Genome-wide association analysis of cognitive function in Danish long-lived individuals

Marianne Nygaard[1,2,*], **Joseph Dowsett**[1,3,*], **Matt McGue**[1,4], **Kaare Christensen**[1,2,5], **Lene Christiansen**[1,3], **Qihua Tan**[1,2], **Jonas Mengel-From**[1,2]

[1]The Danish Twin Registry and The Danish Aging Research Center, Department of Public Health, University of Southern Denmark, J.B. Winsloews Vej 9B, 5000 Odense C, Denmark

[2]Department of Clinical Genetics, Odense University Hospital, J.B. Winsloews Vej 4, 5000 Odense C, Denmark

[3]Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen OE, Denmark

[4]Department of Psychology, University of Minnesota, 75 East River Road, Minneapolis, MN 55455, USA

[5]Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Kløvervænget 47, 5000 Odense C, Denmark

Abstract

Cognitive function is a substantially heritable trait related to numerous important life outcomes. Several genome-wide association studies of cognitive function have in recent years led to the identification of thousands of significantly associated loci and genes. Individuals included in these studies have rarely been nonagenarians and centenarians, and since cognitive function is an important component of quality of life for this rapidly expanding demographic group, there is a need to explore genetic factors associated with individual differences in cognitive function at advanced ages.

In this study, we pursued this by performing a genome-wide association study of cognitive function in 490 long-lived Danes (age range 90.1–100.8 years). While no genome-wide significant SNPs were identified, suggestively significant SNPs ($P < 1 \times 10^{-5}$) were mapped to several interesting genes, including *ZWINT*, *CELF2*, and *DNAH5*, and the glutamate receptor genes *GRID2* and *GRM7*. Additionally, results from a gene set over-representation analysis indicated potential roles of gene sets related to G protein-coupled receptor (GPCR) signaling, interaction

*Corresponding author: Marianne Nygaard, The Danish Twin Registry and The Danish Aging Research Center, Department of Public Health, University of Southern Denmark, J.B. Winsloews Vej 9B, 5000 Odense C, Denmark. Tel: +45 65509267, Fax: +45 65503682, mnygaard@health.sdu.dk.
*These authors contributed equally to this work

Conflicts of Interest

The authors declare no conflicts of interest.

between L1 and ankyrins, mitogen-activated protein kinase (MAPK) signaling, RNA degradation, and cell cycle.

Larger studies are needed to shed further light on the possible importance of these suggestive genes and pathways in cognitive function in nonagenarians and centenarians.

Keywords

Cognitive function; long-lived individuals; genome-wide association study; gene-based analysis; pathway analysis

1. Introduction

General cognitive function is a major contributor to economic and health-related life outcomes, and is known to be positively correlated with education, occupational status, income, health, and longevity, among others (Deary, 2012). Thus, understanding the factors that contribute to cognitive function and its underlying biology is of great importance.

Cognitive function is a complex trait affected by both genetic and environmental factors. Twin studies have estimated that the heritability of cognitive function is around 50–80%, increasing from childhood to approximately 65 year of age (Polderman et al., 2015). The heritability after age 65 has been debated, as some studies have shown that the heritability decreases in very old age (Finkel and Reynolds, 2009; Lee et al., 2010), while others have shown that it remains stable (McGue and Christensen, 2013). Regardless, the heritability remains above 50%, indicating a substantial genetic contribution to individual differences in cognitive functioning. In recent years, a number of genome-wide association studies (GWASs) of cognitive function or similar phenotypes, e.g. intelligence, have been published, resulting in identification of thousands of significantly associated genetic loci and genes (e.g. (Davies et al., 2015; Davies et al., 2018; Davies et al., 2016; Davies et al., 2011; Hill et al., 2019; Lam et al., 2017; Savage et al., 2018; Sniekers et al., 2017; Trampush et al., 2017; Xu et al., 2017)). The most recent studies included genome-wide association meta-analyses of general cognitive function and intelligence (Davies et al., 2018; Savage et al., 2018), and a study combining two large GWASs of intelligence and education (Hill et al., 2019). The meta-analysis of general cognitive function by Davies et al. included 300,486 individuals of European ancestry from 57 population-based cohorts and identified 148 significantly associated independent loci, 709 significantly associated genes, and 7 significant gene sets related to neurogenesis, regulation of nervous system development, neuron projection, neuron differentiation, regulation of cell development, and dendrite (Davies et al., 2018). In the meta-analysis of intelligence by Savage et al., including 269,867 individuals from 14 independent epidemiological cohorts of European ancestry, the authors found 205 significantly associated genomic loci, 1,016 significantly associated genes, and 6 significant gene sets: neurogenesis, neuron differentiation, central nervous system neuron differentiation, regulation of nervous system development, positive regulation of nervous system development, and regulation of synapse structure or activity (Savage et al., 2018). In the study of 248,482 individuals by Hill et al., the authors combined a meta-GWAS of intelligence with a GWAS of education (Okbay et al., 2016), resulting in significant

associations with 187 loci, 538 genes, and gene sets related to neurogenesis, regulation of nervous system development, regulation of cell development, neuron projection, central nervous system neuron differentiation, synapse, neuron differentiation, and oligodendrocyte differentiation (Hill et al., 2019). While the GWASs of cognitive function and related phenotypes published so far have included impressive numbers of individuals, most participants have been young, middle-aged, and elderly individuals, and thus there is a general lack of studies focusing on more extreme age groups like nonagenarians and centenarians. With an increasingly aging population (Vaupel, 2010), cognitive impairment is a major concern, both for the individual and for society. Retaining cognitive abilities in old age is important for the ability to live independently, and is a key element of successful aging and of quality of life (McGue and Christensen, 2001, 2002). It has also been shown that greater variation is observed for cognitive function in older compared to younger individuals (Harris and Deary, 2011), and that this variation becomes increasingly important with age as the level of cognitive function approaches the threshold required for everyday functioning (Tucker-Drob, 2011). Another aspect is that the selection pressure has been and is markedly different for nonagenarians and centenarians compared to younger individuals. At advanced ages individuals are increasingly subjected to selective mortality, i.e. the most frail and disabled individuals tend to die first, leaving the best functioning individuals in the population (Christensen et al., 2008), and it is therefore possible that different genes influence cognition at different ages as it has been seen for the *APOE* gene (Davies et al., 2015; Dokkedal et al., 2020) and that the findings of this study may be different from the findings of studies of younger individuals.

Therefore, to explore genetic variants associated with cognitive function among the very old, we performed a GWAS of cognitive function assessed by a cognitive composite score derived from 5 cognitive tests in a homogenous study population of 490 long-lived Danes (mean age 96.8 years, age range 90.1–100.8 years).

2.   Materials and Methods

2.1.   Study population

The study population consisted of 490 long-lived individuals drawn from four population-based, nation-wide surveys conducted at the University of Southern Denmark: the 1905 birth cohort study (N = 182), the 1910 birth cohort study (N = 128), the 1915 birth cohort study (N = 140), and the Longitudinal Study of Ageing Danish Twins (LSADT, N = 40)). All surveys were without exclusion criteria but required that the participant could provide informed consent. Briefly, the 1905 birth cohort study is an in-depth survey of all Danes born in 1905. The study was initiated in 1998 and follow-up surveys of participating survivors were conducted in 2000, 2003, and 2005. The 1910 and 1915 birth cohort studies include all Danes born in 1910 and 1915, respectively, and alive and living in Denmark on September 1st 2010. The 1915 birth cohort study was followed up in 2015, when participants reached 100 years of age. The 1905, 1910, and 1915 birth cohort studies have been described in more details elsewhere (Rasmussen et al., 2017). From the 1905 and 1915 birth cohort studies, participants for the present study were selected among individuals reaching an age of at least 96 years. However, the blood samples and the applied cognitive scores

were collected as part of the intake surveys (for the 1905 birth cohort study either in 1998 at 92–93 years of age or in 2005 at 100 years of age, for the 1910 birth cohort study in 2010 at 100 years of age, and for the 1915 birth cohort study in 2010 at 95 years of age). LSADT includes Danish twins aged 70 years and older and was initiated in 1995 with follow-up surveys carried out in 1997, 1999, 2001, 2003, and 2005 (Pedersen et al., 2019). From LSADT, one individual from each twin pair was randomly selected among participants that had reached an age of at least 90 years, blood samples were collected in 1997, and the applied cognitive score was from the highest age possible/the latest survey (1997 (N = 3), 1999 (N = 2), 2001 (N = 5), 2003 (N = 6), and 2005 (N = 24).

Written informed consents were obtained from all participants. Collection and use of biological material and survey information were approved by the Regional Committees on Health Research Ethics for Southern Denmark, and the study was approved by the Danish Data Protection Agency.

2.2. Cognitive function

Cognitive function was assessed using a five-component cognitive score including tests of verbal fluency, forward and backward digit span, and immediate and delayed recall (McGue and Christensen, 2001). To create the cognitive composite scores, the individual scores of the five tests were standardized using the means and standard deviations obtained in 46–50 year old individuals born from 1949 to 1952, summed and converted into a T-score with a mean of 50 and a standard deviation of 10 in the 46–50 year age group.

To evaluate cognitive impairment among study participants, Mini Mental State Examination (MMSE) scores were included. MMSE ranges from 0 to 30, and individuals with scores between 0 and 17 are graded as severely cognitively impaired, individuals with scores between 18 and 23 as mildly cognitively impaired, and individuals with scores between 24 and 30 as normal/having no cognitive impairment. The MMSE is further described in the previous literature (McGue and Christensen, 2001; Mengel-From et al., 2016).

2.3. Genotyping and quality control

DNA was extracted from whole blood using a manual (Miller et al., 1988) or a semi-automatic (Autopure, Qiagen, Hilden, Germany) salting out method, or from filter cards using the Extract-N-Amp Blood PCR Kit (Sigma Aldrich, St. Louis, MO, USA) followed by amplification using the GenomePlex Complete Whole Genome Amplification (WGA) Kit (Sigma Aldrich, St. Louis, MO, USA).

Samples were genotyped using the Illumina Human OmniExpress Array (Illumina San Diego, CA, USA) following the manufacturer's protocols. Quality control was carried out in GenomeStudio (Illumina San Diego, CA, USA), Plink (https://www.cog-genomics.org/plink/1.9/) (Chang et al., 2015; Purcell et al., 2007) and R version 3.3.1. Post-genotyping/pre-imputation quality control included filtering SNPs on cluster separation < 0.40, the mean of the normalized theta value of the heterozygote cluster < 0.20 or > 0.80, the mean normalized intensity of the heterozygote cluster < 0.25, call rate < 95%, HWE $P < 1 \times 10^{-4}$, and MAF < 1%. In addition, SNPs with a significantly different ($P < 1 \times 10^{-4}$) minor allele frequency between individuals with DNA extracted from whole blood and individuals with

DNA extracted from filter cards followed by whole genome amplification were excluded. Individuals were excluded based on call rate < 95%, relatedness (1 individual from sample pairs with proportion of IBD > 0.1875) and gender mismatch between genetic and reported gender. Pre-phasing and imputation to the 1000 Genomes phase I v.3 reference panel was performed using IMPUTE2 (Howie et al., 2009). After imputation, genotype probabilities were converted to hard-called genotypes in Plink using a cut-off of 90%, meaning that only genotypes with a probability of more than 90% were called. SNPs with no genotype probabilities above 90% were set to missing. Post-imputation quality control included the removal of insertions and deletions, non-autosomal variants, tri-allelic SNPs, and SNPs with a call rate < 95%, HWE $P < 1\times10^{-5}$, MAF < 1%, and an imputation quality (INFO) score < 0.80, resulting in 4.827.900 bi-allelic, autosomal SNPs available for analysis.

2.4. Statistical analyses

2.4.1. SNP-based analysis—A SNP-based genome-wide association analysis was performed in Plink (https://www.cog-genomics.org/plink/1.9/) (Chang et al., 2015; Purcell et al., 2007) applying a linear regression model with the cognitive composite score as the dependent variable and the hard-called genotype (assuming an additive model with genotype coded 0, 1, or 2 depending on the number of minor alleles) as the independent variable. Age and sex were included as covariates. As all study participants are ethnic Danes, no adjustment for population stratification was performed. A Bonferroni-corrected significance level of 5×10^{-8} was used as the genome-wide significance level, whereas a significance level of 1×10^{-5} was used as a suggestive significance level. A post hoc power calculation performed in Quanto (version 1.2.4, http://biostats.usc.edu/Quanto.html) revealed that assuming an additive model and given a N of 490, a minor allele frequency of at least 0.01, a significance level of of 5×10^{-8}, a mean cognitive composite score and corresponding standard deviation of 27.1 and 9.3, respectively, beta-coefficients of 18.5 and 3.7 could be detected with a power of at least 80% at minor allele frequencies of 0.01 and 0.50.

Manhattan and quantile-quantile (Q-Q) plots (see Figure 1) were created in R using the package *qqman*.

2.4.2. Gene-based analysis—A gene-based analysis was conducted using MAGMA (de Leeuw et al., 2015) as implemented in FUMA v1.3.4 (http://fuma.ctglab.nl/) (Watanabe et al., 2017), which is a web-based platform facilitating functional annotation of GWAS results. Default parameters, including the SNP-wide mean model, were applied along with the 1000G Phase3 EUR reference panel (Genomes Project et al., 2015). To allow for regulatory SNPs to be included in the gene-based analysis, all SNPs located within ±10 kb of a gene were used to derive a P-value for the association between that gene and cognitive function. In total, 18339 protein-coding, autosomal genes defined by the NCBI Build 37.3 were investigated, resulting in a Bonferroni-corrected genome-wide significance threshold of $P < 2.73\times10^{-6}$.

2.4.3. Gene-set analyses—A gene-set analysis was also performed in MAGMA (de Leeuw et al., 2015) as implemented in FUMA v1.3.4 (http://fuma.ctglab.nl/) (Watanabe et al., 2017) using 10678 curated gene sets and GO terms from MSigDB v6.2 (Liberzon et al.,

2011). The MAGMA gene-set analysis is competitive in the sense that it tests if genes within a gene set are more strongly associated with cognitive function than the genes within other gene sets. Bonferroni correction was applied to control for multiple testing.

As the MAGMA gene-set analysis uses the full distribution of gene P-values from the MAGMA gene-based analysis, we additionally performed an over-representation analysis to identify gene sets over-represented only among the nominally significant genes (P < 0.05). Thus, these were submitted to MSigDB (http://software.broadinstitute.org/gsea/msigdb/index.jsp) (Liberzon et al., 2011) using the REACTOME and KEGG databases as reference. A false discovery rate (FDR) < 0.05 was used to identify significant gene sets.

2.4.4. Gene-property analysis—A gene-property analysis was carried out using MAGMA (de Leeuw et al., 2015) as implemented in FUMA v1.3.4 (http://fuma.ctglab.nl/) (Watanabe et al., 2017) to examine the tissue-specificity of cognitive function by testing the correlations between tissue-specific expression profiles and the associations between genes and cognitive function. Gene expression values for 53 specific tissues types were obtained from GTEx v6 (Consortium, 2015), and the full distribution of gene P-values from the MAGMA gene-based analysis was used. The control for multiple testing was done using a Bonferroni correction.

2.4.5. Gene mapping—Prior to gene mapping, independent SNPs, lead SNPs, candidate SNPs, and genomic loci were defined from the results of the SNP-based analysis using FUMA v1.3.4 (http://fuma.ctglab.nl/) (Watanabe et al., 2017). Firstly, independent SNPs were defined as SNPs with $P < 1\times10^{-5}$ and independent of other suggestively significant SNPs at $r^2 < 0.6$. Secondly, lead SNPs were defined as a subset of the independent SNPs as those that were independent from each other at $r^2 < 0.1$. Thirdly, candidate SNPs were defined as SNPs with a MAF $\geq$ 0.01 and in LD ($r^2 \geq$ 0.6) with any of the independent SNPs. The candidate SNPs were identified from the 1000G Phase3 EUR reference panel (Genomes Project et al., 2015), and thus they were not necessarily a part of our initially performed GWAS. Only SNPs with a MAF $\geq$ 0.01 in the 1000G Phase 3 EUR reference panel were included in the annotation, for which reason six of the suggestively significant SNPs (rs17265690, rs114933297, rs141363063, rs148488001, rs192345176, and rs138203943; see Table 2) were not taken into account by FUMA. Finally, genomic loci were defined by merging lead SNP-containing loci less than 250 kb apart, and with the borders of the loci defined by the candidate SNPs. LD patterns and MAFs were deduced from the 1000G Phase3 EUR reference panel (Genomes Project et al., 2015).

The identified candidate SNPs were mapped to genes in FUMA using three strategies: 1) positional mapping, where SNPs were mapped to genes based on physical distance (+/− 10 kb); 2) expression quantitative trait loci (eQTL) mapping, where SNPs were mapped to genes with which the SNPs showed a significant (FDR < 0.05) eQTL association (i.e. allelic variation at the SNP is associated with the expression level of that gene); 3) chromatin interaction mapping, where SNPs were mapped to genes when there is a significant (FDR < 1×10^{-6}) 3D DNA-DNA interaction between the SNP region and the gene region.

2.5. Verification

Verification of the results of the SNP-based analysis (independent SNPs and candidate SNPs as defined in the section 'Gene mapping') was sought by *in silico* look-up in the results of the already published meta-GWASs on general cognitive function (Davies et al., 2018) and intelligence (Savage et al., 2018), and in the results of the study combining GWASs of intelligence and education (Hill et al., 2019). For the studies by Hill et al. and Davies et al., information was only available for genome-wide significant ($P < 5\times10^{-8}$) and suggestively significant ($P < 1\times10^{-5}$) SNPs, respectively, and therefore the replication was primarily based on the results of the study by Savage et al. where summary statistics were available for all SNPs included in the study.

2.6. Replication of results of previous studies

Replication of previously reported genome-wide significant findings was sought in the present study by comparing the results of the SNP- and gene-based analyses to the findings of the meta-GWASs published by Davies et al. (Davies et al., 2018), Savage et al. (Savage et al., 2018), and Hill et al. (Hill et al., 2019).

3. Results

Study population characteristics are shown in Table 1. Notably, the study population of long-lived individuals had a mean cognitive score of 27.1, corresponding to more than 2 SDs lower than the average score for 46–50 year old individuals born from 1949–1952, indicating, as expected, a clear cross-sectional age-related decline in cognitive function. The cognitive score range of 4.1 to 63.9 (IQR 20.6–32.6) reveals that there is substantial variation among the study population participants.

No genome-wide significant ($P < 5\times10^{-8}$) SNPs were identified in the SNP-based association analysis. However, 65 SNPs (see Table 2) were found to associate with cognitive function at a suggestive significance level ($P < 1\times10^{-5}$). Manhattan- and QQ-plots for the SNP-based association analysis are shown in Figure 1. According to the genomic loci characterization implemented in FUMA, the 65 SNPs represent 14 independent signals distributed across 12 genomic loci on chromosomes 1, 3, 4, 5, 6, 10, and 18.

The MAGMA gene-based (see Supplementary Table 1), gene-set (see Supplementary Table 2) and gene-property analyses did not reveal any significant results. Interestingly, however, a striking tendency was seen in the gene-property analysis results where the 10 tissue-specific expression profiles most correlated with the results of the gene-based analysis were of various parts of the brain (see Figure 2). Also, the MSigDB over-representation analysis of the nominally significant genes identified in the MAGMA gene-based analysis, revealed significant enrichment (FDR < 0.05) for gene sets related to G protein-coupled receptor (GPCR) signaling (the Reactome gene sets 'Signaling by GPCR', 'GPCR ligand binding', 'Class A/1 (Rhodopsin-like receptors)', 'GPCR downstream signaling', 'Peptide ligand-binding receptors', and 'G alpha (12/13) signaling events'), interaction between L1 and ankyrins, mitogen-activated protein kinase (MAPK) signaling, RNA degradation, and cell cycle (see Table 3).

Using the gene mapping strategies implemented in FUMA, the suggestively significant SNPs were found to map to 60 unique genes; 6 were identified through positional mapping, 4 were identified through eQTL mapping, and 54 were identified through chromatin interaction mapping (see Supplementary Table 3). Two genes, *CELF2* and *DNAH5*, were identified by two mapping strategies, and one gene, *ZWINT*, was identified by all three strategies.

Independent SNPs and candidate SNPs identified based on the independent SNPs were sought verified by *in silico* look-up in three recent meta-GWASs of general cognitive function and intelligence. Overall, 9.7% (P = 7×10^{-4}) of the independent and candidate SNPs were verified (same direction of effect and P < 0.05). The verified SNPs were candidate SNPs identified based on four of the independent SNPs (rs187473706, rs2016129, rs1556397, and rs56139919 on chromosomes 6 and 10; see Table 2).

Replication of genome-wide significant SNP- and gene-based findings previously published in GWASs of general cognitive function and intelligence, revealed that of the genome-wide significant SNPs identified in the studies by Davies et al. 2018, Savage et al. 2018, and Hill et al. 2019, 55.0%, 72.4%, and 72.8%, respectively, were available in the present study, and 5.1%, 4.9%, and 4.4% of these replicated (P < 0.05). When restricting to SNPs showing the same direction of effect, 2.6% and 2.4% of the genome-wide significant SNPs from the studies by Savage et al. 2018 and Hill et al. 2019 replicated in our study. Unfortunately, the publicly available data from the Davies et al. 2018 study did not allow a straightforward evaluation of direction of effect. Between 5.1% and 6.5% of the genome-wide significant results of gene-based analyses were found to replicate in the present study.

4. Discussion

To further our understanding of the underlying biology of the genetic component of cognitive function among nonagenarians and centenarians, we carried out a genome-wide association study of cognitive function in 490 long-lived Danes.

While the SNP-based association analysis in the present study did not reveal any genome-wide significant findings, a number of SNPs with a suggestively significant association to cognitive function among the very old were identified. Based on the independent SNPs identified from these suggestively significant SNPs and the derived candidate SNPs, 60 genes were found to be implicated in cognitive function using three gene mapping strategies; positional mapping, eQTL mapping, and chromatin interaction mapping (see Supplementary Table 3). The *ZWINT* gene was identified by all three mapping strategies, while *CELF2* and *DNAH5* were identified by positional mapping and chromatin interaction mapping. The *ZWINT* gene encodes the ZW10 interacting kinetochore protein, which regulates centromere division and was recently suggested as a potential target for lung cancer therapy (Peng et al., 2019). The role of *ZWINT* in cognitive function is thus less clear, although the protein encoded by *ZWINT* is reported by the Biological General Repository for Interaction Datasets (BioGRID; https://thebiogrid.org) (Oughtred et al., 2019) to interact with the products of the *APP* and *CLU* genes that are well-known in relation to Alzheimer's disease and cognitive function (Kunkle et al., 2019). Similarly, the *CELF2* gene product, the

CUGBP Elav-like family member 2 protein, has been found to interact with the *APP* gene product (BioGRID; https://thebiogrid.org) (Oughtred et al., 2019). In addition, *CELF2* is highly expressed in the brain (Li et al., 2001) and genetic variants in *CELF2* have been found to be involved in late-onset Alzheimer's disease in *APOE ε4* homozygotes (Wijsman et al., 2011), as well as significantly associated with educational attainment, cognitive function, and mathematical ability (Kichaev et al., 2019; Lee et al., 2018). The *DNAH5* gene encodes an axonemal dynein heavy chain protein, which is part of a microtubule-associated motor protein complex in motile cilia (Zariwala et al., 2007), and plays a role in primary ciliary dyskinesia that is characterized by abnormal ciliary motility and affects the lungs, reproductive organs, and organ laterality (Olbrich et al., 2002; Zariwala et al., 2007). Of more relevance, *DNAH5* has also been suggested to play a role in mathematical ability (Docherty et al., 2010).

In addition, the mapped genes include *CCSER1*, *TFB1M*, *KIAA1217*, *PDE4B*, *NOX3*, *ARID1B*, *ZCCHC2*, *ECHDC3*, and *LYZL1* that have all been implicated in Alzheimer's disease, educational attainment, cognitive function, mathematical ability, and/or intelligence (Davies et al., 2018; Hill et al., 2019; Jun et al., 2017; Kichaev et al., 2019; Lee et al., 2018; Savage et al., 2018; Witoelar et al., 2018); the *DUSP22* gene, which has been found to be down-regulated in the hippocampus of Alzheimer's disease patients and is suggested to be important for Tau phosphorylation and CREB signaling (Sanchez-Mut et al., 2014); the *BCL2* gene, which encodes an anti-apoptotic protein suggested to link autophagy and Alzheimer's disease (Uddin et al., 2018); *GRID2* that encodes the glutamate ionotropic receptor delta type subunit 2 protein, which is expressed selectively in cerebellar Purkinje cells (Araki et al., 1993), and *GRM7* that encodes the glutamate metabotropic receptor 7 protein, which is expressed in many brain tissues, in particular in the hippocampus, cerebral cortex, and cerebellum (Makoff et al., 1996). Both ionotropic and metabotropic glutamate receptors are activated by glutamate. Glutamate is the major excitatory neurotransmitter in the central nervous system, and glutamatergic neurotransmission is involved in most aspects of normal brain function. Ionotropic glutamate receptors are ligand-gated ion channels that produce excitatory currents upon activation by glutamate, while metabotropic glutamate receptors are G protein-coupled receptors (GPCRs) that control cellular processes through G protein signaling cascades (Reiner and Levitz, 2018). A number of GWASs have found *GRID2* to be involved in educational attainment, intelligence and cognitive function (Hill et al., 2019; Kichaev et al., 2019; Lee et al., 2018; Savage et al., 2018), whereas *GRM7* was recently suggested as a novel gene significantly associated with Alzheimer's disease (Squillario et al., 2020).

The role of GPCRs in cognitive function in nonagenarians and centenarians was further supported by the gene set over-representation analysis. Using the list of genes found to associate with cognitive function among nonagenarians and centenarians at a nominal significance level from the MAGMA gene-based analysis, the analysis identified several significantly over-represented gene sets related to GPCR signaling. The GPCRs comprise the largest family of transmembrane proteins in humans and share a common structure of seven transmembrane helical regions. Roughly 50% of GPCRs have sensory functions, and around 90% of the non-sensory GPCRs are expressed in the brain, where they play important roles in numerous neuronal functions, including regulation of neuronal

communication at the synapse (Huang and Thathiah, 2015) as well as structural and synaptic plasticity, which have been associated with learning and memory and thereby cognitive function (Leung and Wong, 2017). Furthermore, GPCRs have been implicated in a number of neurodegenerative diseases like Alzheimer's disease, vascular dementia, frontotemporal dementia, Parkinson's disease, and Huntington's disease (Huang et al., 2017). Interestingly, the Reactome gene set 'Interaction between L1 and Ankyrins' was also among the gene sets in which genes associated with cognitive function at a nominal significance level were significantly over-represented. L1-type proteins are transmembrane cell adhesion molecules (CAMs) that play important roles in several neural processes, including axonal formation, growth and branching, synapse development, and regulation of synaptic plasticity (Skaper, 2012). Several of these processes might be regulated by interaction between L1-type CAMs and ankyrins (Hortsch et al., 2009).

The 'G alpha (12/13) signaling events' pathway replicated at a nominal significance level (P < 0.05) in the study by Davies et al. (Davies et al., 2018), and the more generic pathways 'MAPK signaling pathway' and 'Cell cycle' both replicated in at least two of the studies by Davies et al., Hill et al., or Savage et al. (Davies et al., 2018; Hill et al., 2019; Savage et al., 2018).

Using a Bonferroni-corrected significance level threshold, which is the default in the MAGMA gene-set analysis performed as part of FUMA, no gene sets were found to be significant. However, in connection with another gene-set analysis algorithm it has been argued that using a Bonferroni-corrected significance level is too stringent as the gene sets are not independent. Instead, a significance level of $P < 1 \times 10^{-5}$ has been suggested to be sufficient to ensure no inflation of the type 1 error rate (Mishra and Macgregor, 2015). Using this significance level, one gene set, the 'hirsch cellular transformation signature dn' gene set (Hirsch et al., 2010) was found to be significantly associated with cognitive function (see Supplementary Table 2). This gene set was previously found to be associated with general cognitive function and intelligence with the same direction of effect as seen in this study at a nominal significance level in the studies by Davies et al. and Hill et al. (Davies et al., 2018; Hill et al., 2019).

In addition to uncovering several suggestive findings of the genetic basis of cognitive function among long-lived individuals, the present study also added support for several previously reported associations. While this suggests that there is overlap between the genetic contribution to cognitive function in younger and long-lived individuals, the proportion of replicated findings (around 5%) is somewhat smaller than in other studies (typically more than 10%), which is likely to be explained by differences, primarily in the age of the study populations, but potentially also in degree of genetic homogeneity, phenotypes and analysis methods.

This study has some methodological limitations that should be taken into account when considering the significance and generalizability of the results. First, the sample size is limited, which means that the power, especially in the SNP-based association analysis, is challenged and thus chance findings cannot be excluded. However, a quite sizeable percentage (nearly 10%) of the independent and candidate SNPs were *in silico* verified in

large and well-powered studies and also, the gene-property analysis results showed a clear tendency as the 10 tissue-specific expression profiles most correlated with the results of the gene-based analysis were from various parts of the brain (see Figure 2). These results support the biological importance of our findings, despite the general lack of statistical significance. Second, the individuals included in this study are selected in the sense that individuals with severe cognitive impairment are likely to have been represented among those without a cognitive assessment. This is supported by the proportion of study participants with MMSE scores between 0 and 17 (15.9%, see Table 1) being lower than among all participants in the 1905 birth cohort study (22% and 39.2% with severe cognitive impairment at age 92–93 and 100, respectively (Christensen et al., 2013; Engberg et al., 2008) and the 1915 birth cohort study (17% with severe cognitive impairment at age 95 (Christensen et al., 2013)). In addition, individuals donating a blood sample, and who can hence be included in genetic studies, have a higher functional level than those not donating a blood sample (Mengel-From et al., 2011). However, if anything, these limitations and biases are likely to attenuate the association between genetic variants and cognitive function, and thus make it harder to find significant associations, which increase the confidence in our significant findings.

In conclusion, this study presents suggestive evidence that several genes and gene sets implicated in neurological and brain-related processes, especially G protein-coupled receptor signaling, contribute to variation in cognitive function among long-lived individuals. We acknowledge the limited size of the study, and consequently conclude that larger studies of nonagenarians and centenarians are needed to validate the findings of this study, with the overall goal of clarifying if the genetic landscape of cognitive function in long-lived individuals diverges from that found in predominantly younger individuals, and providing new insight into the genetic background of cognitive aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

- A significant role in cognition of gene sets related to GPCR signaling is found

- Other significant gene sets are found, e.g. interaction between L1 and ankyrins

- Suggestively significant SNPs are mapped to several interesting genes

- These include *ZWINT*, *CELF2*, *DNAH5*, and the glutamate receptor genes *GRID2* and *GRM7*

a.
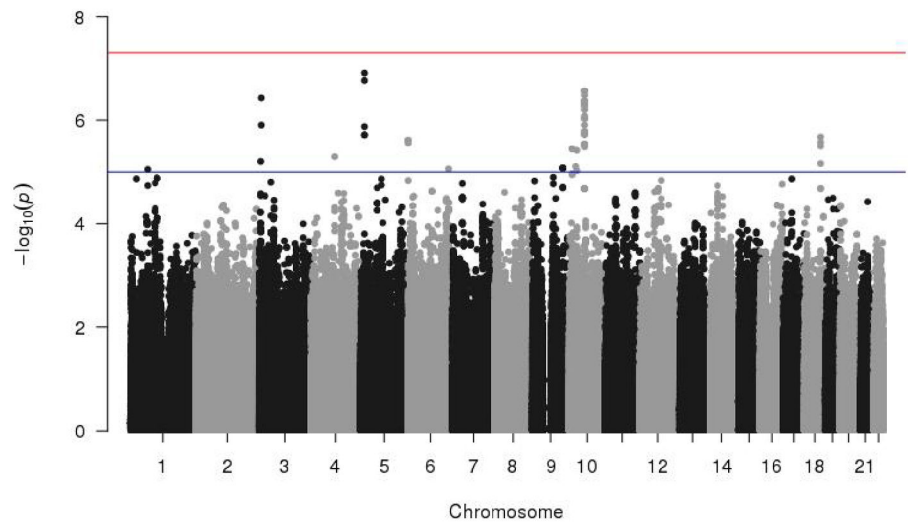
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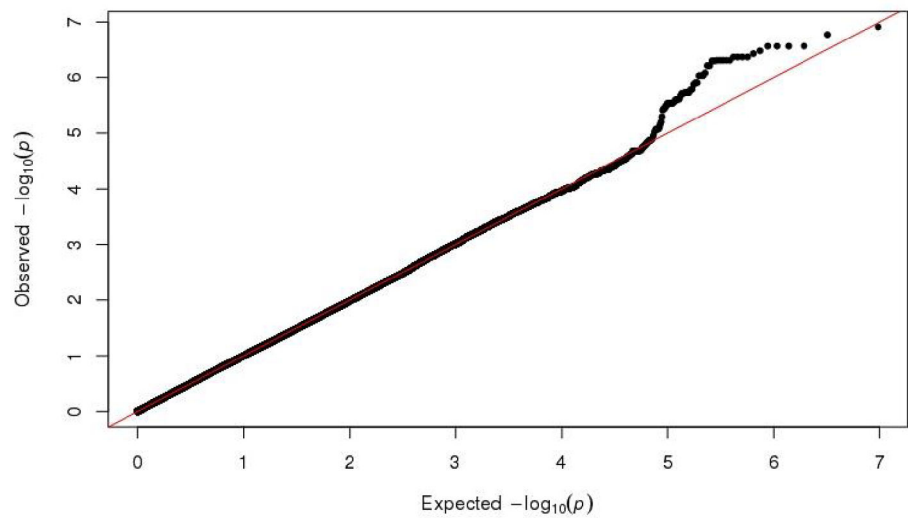
Figure 1.
Manhattan (a) and Q-Q (b) plots of P-values of the association between single nucleotide polymorphisms (SNPs) and cognitive function. The threshold for genome-wide significance ($P < 5 \times 10^{-8}$) is indicated by the red line and the threshold for suggestive significance ($P < 1 \times 10^{-5}$) is indicated by the blue line.
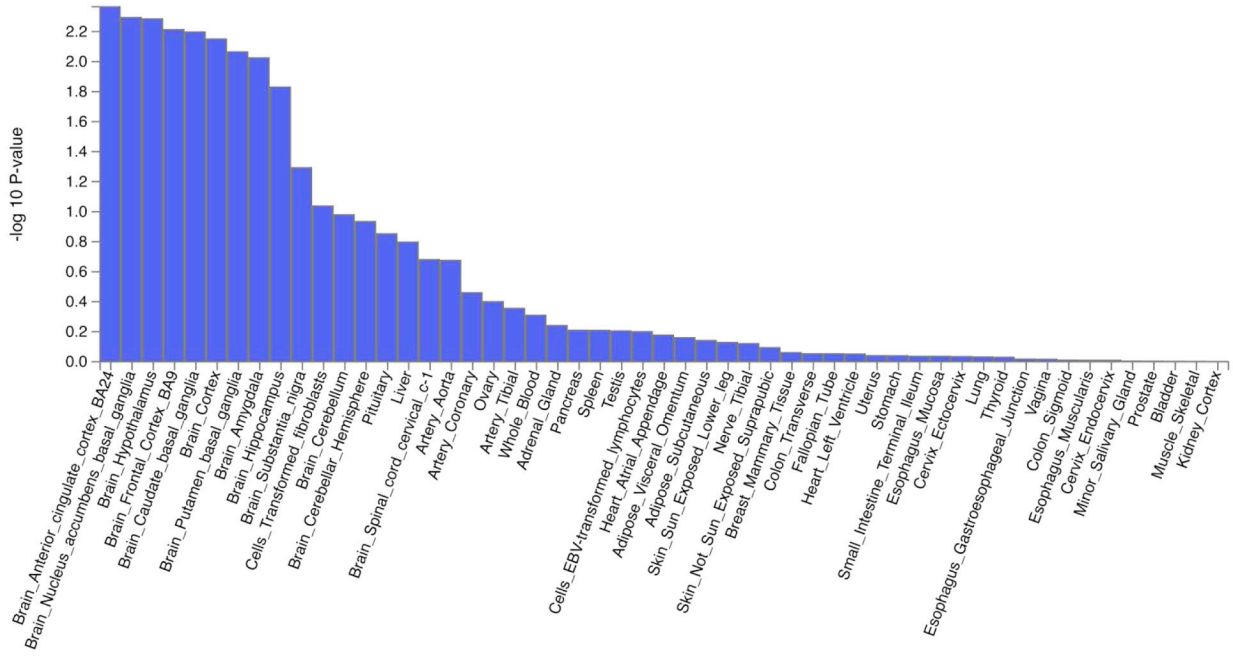
Figure 2.
Results of the gene-property analysis carried out using MAGMA examining the correlations between tissue-specific expression profiles for 53 specific tissues types obtained from GTEx v6 and the associations between genes and cognitive function.

Table 1.

Study population characteristics.

N	490
N Women (%)	383 (78.2)
Age, mean (SD) [*]	96.8 (3.1)
Age, range [*]	90.1–100.8
Birth Year, range	1901–1915
Cognitive Score, mean (SD)	27.1 (9.3)
Cognitive Score, range	4.1–63.9
N with severe cognitive impairment, MMSE 0–17 (%)	78 (15.9)
N with mild cognitive impairment, MMSE 18–23 (%)	169 (34.6)
N with no cognitive impairment, MMSE 24–30 (%)	242 (49.5)

[*] Age at cognitive assessment.

Table 2.

Summary statistics for the 65 SNPs associated with cognitive function at a suggestive significance level (P < $1{\times}10^{-5}$). SNP positions and distance to nearest gene are based on the GRCh37/hg19 genome build. Nearest gene refers to nearest RefSeq gene. SNPs identified by FUMA as independent SNPs are highlighted in bold. Independent SNPs in LD with candidate SNPs found to replicate (P < 0.05) are marked with *.

SNP	Chr	Position	Locus	Ma	MAF	Beta	P	Nearest Gene	Distance
rs17265690	5	13816888	5p15.2	G	0.0138	13.63	1.22E-07	*DNAH5*	0 (intron)
rs114012368	5	13845391	5p15.2	G	0.0153	11.14	1.70E-07	*DNAH5*	0 (intron)
rs187473706*	10	58207108	10q21.1	A	0.1277	−4.579	2.73E-07	*ZWINT*	86074
rs4405235	10	58103101	10q21.1	G	0.1313	−4.577	2.74E-07	*ZWINT*	14098
rs11005326	10	58117124	10q21.1	G	0.1313	−4.577	2.74E-07	*ZWINT*	75
rs11005338	10	58125900	10q21.1	G	0.1313	−4.577	2.74E-07	*ZWINT*	4866
rs11005320	10	58096959	10q21.1	C	0.1319	−4.564	3.32E-07	*ZWINT*	20240
rs28502528	3	7210895	3p26.1	G	0.3073	3.391	3.74E-07	*GRM7*	0 (intron)
rs58796432	10	58208170	10q21.1	T	0.1304	−4.493	4.30E-07	*ZWINT*	87136
rs11005358	10	58209898	10q21.1	A	0.1304	−4.493	4.30E-07	*ZWINT*	88864
rs74137635	10	58210671	10q21.1	C	0.1304	−4.493	4.30E-07	*ZWINT*	89583
rs12266849	10	58218034	10q21.1	G	0.1304	−4.493	4.30E-07	*ZWINT*	97000
rs12257459	10	58208672	10q21.1	G	0.1303	−4.487	4.94E-07	*ZWINT*	87638
rs2087837	10	58222830	10q21.1	A	0.1303	−4.487	4.94E-07	*ZWINT*	101796
rs11005363	10	58237680	10q21.1	C	0.1302	−4.487	4.94E-07	*ZWINT*	116646
rs58386275	10	58241470	10q21.1	C	0.1301	−4.487	4.94E-07	*ZWINT*	120436
rs11005366	10	58242596	10q21.1	T	0.1301	−4.487	4.94E-07	*ZWINT*	121562
rs4935645	10	58021087	10q21.1	C	0.1391	−4.405	5.04E-07	*ZWINT*	96112
rs2393046	10	58023980	10q21.1	T	0.1388	−4.405	5.04E-07	*ZWINT*	93219
rs74137642	10	58243119	10q21.1	G	0.1306	−4.427	6.16E-07	*ZWINT*	122085
rs7091142	10	58244433	10q21.1	T	0.1306	−4.427	6.16E-07	*ZWINT*	123399
rs141232317	10	58206613	10q21.1	T	0.1208	−4.453	8.32E-07	*ZWINT*	85579
rs2263916	10	58161893	10q21.1	G	0.1317	−4.386	9.30E-07	*ZWINT*	40859
rs10763369	10	58175630	10q21.1	A	0.1315	−4.386	9.30E-07	*ZWINT*	54596
rs10733958	10	58179399	10q21.1	G	0.1315	−4.386	9.30E-07	*ZWINT*	58365
rs1516302	3	7208953	3p26.1	A	0.3005	3.199	1.24E-06	*GRM7*	0 (intron)
rs11005287	10	58024579	10q21.1	A	0.1378	−4.246	1.24E-06	*ZWINT*	92620
rs114240283	5	13799563	5p15.2	A	0.0148	11.63	1.33E-06	*DNAH5*	0 (intron)
rs12264147	10	58025087	10q21.1	T	0.1387	−4.187	1.62E-06	*ZWINT*	92112
rs2393054	10	58056974	10q21.1	C	0.1395	−4.167	1.66E-06	*ZWINT*	60225
rs12257337	10	58062019	10q21.1	T	0.1390	−4.146	1.86E-06	*ZWINT*	55180
rs7099218	10	58067127	10q21.1	C	0.1390	−4.146	1.86E-06	*ZWINT*	50072
rs12255620	10	58076723	10q21.1	G	0.1387	−4.146	1.86E-06	*ZWINT*	40476
rs73280169	10	58077515	10q21.1	T	0.1388	−4.146	1.86E-06	*ZWINT*	39684

SNP	Chr	Position	Locus	Ma	MAF	Beta	P	Nearest Gene	Distance
rs114740310	5	13870802	5p15.2	T	0.0144	11.17	1.92E-06	*DNAH5*	0 (intron)
rs114933297	5	13874625	5p15.2	T	0.0145	11.17	1.92E-06	*DNAH5*	0 (intron)
rs595286	18	60280097	18q21.33	G	0.4679	−2.964	2.10E-06	*ZCCHC2*	26121
rs9392881	6	791124	6p25.3	G	0.1214	−4.441	2.42E-06	*LOC101927691*	77374
rs9392150	6	791318	6p25.3	A	0.1211	−4.421	2.46E-06	*LOC101927691*	77180
rs62384998	6	791566	6p25.3	C	0.1212	−4.426	2.47E-06	*LOC101927691*	76932
rs7763687	6	791850	6p25.3	T	0.1212	−4.426	2.47E-06	*LOC101927691*	76648
rs306213	18	60291954	18q21.33	A	0.3434	−3.146	2.67E-06	*ZCCHC2*	37978
rs62385000	6	792759	6p25.3	A	0.1214	−4.432	2.79E-06	*LOC101927691*	75739
rs11005317	10	58088163	10q21.1	G	0.1407	−4.021	2.90E-06	*ZWINT*	29036
rs3750796	10	58088534	10q21.1	G	0.1407	−4.021	2.90E-06	*ZWINT*	28665
rs7094892	10	58088575	10q21.1	T	0.1407	−4.021	2.90E-06	*ZWINT*	28624
rs2393065	10	58089291	10q21.1	C	0.1407	−4.021	2.90E-06	*ZWINT*	27908
rs9971280	10	58090264	10q21.1	C	0.1406	−4.021	2.90E-06	*ZWINT*	26935
rs59704796	10	58090554	10q21.1	C	0.1406	−4.021	2.90E-06	*ZWINT*	26645
rs637216	18	60282910	18q21.33	G	0.4677	−2.919	3.18E-06	*ZCCHC2*	28934
rs3750795	10	58088644	10q21.1	G	0.1403	−4.012	3.30E-06	*ZWINT*	28555
rs72773940	10	11148581	10p14	T	0.0287	−8.131	3.58E-06	*CELF2*	0 (intron)
rs117481327	10	11154826	10p14	G	0.0293	−8.121	3.61E-06	*CELF2*	0 (intron)
rs2016129*	10	29288295	10p12.1	T	0.0682	5.091	3.84E-06	*C10orf126*	117468
rs114594327	4	91942029	4q22.1	T	0.0268	7.955	5.09E-06	*CCSER1*	0 (intron)
rs74897566	3	5083637	3p26.1	C	0.0196	10.88	6.29E-06	*BHLHE40*	56771
rs306216	18	60295543	18q21.33	G	0.3009	−3.131	6.92E-06	*ZCCHC2*	41567
rs1556397*	10	24851710	10p12.1	A	0.3311	−2.923	7.86E-06	*KIAA1217*	14938
rs141363063	9	116670492	9q32	T	0.0110	11.55	8.44E-06	*ZNF618*	0 (intron)
rs148488001	9	116691092	9q32	G	0.0110	11.55	8.44E-06	*ZNF618*	0 (intron)
rs192345176	9	116700201	9q32	G	0.0109	11.55	8.44E-06	*ZNF618*	0 (intron)
rs138203943	9	116704225	9q32	A	0.0109	11.55	8.44E-06	*ZNF618*	0 (intron)
rs56139919*	6	155914380	6q25.3	A	0.0207	8.531	8.72E-06	*LOC105378068*	67228
rs489500	1	65525901	1p31.3	G	0.0139	9.476	8.94E-06	*JAK1*	0 (intron)
rs11007349	10	29287378	10p12.1	T	0.0674	5.102	9.50E-06	*C10orf126*	116551

Table 3.

Significant (FDR < 0.05) gene sets identified in the MSigDB over-representation analysis using the REACTOME and KEGG databases as reference.

Gene set	# Genes in gene set (K)	# Genes in overlap (k)	k/K	P	FDR q-value
REACTOME_SIGNALING_BY_GPCR	920	42	0.0457	8.24E-9	4.22E-6
REACTOME_GPCR_LIGAND_BINDING	408	26	0.0637	9.81E-9	4.22E-6
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	305	21	0.0689	6.98E-8	2.00E-5
REACTOME_GPCR_DOWNSTREAM_SIGNALING	805	33	0.0410	3.38E-6	7.26E-4
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	188	14	0.0745	4.22E-6	7.26E-4
REACTOME_INTERACTION_BETWEEN_L1_AND_ANKYRINS	23	5	0.2174	3.48E-5	4.30E-3
REACTOME_G_ALPHA1213_SIGNALLING_EVENTS	74	8	0.1081	3.50E-5	4.30E-3
KEGG_MAPK_SIGNALING_PATHWAY	267	15	0.0562	5.51E-5	5.66E-3
KEGG_RNA_DEGRADATION	59	7	0.1186	5.92E-5	5.66E-3
REACTOME_CELL_CYCLE	421	19	0.0451	1.16E-4	9.99E-3