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No Association between Variation in Longevity Candidate Genes and Aging-related Phenotypes in Oldest-old Danes

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Abstract

In this study we explored the association between aging-related phenotypes previously reported to predict survival in old age and variation in 77 genes from the DNA repair pathway, 32 genes from the growth hormone 1/insulin-like growth factor 1/insulin (GH/IGF-1/INS) signalling pathway and 16 additional genes repeatedly considered as candidates for human longevity: *APOE*, *APOA4*, *APOC3*, *ACE*, *CETP*, *HFE*, *IL6*, *IL6R*, *MTHFR*, *TGFB1*, *SIRTs 1*, *3*, *6*; and *HSPAs 1A*, *1L*, *14*. Altogether, 1,049 single nucleotide polymorphisms (SNPs) were genotyped in 1,088 oldest-old (age 92–93 years) Danes and analysed with phenotype data on physical functioning (hand grip strength), cognitive functioning (mini mental state examination and a cognitive composite score), activity of daily living and self-rated health.

Five SNPs showed association to one of the phenotypes; however, none of these SNPs were associated with a change in the relevant phenotype over time (7 years of follow-up) and none of the SNPs could be confirmed in a replication sample of 1,281 oldest-old Danes (age 94–100).

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7. CONFLICT OF INTEREST

The authors have no conflicts to declare.

Hence, our study does not support association between common variation in the investigated longevity candidate genes and aging-related phenotypes consistently shown to predict survival. It is possible that larger sample sizes are needed to robustly reveal associations with small effect sizes.

Keywords

human aging; oldest-old; single nucleotide polymorphisms; association study

1. INTRODUCTION

It has been estimated that approximately 25% of the variation in human life span is caused by genetic variation (Herskind et al., 1996), whereas the genetic contribution to aging-related phenotypes is even larger: it has for instance been estimated to be about 76% for the overall cognitive functioning in elderly (McGue and Christensen, 2002) and around 52% for hand grip strength (Frederiksen et al., 2002). Therefore, as aging-related phenotypes are more heritable than longevity itself and are recognized to predict survival (Nybo et al., 2003 and Yaffe et al., 2010), the study of aging-related phenotypes might increase the probability of identifying genetic variants of relevance to both aging and longevity. Some studies have suggested that the same genomic regions could indeed be involved in both processes (e.g. Montesanto et al., 2013).

Although only the *APOE* and *FOXO3A* genes have been consistently found to associate to human longevity in a number of candidate gene studies (e.g. Schachter et al., 1994 and Wilcox et al., 2008) and genome-wide association studies (reviewed in Broer and van Duijn, 2015), several additional genes are considered potential candidates (e.g. Argon and Gidalevitz, 2015). In this study we explore 125 genes that take part in biological processes suggested to be involved in human longevity (e.g. Argon and Gidalevitz, 2015 and Soerensen et al., 2012). The selected genes are implicated in DNA repair, growth hormone 1/insulin-like growth factor 1/insulin (GH/IGF-1/INS) signalling, lipoprotein metabolism (including the apolipoprotein E (*APOE*) gene), heat shock protein and cytokine activities. Furthermore, we investigate the sirtuins 1, 3 and 6 genes, the angiotensin I-converting enzyme gene (*ACE*), the iron absorption regulatory hemochromatosis gene (*HFE*) and the methylenetetrahydrofolate reductase gene (*MTHFR*). The included genes and single nucleotide polymorphisms (SNPs) are listed in Supplementary Table 1.

Common variation in some of the genes under study has previously been suggested to be associated to aging-related phenotypes; e.g. variation in *ACE* and insulin-like growth factor 2 (*IGF2)* to physical functioning (Baessler et al., 2007 and Pereira et al., 2013) and variation in *APOE* and *MTHFR* to cognitive functioning (Wisdom et al., 2011 and Peng et al., 2015).

Here we assessed the association of the candidate genes with phenotypes representing cognitive functioning, physical functioning (hand grip strength), self-rated health and activity of daily living (ADL). The discovery sample was drawn from the cohort of oldest-olds in which the baseline values for these phenotypes were previously reported to predict survival during old age (Nybo et al., 2003 and Thinggaard et al., 2016). As the genetic

contribution to aging-related phenotypes and longevity has been suggested to be gender-specific (e.g. Lehmann et al., 2006), we also performed a gender-stratified analysis.

2. MATERIALS AND METHODS

2.1 Discovery and replication samples

The discovery population consisted of 1,088 oldest-old individuals randomly drawn from the Danish 1905 Cohort Study, a nationwide survey of the entire Danish 1905 birth cohort initiated in 1998 (Nybo et al., 2001): 3,600 individuals were still alive, 2,262 participated in the baseline survey in 1998. Follow-up surveys of survivors were conducted in 2000, 2003 and 2005. The age range at baseline of the discovery sample was 92.2–93.8 years and the gender distribution was 29% males and 71% females. The replication population was 1,281 individuals from the Danish 1910 and 1915 birth cohort studies (Christensen et al., 2013 and Vestergaard et al., 2015) with an age range of 94.7–100.9 years and a gender distribution of 27% males and 73% females. The three cohort studies were conducted in a similar way and the questions regarding the phenotypes under study were identical. Permission to collect blood samples and usage of register-based information was granted by The Danish Regional Committees on Biomedical Research Ethics.

2.2. Phenotypes

The surveys included an extensive home-based interview focusing on health issues and assessments of cognitive and functional abilities (see Nybo et al., 2001 and Supplementary Information). Two cognitive state phenotypes were considered here: the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and a cognitive composite score (McGue and Christensen, 2002). Hand grip strength was measured using a hand-held dynamometer (SMEDLEY' dynamometer, Scandidact, Kvistgaard, Denmark), using the maximum of three measurements with the strongest hand. Self-rated health was evaluated by asking the participants "How do you consider your health in general?" with five response categories: very poor, poor, acceptable, good, and excellent. ADL was assessed by a five-item ADL disability score based on the Katz ADL index on bathing, dressing, toileting, transfer and feeding (Katz et al., 1970). To inspect physical functioning and endurance we used an 11-item ADL strength score related to, among others, the ability to walk, run, climb the stairs and carrying weights (Christensen et al., 2000). The descriptives regarding the phenotypes investigated in the discovery and replication samples are shown in Table 1.

2.3. Genotype data

The selection of genes and gene variants, and the generation of genotype data in the discovery sample are described in detail in (Soerensen et al., 2012, Soerensen et al., 2013 and Supplementary Information). The SNPs were primarily chosen to be tagging SNPs with an $R^2>0.8$. Subsequent investigation of the linkage disequilibrium (LD) in the discovery sample showed that 94% of the chosen SNPs displayed a pairwise R^2 below 0.8 and were thus rather independent. In total, data on 1,049 SNPs in 125 genes were available for the present study after data cleaning.

The 311 SNPs of the oxidative stress pathway from the original dataset (Soerensen et al., 2012), as well as 15 SNPs in the *FOXO3A* gene of the GH/IGF-1/INS signalling pathway, have previously been investigated with respect to aging phenotypes in separate studies (Dato et al., 2014 and Soerensen et al., 2015), and these SNPs are therefore not included in the present study. Also, 19 SNPs in the *KL* gene of the GH/IGF-1/INS signalling pathway were recently explored by growth curve models with respect to cognitive state and decline (Mengel-From et al., 2015). Hence, in the present study *KL* is not investigated with respect to cognition. Nonetheless, neither *FOXO3A* nor *KL* displayed significance levels in the previous studies, which would have passed correction for multiple testing in the present study.

DNA of the replication sample was purified from blood spot cards using the Extract-N-Amp™Blood PCR Kit (Sigma-Aldrich, St. Louis, MO, USA) and genotyping was done using predesigned TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA).

2.4 Statistical analysis

Association studies in the discovery sample were performed for single SNPs and the baseline values of each of the six phenotypes for both genders combined and for males and females separately. The continuous phenotypes cognitive composite score and hand grip strength were investigated by linear regression and assuming an additive genotype model: the cognitive composite score was analysed in Plink (http://pngu.mgh.harvard.edu/purcell/plink, (Purcell et al., 2007)), while hand grip strength was analysed by a robust linear regression in R (www.r-project.org) with the rlm command from the MASS package. A robust linear regression was used for hand grip strength due to violation of the assumption of variance homogeneity. As the ADL strength score data and the MMSE data were not normally distributed, we applied the quantiles of the ADL score, while the MMSE data were divided in three groups: MMSE<18, MMSE ≥ 18 to <24, and MMSE ≥ 24, compatible to severe cognitive impairment, mild cognitive impairment and no cognitive impairment (Nybo et al., 2003). The ADL strength score quantiles and the MMSE groups were, as the two additional categorical phenotypes self-rated health and ADL disability score, analysed by ordinal logistic regression using the polr command from the MASS package in R. All analyses were adjusted for age and gender (both genders combined) or for age (gender-stratified analyses). All p-values were corrected for multiple testing by permuting the phenotype labels 10,000 times for each analysis. The replication studies, including pooled analyses of both study populations, were done using STATA 11.1 (Stata Corporation, College Station, TX, USA) and the same statistical methods as above.

The 5 SNPs found to associate to the phenotypes at baseline were investigated in a longitudinal analysis. The 1,088 individuals from the discovery sample were all participants in the baseline survey in 1998, whereas those of the 1,088 individuals who survived to year 2000, 2003 or 2005 were contacted for follow-up surveys in those years. In the longitudinal analysis data from all four waves of survey were used. The changes in the analysed phenotypes during follow-up are shown in Supplementary Figure 1. For each of the 5 SNPs a random effects model was applied using STATA 11.1 (by the xtmixed command),

including a SNP effect, a time effect and a SNP-time interaction. The SNP effect was assumed to depend additively on the number of minor alleles, and, thereby, the interaction term describes the change in phenotype over a follow-up period of 7 years, which can be attributed to each copy of the minor allele.

2.5 Power calculations

Power calculations in the discovery sample were performed in Quanto (http://biostats.usc.edu/cgi-bin/DownloadQuanto.pl (Quanto1_2_4a)) assuming an additive model, a power>0.8 and a Bonferroni-corrected significance level of 0.05/1,049, i.e. considering the number of SNPs tested in the discovery sample as independent. The calculations indicated that an effect size of 0.22 of a standard deviation (SD) or greater for the cognitive composite score (β-coefficients>0.74) and for the hand grip strength (β-coefficients>1.50) should reach statistical significance with a probability of at least 0.8 for SNPs with a minor allele frequency (MAF) of 0.4. For SNPs with a MAF of 0.1, the corresponding result was 0.36 of a SD, corresponding to β-coefficients >1.21 for the cognitive composite score and β-coefficients>2.45 for the hand grip strength.

Similarly, power calculations considering a significance level of 0.05 for the replication sample indicated that even smaller effect sizes should reach statistical significance: 0.12 of a SD for a MAF of 0.4 (corresponding to β-coefficients of >0.012 and >0.025 for the cognitive composite score and hand grip strength, respectively) and 0.20 of a SD for a MAF of 0.1 (corresponding to β-coefficients of >0.019 and >0.04 for the cognitive composite score and hand grip strength, respectively).

3. RESULTS

The descriptives regarding the phenotypes investigated in the discovery and replication samples are shown in Table 1. Regression analyses of the phenotype and genotype data of the 1,088 oldest-old individuals in the discovery sample showed the minor alleles of 3 SNPs to be significantly associated with either a decreased cognitive functioning (the cognitive composite score) or an increased ability (the ADL strength score) for both genders combined. The minor alleles of 2 additional SNPs revealed association with either a decreased hand grip strength or an increased self-rated health in the gender-stratified analysis. The data are summarized in Table 2, and the results of all analyses are shown in Supplementary Table 2.

None of these 5 SNPs were associated with the change in the relevant phenotypes over time using a follow-up period of seven years (see Supplementary Table 3).

In a replication study of the initial findings (cf. Table 2) using a sample of 1,281 oldest-old Danes, no significant replication was observed. Pooled replication analyses of both study populations showed similar or larger p-values as found in the discovery sample, hence not clearly supporting replication. The data are summarized in Supplementary table 4.

4. DISCUSSION

In this study we explored SNPs in 125 longevity candidate genes that take part in biological processes such as DNA repair, GH/IGF-1/INS signalling and lipoprotein metabolism. Initially five SNPs were found to each associate to one of the four phenotypes: cognitive composite score, the ADL strength score, hand grip strength or self-rated health, while no SNPs showed association to the MMSE or ADL disability scores. The fact that we find no association for the MMSE or ADL disability scores might indicate that the investigated genes are not related to these phenotypes. We can, however, not exclude that more statistical power (a larger sample size) is needed to detect additional potential associations to the six phenotypes investigated here, especially if these are of small effect size. Furthermore, the relevant genetic variation might also be of a different nature than the SNPs investigated here; they might be less frequent or they might be structural such as copy number variants. Finally, we cannot exclude that the potential associations could have their most pronounced effects in an age span different from that of the nonagenarians investigated here.

One drawback of the present study is that the most intensively studied *APOE* variants, the *APOE* epsilon variants, were not investigated due to the format of the GoldenGate array. However, these variants were investigated in a previous study of the discovery cohort with respect to cognitive functioning and were not found to significantly associate with cognitive status (Bathum et al., 2006). A post hoc analysis of the *APOE* epsilon variants and the remaining phenotypes of the present study did not show significance when considering the number of variants tested (data not shown). Moreover, *APOE*-rs769449, which was associated with longevity in the discovery cohort in our previous study (Soerensen et al., 2013), and was found to be in modest LD (R^2=0.55) with the *APOE*ε4 defining variant rs429358 (Soerensen et al., 2013), displayed no significant associations in the present study when considering correction for multiple testing (see Supplementary Table 2). Hence, the lack of association to the aging phenotypes of the present study could imply that rs769449 and the *APOE* epsilon variants mediate their effects on longevity via other aging related phenotypes than those investigated in the present study, or it could indicate that the effect sizes are too modest to display significance.

Considering the anticipated importance of the biological processes under study in the aging process and longevity (e.g. Argon and Gidalevitz, 2015) it might appear surprising that we did not observe significant replicable association to the included aging-related phenotypes. Furthermore, as both the phenotypes and a number of the genes explored in the present study were previously linked to survival during old age (Nybo et al., 2003, Yaffe et al. 2010, Jacobsen et al. 2010, Soerensen et al., 2012 and Soerensen et al., 2013), the lack of replicable association in the present study could imply that different genetic loci are involved in longevity and in the aging-related phenotypes under study. Nevertheless, the replication of initial findings in genetic epidemiology is in general considered a difficult task (Lui et al., 2008). Lack of power, population stratification, differences in phenotypes and between-study heterogeneity have been put forward as factors affecting the success of replication. The present replication sample was included due to the similar survey methods (including identical survey questions giving identical phenotype definitions), the very comparable ethnicity, homogeneity of the study populations, and the size of the replication sample (cf.

the power described in section 2.5). However, differences in age or the birth cohort of the individuals under study can also influence replication (Lasky-Su et al., 2008, Nygaard et al., 2014 and Sebastiani et al., 2015). Therefore, as the individuals in the discovery sample of the present study were younger (age 92–93) as compared to the replication cohort (age 94–100), and as the individuals belonged to different birth cohorts (1905 vs. 1910 and 1915), age and cohort effects might be especially important in the present study. Accordingly, subtle differences in allele frequencies between individuals of similar age in the two study populations were observed (data not shown) and the distribution of some of the phenotypes differed. For example, did the individuals of the replication sample have somewhat better self-rated health than the individuals of the discovery sample (see Supplementary Table 2). In any case, the lack of replication in the present study does not support association between the investigated genes and the aging-related phenotypes in the oldest-olds.

5. CONCLUSION

The present study does not show replicable association of longevity candidate genes to aging-related phenotypes previously shown to predict survival. This could indicate that different genomic positions are involved in aging and in longevity. Alternatively, the lack of replication in the present study could indicate that larger sample sizes are needed to robustly reveal associations, or that age or cohort effects are relevant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- No association of 125 longevity candidate genes to aging-related phenotypes.
- Previously the phenotypes were found to predict survival in the present cohort.
- Some of the genes were previously linked to longevity in the individuals studied.
- The lack of replication may be due to lack off power or age/cohort specific effects.

TABLE 1

Characteristics of the discovery and replication samples with respect to the phenotypes investigated.

Discovery sample (N = 1,088)		
Phenotype	**n**	
Cognitive composite score (mean (SE))	1,039	0.32 (0.11)
Hand grip strength (mean (SE))	997	16.44 (0.21)
MMSE (no. individuals (%))		
Cognitive impairment	1,042	207 (19.9)
Mild cognitive impairment		338 (32.4)
No cognitive impairment		497 (47.7)
Activity of daily living (no. individuals (%))		
Disabled	1,086	117 (10.8)
Moderately disabled		403 (37.1)
Not disabled		566 (52.1)
Activity of daily living strength score (no. individuals (%))		
1st quantile (lowest strength)	1,075	276 (25.7)
2nd quantile		266 (24.7)
3rd quantile		301 (28.0)
4th quantile (highest strength)		232 (21.6)
Self-rated health (no. individuals (%))		
Very poor	1,046	17 (1.6)
Poor		78 (7.5)
Acceptable		350 (33.4)
Good		417 (39.9)
Excellent		184 (17.6)
Replication sample (N = 1,281)		
Phenotype	**n**	
Cognitive composite score (mean (SE))	1,254	0.47 (0.10)
Hand grip strength (mean (SE))	1,101	15.94 (0.20)
Activity of daily living strength score (no. individuals (%))		
1st quantile (lowest strength)	1,263	318 (25.2)
2nd quantile		314 (24.9)
3rd quantile		334 (26.4)
4th quantile (highest strength)		297 (23.5)
Self-rated health (no. individuals (%))		
Very poor	1,275	21 (1.6)

Discovery sample (N = 1,088)		
Phenotype	**n**	
Poor		53 (4.2)
Acceptable		317 (24.9)
Good		528 (41.4)
Excellent		356 (27.9)

Notes: n: number of individuals with phenotype data, SE: standard error.

TABLE 2

SNPs associated with aging phenotypes after correction for multiple testing in the Danish 1905 Cohort (discovery sample).

Both genders (n=1,088)									
Phenotype	**Gene**	**SNP**	**Chr./position**	**Position in gene**	**Minor/major allele**	**MAF**	**β coefficient**	**p-value**	**Corrected p-value**
Cognitive composite score	*RAD23B*	rs10739239	9/109101964	Intronic	T/A	0.42	−0.70	$5.34*10^{-6}$	0.003
	RAD23B	rs10816492	9/109123748	Intronic	A/G	0.47	−0.59	$8.64*10^{-5}$	0.046
							OR		
ADL strength score	*RECQ1L*	rs1061627	12/21545674	5′ UTR	G/A	0.17	1.54	$2.63*10^{-5}$	0.014
Females (n=775)									
Phenotype	Gene	SNP	Chr./position	Position in gene	Minor/major allele	MAF	β coefficient	p-value	Corrected p-value
Hand grip strength	*ERCC5*	rs2227869	13/102313086	Coding (non-syn)	G/C	0.04	−2.43	$4.55 * 10^{-5}$	0.039
Males (n=313)									
Phenotype	Gene	SNP	Chr./position	Position in gene	Minor/major allele	MAF	β coefficient	p-value	Corrected p-value
Cognitive composite score	*RAD23B*	rs10739239	9/109101964	Intronic T/A		0.42	−1.20	$2.83 * 10^{-5}$	0.015
							OR		
Self-rated health	*APOA4/APOC3*	rs2849174	11/116202276	5′UTR	A/G	0.29	2.10	$4.71*10^{-5}$	0.005

Notes: chr: chromosome, MAF: minor allele frequency, corrected p-values: corrected for multiple testing by permutation, OR: odds ratio, UTR: untranslated region and non-syn: non-synonymous SNP.