4*	3.94E-06	+	0.7336	−	133	91	8	12	21	1
5	rs10259086	7	*ORC5L*	5.16E-06	+	0.03266	+	239	154	56	21	4	4
6	rs2769255	1	*KCNQ4*	5.17E-06	+	0.01322	+	287	151	68	56	7	5
7	rs17291546	6	*LOC340156*	7.65E-06	−	0.01624	−	29	19	9	1	0	0
8	rs12606100	18	*NETO1*	8.72E-06	+	0.02853	+	23	16	5	2	0	0
9	rs1274214	11	*GRAMD1B*	8.87E-06	−	0.0567	−	101	39	28	17	17	0
Time to event													
1	rs16852912	3	*MECOM*	0.00589	+	3.37E-06	+	169	67	49	49	2	2
2	rs8001976	13	*SUCLA2*	0.01473	+	3.43E-06	+	433	198	91	46	59	39
3	rs4764043	12	*GRIN2B*	0.0017	+	6.10E-06	+	45	42	2	1	0	0
4	rs10202497	2	*COL6A3*	0.00035	−	8.22E-06	+	135	83	27	12	9	4
5	rs2367725	1	*ST3GAL3*	0.0274	+	9.31E-06	+	459	317	56	37	31	18

p-values are for the inverse variance-weighted meta-analysis. Total represents the number of SNPs in time to death (time to event) analysis within 500 kilo base pair of SNPs from the time to event (time to death) analysis that are in LD with the top SNPs from the time to death (time to event) analysis in the HapMap CEU release 22 ($r^2 \geq 0.10$) and have association *p*-value < 0.05.
Key: Chr, chromosome; Effect, meta-analysis direction of effect; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.
[a] Only SNPs that were nominally significant ($p < 0.05$) for both traits are shown.

Table 4
Results from the gene annotation analysis using PANTHER

Biological process	H. sapiens (reference)	Number of genes observed	Number of genes expected	−/+	*p*-value unadjusted	*p*-value adjusted [a]
Time to death:						
Biological process unclassified	11321	238	367.71	−	1.29E-20	4.00E-19
Developmental processes	2152	152	69.9	+	1.39E-19	4.32E-18
Neuronal activities	569	65	18.48	+	8.94E-18	2.77E-16
Signal transduction	3406	199	110.63	+	9.09E-17	2.82E-15
Neurogenesis	587	64	19.07	+	1.43E-16	2.84E-14
Ectoderm development	692	68	22.48	+	2.33E-15	3.38E-13
Cell adhesion	622	57	20.2	+	7.00E-12	2.17E-10
Time to event:						
Developmental processes	2152	115	57.46	+	1.02E-12	3.16E-11
Biological process unclassified	11321	214	302.27	−	2.93E-12	9.08E-11
Neuronal activities	569	47	15.19	+	2.28E-11	7.08E-10

Candidate genes (genes observed) were in the neighborhood of single nucleotide polymorphisms (SNPs) associated with p value $< 1 \times 10^{-3}$. For time to death 862 candidate genes were identified; 826 could be matched to the Protein ANalysis THrough Evolutionary Relationships (PANTHER) gene list. For time to event 704 candidate genes were identified; 679 could be matched to the PANTHER gene list. Extended lists of PANTHER pathways, biological processes, and molecular functions are listed in the Supplementary Tables (S13, S14).

[a] Bonferroni correction multiplying the single-test *p*-value by the number of independent tests to obtain an expected error rate.

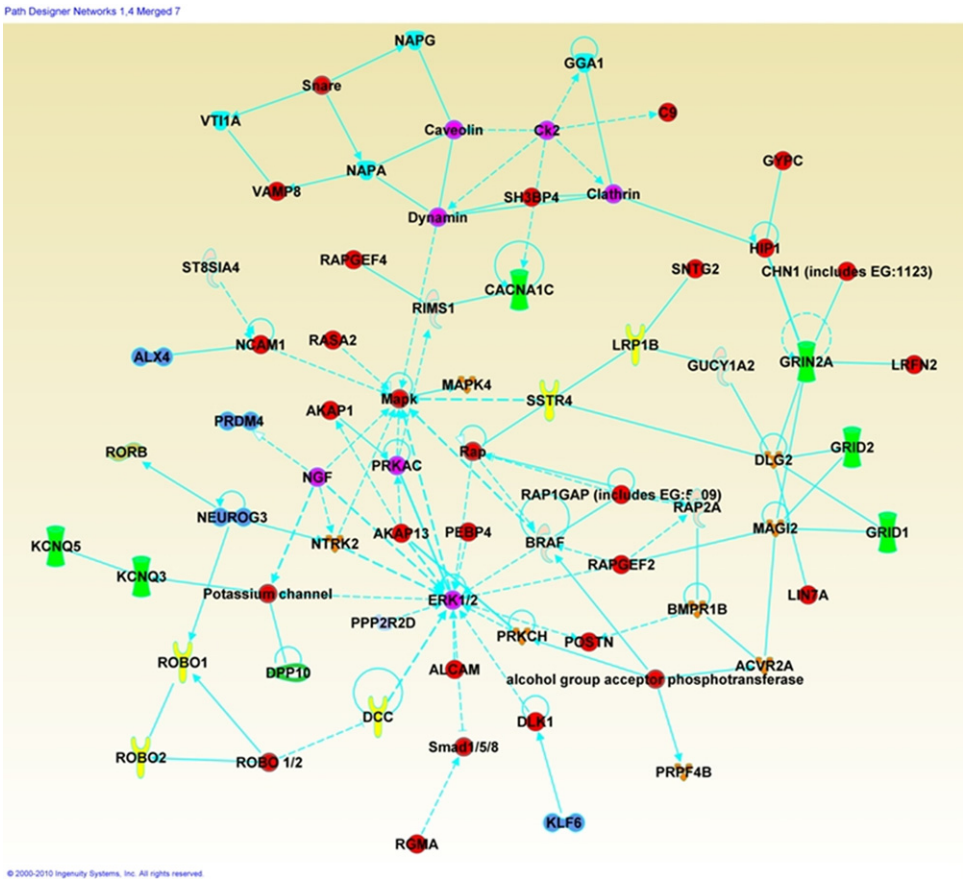

Fig. 2. Network describing neuronal activities related to time to death. Pathway analysis of genes (single nucleotide polymorphisms; SNPs) associated with time to death. Genes are represented as nodes; edges indicate known interactions (solid lines depict direct and hatched lines depict indirect interaction). Human gene functions are color-coded as follows: red = unknown, yellow = transmembrane receptor and G protein coupled receptor, magenta (pink-purple) = group/complex/other, bright green = ion channel, hunter green (dark green) = peptidase, navy blue = transcription regulator, light blue = transporter, beige = enzyme, orange = kinase, light green = cytokine, light purple = phosphate, gray = translation regulator, olive green = ligand-dependent nuclear receptor.

protein, *GRID2* (glutamate receptor, ionotropic, delta 2)—a relatively new member of the family of ionotropic glutamate receptors which are the predominant excitatory neurotransmitter receptors in the mammalian brain, and have a role in neuronal apoptotic death, and *RIMS1* (regulating synaptic membrane exocytosis 1), which regulates synaptic vesicle exocytosis and may be part of the protein scaffold of the cell.

Among the genes that were highlighted through the IPA analysis in the analysis of time to event was *MYC* (v-myc myelocytomatosis viral oncogene homolog)—a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis, and cellular transformation. *MYC* functions as a transcription factor that regulates transcription of specific target genes. Second in line were *E2F1* (E2F transcription factor 1), *EGFR* (epidermal growth factor receptor), and *CEBPA* (CCAAT/enhancer binding protein [C/EBP], alpha). *EF21*, a transcription factor, plays a crucial role in the control of cell cycle and action of tumor suppressor proteins can mediate both cell proliferation and p53-dependent/independent apoptosis. *EGFR* is a transmembrane glycoprotein that serves as a receptor for members of the epidermal growth factor family and supports cell proliferation. *CEBP*-Alpha, a bZIP transcription factor, can bind as a homodimer to certain promoters and enhancers. *CEBPA* also forms heterodimers with the related proteins CEBP-beta and CEBP-gamma and modulates the expression of leptin, interacts with *CDK2* and *CDK4*, and thereby inhibits these kinases and causes growth arrest in cultured cells.

4. Discussion

In our analyses of over 25,000 individuals of 55 years and older followed for an average of 11 years, we did not identify genome-wide significant associations for all-cause mortality and survival free of major diseases. However, both traits highlighted loci with suggestive significance that were in the neighborhood of genes related to neural regulation. In addition, our pathway and network analyses identified an enrichment of genes associated with cellular and neural development and function, and cell communication that may contribute to variation in human aging. Brain development might be responsible for the creation of redundancy in brain circuitry, which is associated with functional reserve and resiliency. Brain function regulates most of the compensatory strategy supporting maintenance of homeostatic equilibrium. Both of these processes are essential to healthy aging and longevity.

Several explanations are possible for the lack of genome-wide significant findings. First, mortality is arguably 1 of the most complex phenotypes, and several trajectories toward extreme old age have been identified (Evert et al., 2003). Multiple genes could mediate the aging process but would have their effects through numerous different pathophysiological processes and diseases that act as intermediate factors on the pathway to death (de Magalhaes et al., 2010). Therefore, any common variation in genes associated with aging probably has a small effect.

Second, the largely negative findings of this and other studies contrast with the intriguing animal studies of longevity. Very large effects of single genes on lifespan have indeed been observed in laboratory animals, but humans often have several homologues of these genes which might significantly differ in function or compensate for mutated genes through redundant mechanisms (Kuningas et al., 2008). This could explain why our top findings did not include genes in these pathways found in animal models. Animal models also represent genetically homogenous populations and are exposed to controlled environmental influences. The lack of replication of animal model findings in humans suggests that the use of knockout animals may not provide the optimal approach to understanding the variation in survival in humans as interactions with environmental factors may obscure the associations and prevent the identification of loci in humans.

Third, our study is based on common genetic variants and therefore we cannot exclude effects due to low frequency and rare variants ($< 5\%$) or due to the presence of structural variation, such as copy number polymorphisms. Our discovery set may lack the power to identify all the relevant loci, even though we had sufficient power to detect common SNPs (minor allele frequency = 5% or more) with a relative risk of 1.10 (Supplementary Table S2).

Last, we cannot exclude that phenotypic heterogeneity influenced our findings. While all cohorts had prospectively collected information on major health events and diagnoses, heterogeneity in the methods of assessment and classification might have limited the ability to identify true effects.

Complex diseases may result from the effects of a large number of low frequency variants, with substantial allelic heterogeneity at disease-causing loci (Pritchard, 2001; Pritchard and Cox, 2002; Swarbrick and Vaisse, 2003). Theoretical modeling that incorporates mutation, random genetic drift, and purifying selection suggests that many of the variants that affect complex traits may be in the 1%–5% frequency range (Pritchard, 2001). Indeed, sequencing of candidate genes in an attempt to capture such low frequency variants, has led to the identification of rare variants with modest effects on body mass index (Ahituv et al., 2007; Challis et al., 2002; Cone, 2000), triglyceride levels (Romeo et al., 2007), high-density lipoprotein (HDL; Cohen et al., 2004; Romeo et al., 2007) and low-density lipoprotein (LDL) cholesterol levels (Cohen et al., 2005, 2006; Kotowski et al., 2006).

It is impossible to determine the functional variant of a gene by GWAS. Moreover, we cannot conclude from the location of an SNP that this variation is involved in the expression of the closest gene. However, our top results suggested a possible role of genes involved in neurological

processes in human longevity and aging. Ten of the 22 suggestive associations identified in our analyses are in or near genes that are highly expressed in the brain (*HECW2* [Rotin and Kumar, 2009], *HIP1* [Blanpied et al., 2003], *BIN2*, *GRIA1*), were previously related to the regulation of neuronal excitability and plasticity (*KCNQ4* [Van Eyken et al., 2006], *LMO4* [Joshi et al., 2009; Leuba et al., 2004], *GRIA1*), and the maintenance of neural circuitry and synaptic plasticity (*NETO1*), or are associated with neurological diseases such as Alzheimer's disease (*LMO4* [Leuba et al., 2004], *BIN2*, GRIA1, GRIN2B), and amyotrophic lateral sclerosis (*GRIN2B*). In addition, 6 of the 22 SNPs were in close proximity to genes associated with other phenotypes of aging such as autophagy (*ATG4C* [Kenyon, 2010]), cancer (*ATG4C* [Maiuri et al., 2009], *HIP1* [Bradley et al., 2007], *HECW2* [Rotin and Kumar, 2009], *VWA5A* [Zhou et al., 2009], *MECOM*), and mitochondrial depletion syndrome (*SUCLA2*). Notably, *BIN2*, *ATG4C*, *KCNQ4*, *MECOM*, and *SUCLA2* showed associations with both traits in our study.

Using the expression quantitative trait loci (eQTL) browser (eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) we detected eQTL associated with *HIP1*, *COL5A1*, *LOC340156*, and *SMARCA2* in time to death only.

Interestingly, SNPs known to be associated with longevity and disease in the neighborhood of APOE (Deelen et al., 2011) or *FOXO3A* (Flachsbart et al., 2009; Willcox et al., 2008) only reached nominal significance (results not shown). These genes were originally identified in studies of centenarians; it is possible that our study of cohorts comprised of individuals from the general populations did not have sufficient statistical power to identify these genes with certainty. (Tan et al., 2008).

While meta-analysis of GWAS has the power to detect small changes of allele frequencies between groups with the analyzed trait, true association signals may not be revealed based on a stringent genome-wide significance threshold. This situation, although limiting false positive findings, performs poorly in identifying false negatives as they may fall below the threshold. Network analyses using a less stringent significance threshold do not amend the overall negative finding of this study. However, it is well-recognized that within the many associations that failed to attain this level of significance lie true positive associations. Network analyses can provide useful information exploring multiple gene effects and their interactions.

In fact the interpretation of most GWAS results is difficult because individual results may involve many seemingly unrelated genes. Because PANTHER and IPA are built on different conceptual approaches, database sources and different pathway classifications, they can be seen as complementary approaches. Our pathway and network analyses highlighted neuronal activities and organism developmental processes as major biological processes involved aging. In addition, it highlighted Wnt signaling and showed that those genes that were involved in most pathways indeed had substantial effects within the analyzed trait. *NTRK2* (Rico et al., 2002), *NCAM1* (Rutishauser et al., 1988), *GRID2* (Hirai et al., 2003), and *RIMS1* (Johnson et al., 2003; Schoch et al., 2002) are associated with neuronal development and disease pathways that were highlighted in the analysis of time to death. *MYC* (Cole, 1986; Goga et al., 2007), *E2F1* (Nevins, 2001), *EGFR* (Wang et al., 2004), and *CEBPA* (Ménard et al., 2002; Wang et al., 2001) are associated with "cancer," "cell function," and "development" pathways.

Few if any of the top hits from the GWAS were involved in common pathways of aging, typically addressed in candidate gene studies. For example, there was no specific evidence for genes involved in IGF-insulin signaling. However, this negative finding cannot be interpreted as evidence against the importance of IGF-insulin signaling, as well as other processes such as inflammation, oxidative stress, cellular damage and repair, growth hormone, and cell proliferation in aging. Moreover, it is possible that polymorphisms in related genes have an effect in the oldest old, who were represented by fewer numbers in our study population such that our study design would be underpowered to detect it. It is also conceivable that the neurological pathways identified by our analysis interact with the known candidate genes involved in aging (Bishop et al., 2010; Finch and Ruvkun, 2001). It is feasible that the traditional aging pathways are hierarchically controlled by neurons and that the brain might be the location coordinating physiological changes (Bishop et al., 2010; Finch and Ruvkun, 2001). Because neurons are particularly susceptible to damage caused by reactive oxygen species, limitations in cellular maintenance and repair might reinforce these pathways and accelerate aging (Finch and Ruvkun, 2001). An increased ability of neuronal cells to prevent or repair oxidative damage might result in beneficial hormonal signaling, otherwise deregulated with age, thus delaying the onset of age-related disease and directly regulating cognitive aging and life span (Bishop et al., 2010; Cutler and Mattson, 2006; de Magalhães and Sandberg, 2005).

In conclusion, our analysis did provide suggestive evidence that aging is under neuronal control. Unfortunately, we have no relevant tissue or expression experiment available to further underscore or validate our findings. Future investigations of changes of gene expression with age at cellular and population levels are warranted.

Disclosure statement

The authors declare that no competing interests exist.

All participants included in this analysis provided written informed consent.

Replication Samples

Whitehall II: Whitehall II has been supported by grants from the Medical Research Council; Economic and Social Research Council; British Heart Foundation; Health and Safety Executive; Department of Health; National Heart Lung and Blood Institute (HL36310), US NIH National Institute on Aging (AG13196), US NIH Agency for Health Care Policy Research (HS06516); and the John D. and Catherine T. MacArthur Foundation Research Networks on Successful Midlife Development and Socio-economic Status and Health.

English Longitudinal Study of Aging: Samples from the English Longitudinal Study of Ageing (ELSA) DNA Repository (EDNAR), received support under a grant (AG1764406S1) awarded by the National Institute on Ageing (NIA). ELSA was developed by a team of researchers based at the National Centre for Social Research, University College London and the Institute of Fiscal Studies. The data were collected by the National Centre for Social Research.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2011.05.026.

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