European Journal of Human Genetics (2012) 20, 341–347

www.nature.com/ejhg

ARTICLE

Evolutionary conserved longevity genes and human cognitive abilities in elderly cohorts

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Genetic influences have an important role in the ageing process. The genetic factors that influence success in bodily ageing may also contribute to the successful ageing of cognitive abilities. A comparative genomics approach found longevity genes conserved between yeast *Saccharomyces cerevisiae* and nematode *Caenorhabditis elegans*. We hypothesised that these longevity genes influence variance in cognitive ability and age-related cognitive decline in humans. Here, we investigated six of these genes that have human orthologs and show expression in the brain. We tested *AFG3L2* (MIM: 604581, AFG3 ATPase family gene 3-like 2 (yeast)), *FRAP1* (MIM: 601231, a FK506 binding protein 12-rapamycin associated protein), *MAT1A*, *MAT2A* (MIM: 610550 and 601468, methionine adenosyltransferases I alpha and II alpha, respectively), *SYNJ1* and *SYNJ2* (MIM: 604297 and 609410, synaptojanin-1 and synaptojanin-2, respectively) in approximately 1000 healthy older Scots: the Lothian Birth Cohort 1936 (LBC1936). They were tested on general cognitive ability at age 11 years. At a mean age of 70 years, they re-sat the same general cognitive ability test and underwent an additional battery of diverse cognitive tests. In all, 70 tag and functional SNPs in the six longevity genes were genotyped and tested for association with cognition and cognitive ageing in LBC1936. Suggestive associations were detected between SNPs in *SYNJ2*, *MAT1A*, *AFG3L2* and *SYNJ1* and a general memory factor and general cognitive ability at age 11 and 70 years. Replication studies for cognitive ability associations were performed in 2506 samples from the Cognitive Ageing Genetics in England and Scotland consortium. A meta-analysis replicated the *SYNJ2* association with cognitive abilities (lowest *P*=0.00077). *SYNJ2* is a novel gene in which variation is potentially associated with cognitive abilities.

European Journal of Human Genetics (2012) **20,** 341–347; doi:10.1038/ejhg.2011.201; published online 2 November 2011

Keywords: cognition; cognitive ageing; longevity genes; gene association; *SYNJ2*

INTRODUCTION

With the growing absolute numbers and proportions of older people in societies, the changes wrought by ageing have become a research priority.[1] Very high on this list is the problem of cognitive ageing. Cognitive ageing is a much-feared aspect of growing old and is the major cause of older people's losing independence and lowering their quality of life.[2] There are marked individual differences in age-related cognitive changes,[3,4] and among the causes of this variation are genetic, medical, psychological, and social and lifestyle factors.[5,6] Beyond *APOE*, and excluding the dementias, there are no solid associations between genetic variants and cognitive functions in old age, despite many suggestions and replication attempts.[7]

There are well-established phenotypic associations between cognition, health and longevity.[8,9] The 'common-cause' hypothesis of cognitive ageing states that there are general bodily factors that affect both physical and mental changes with age.[10] Human longevity has been associated with higher cognitive abilities,[11] even when intelligence is measured in childhood or early adulthood and the assessment of survival has been conducted several decades later.[12,13] Behavioural genetic studies have shown that genetic factors influence both longevity[14] and cognitive traits,[7] alongside other stochastic variation.[15] Given the well-replicated phenotypic association between cognitive abilities and longevity, it is plausible that part of this is explained by shared genetic factors (genetic correlation); therefore, to explore this, it is useful to examine genes involved in longevity for their association with cognitive abilities and cognitive ageing. We can study this cognitive function and lifetime cognitive change in older people as we have the advantage of having an ageing sample with a measure of cognitive ability from youth.

In an effort to harness the 'new biology of ageing research',[16] we chose evolutionary-conserved, longevity genes uncovered by a

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Received 11 March 2011; revised 25 July 2011; accepted 6 September 2011; published online 2 November 2011

comparative functional genomics investigation on ageing between two divergent eukaryotic species, the yeast *Saccharomyces cerevisiae* and the nematode *Caenorhabditis elegans*.[17] The investigators had reported the replicative lifespan phenotypes (the number of times that a mother cell can bud to form a daughter) for single-gene deletions of the yeast orthologs of worm ageing genes. They identified 25 genes that modulate yeast replicative lifespan and suggested that 'many of these genes, and the pathways within which they function, are likely to modulate ageing in mammals, and that mammalian orthologs of these gene pairs are reasonable candidates as potential therapeutic targets for age-associated diseases' (Smith *et al*,[17] 568). From this list of 25 genes, we narrowed our focus to brain-expressed[18] genes in humans, resulting in six genes – *AFG3L2* (MIM: 604581, AFG3 ATPase family gene 3-like 2 (yeast)), *FRAP1* (MIM: 601231, a FK506 binding protein 12-rapamycin-associated protein), *MAT1A*, *MAT2A* (MIM: 610550 and 601468, methionine adenosyltransferases I alpha and II alpha, respectively), *SYNJ1* and *SYNJ2* (MIM: 604297 and 609410, synaptojanin-1 and synaptojanin-2, respectively). These genes are novel candidate genes for cognition and do not fall in any of the linkage regions for cognitive traits.[7] We proposed that variation in these evolutionary conserved genes for longevity may be associated with individual differences in human cognitive ageing. We tested this hypothesis in a Scottish cohort, assessed for cognitive ability at age 11 and 70 years, by genotyping haplotype-tagging genetic variants in the six genes. We sought replication in newly available genome-wide association genotype data from cohorts of older individuals from England and Scotland in the Cognitive Ageing Genetics in England and Scotland (CAGES) consortium: the Lothian Birth Cohort of 1921 (LBC1921),[19] the Aberdeen Birth Cohort of 1936 (ABC1936)[19,20] and the Manchester and Newcastle Longitudinal Studies of Cognitive Ageing.[21]

MATERIALS AND METHODS

Subjects

There are 1091 individuals (543 females) in the LBC1936. All were born in 1936 and attended school in Scotland in 1947. At an average age of 11 years, they took a valid IQ-type test – a version of the Moray House Test No. 12 (MHT) – in the nationwide Scottish Mental Survey 1947 (SMS1947; N=70 805).[22] At age ~70 years, the LBC1936 were recruited as surviving and still relatively healthy participants of the SMS1947 who were living in Edinburgh and the surrounding areas (Lothians) of Scotland. They re-sat the same mental test and other cognitive and medical tests, as described elsewhere in detail.[23] All participants in the study lived independently in the community and travelled to the Wellcome Trust Clinical Research Facility (WTCRF) at the Western General Hospital, Edinburgh, UK for testing. DNA samples were available for 1078 participants. In all, 12 participants were excluded owing to possible dementia: 11 scored <24 on the Mini-Mental State Examination (MMSE)[24,25] and one had an incomplete MMSE test. The final sample with phenotype data used in the analyses was 1038 individuals (521 females), with a mean age of 69.5 years (range 67.6–71.3). They had a mean age of 10.9 years (range 10.4–11.4) when tested in the SMS1947.

The replication cohorts were from the CAGES project: the LBC1921 (N=517 (303 females), mean age 79.1 years (SD=0.6)),[19] the ABC1936 (N=426, (208 females), mean age 64.4 years (SD=0.9))[19,20] and the Manchester and Newcastle Longitudinal Studies of Cognitive Ageing (Manchester N=805 (572 females), Newcastle N=758 (536 females), median age of 65 years, range 44–93 years),[21] as described previously.[26] All four cohorts comprised non-clinical samples of relatively healthy people from middle to older adulthood.

Cognitive tests

A full description of the cognitive tests applied to LBC1936 is available elsewhere.[23] The tests pertinent to this study are described in brief below. A general measure of cognitive ability with an emphasis on verbal reasoning (MHT) was administered when participants were a mean age of 11 years in the SMS1947 (Scottish Council for Research in Education).[22] The MHT was re-administered at a mean age of almost 70 years for LBC1936, using the same instructions and 45 minute time limit that were applied at age 11 years. Cognitive tests assessing reasoning and different aspects of memory were administered to the LBC1936 at age 70 years. These include: logical memory, backward digit span, spatial span and verbal paired associates from the Wechsler Memory Scale-III^{UK};[27] letter-number sequencing, matrix reasoning and block design from the WAIS-III^{UK};[27] The information processing speed battery comprised two psychometric tests from the WAIS-III^{UK} (digit symbol and symbol search) and two elementary cognitive tasks: simple and four choice reaction time (RT), and inspection time, which is a psychophysical assessment of the efficiency of the early stages of visual decision-making.[19,28]

The replication was performed in four cohorts from the CAGES study: LBC1921,[19] ABC1936[19,20] and the Manchester and Newcastle Longitudinal Studies of Cognitive Ageing,[21] previously described in detail. Individuals with MMSE <24 were removed from LBC1921 and ABC1936. In LBC1921, scores for the MHT taken at age 11 and 79 years, logical memory, Raven's Progressive Matrices and verbal fluency, all taken at age 79 years were available. In ABC1936, scores for the MHT taken at age 11 years, block design, auditory verbal learning test (AVLT), and Raven's Progressive Matrices, digit symbol and Uses of Common Objects taken at age 64 years were used. For Manchester and Newcastle, the cognitive tests applied were described previously.[21,26]

SNP selection

Tagging SNPs were selected to tag haplotypes from the specific gene regions and 5 kb either side of the gene. Genotype data were downloaded from the HapMap CEPH population (Release 22). The tagging SNPs were chosen by Tagger[29] in Haploview v. 4.1[30] using the pairwise tagging method with the default settings (r^2=0.8), with one exception (minimum minor allele frequency (MAF) 0.05). In all, 70 tag SNPs act as direct proxies to all other SNPs in the six genes because they are highly correlated with one another ($r^2 \geq 0.8$). Coding non-synonymous SNPs were also chosen (rs2502601 in *SYNJ2* and rs2254562 in *SYNJ1*) as the allele changes (A→G) in both SNPs cause missense mutations and residue changes (Glu→Gly, Lys→Arg, respectively). These two SNPs predict important functionality of the *SYNJ2* and *SYNJ1* genes, and consequently are informative as potential causative variants. In total, 70 SNPs were genotyped. This SNP selection was carried out before genome-wide SNP data becoming available on the LBC1936 sample.

Conventionally significantly associated SNPs ($P<0.05$) were chosen for replication for cognitive abilities in the CAGES cohort: N=21 SNPs. The SNPs were extracted from genome-wide data as described previously[26] and genotype data were imputed to HapMap phase II CEU data as the reference sample, using NCBI build 36 (UCSC hg18) in the MACH software.[31] Before imputation, SNPs were removed that diverged from Hardy–Weinberg equilibrium (HWE) with a significance $P<1\times10^{-3}$ and SNPs with an MAF <0.01.[32]

Genotyping

Genomic DNA was isolated from whole blood by standard procedure at the WTCRF Genetics Core, Western General Hospital, Edinburgh. In total, 49 markers were genotyped using a competitive allele-specific PCR system (KASPar) by KBiosciences (Herts, UK). *SYNJ2* was not fully covered in the first SNP selection owing to a genome browser change and human error. To rectify this, 21 SNPs in *SYNJ2* (rs1750043–rs13217929) were extracted from a whole genome scan on LBC1936 as described previously.[33] The 'force-include' option in Tagger was used to force the inclusion of the SNPs genotyped in the whole genome scan as tagging SNPs, and the same level of tagging coverage for *SYNJ2* ($r^2 \geq 0.8$) was achieved.

Statistical analyses

Cognitive phenotypes. The cognition data were prepared by removing outliers in the cognitive variable data with Z scores greater than ±3. A general cognitive ability factor was derived from principal components analysis of one WMS-III subtest (digit-span backwards) and five WAIS-III subtests (matrix reasoning, letter-number sequencing, block design, symbol search and digit symbol), as described previously.[34] A general speed factor was separately derived from principal components analysis of speed measures (choice RT

mean, simple RT mean (log-transformed), digit symbol, inspection time and symbol search).[34] A general memory factor was derived from principal components analysis of WMS III logical memory I total recall score (A+B+B2), WMS III logical memory II delayed recall total score (A+B), WMS III spatial span forward, WMS III spatial span backward, WMS III verbal paired associates I (List A+B+C+D), WMS III verbal paired associates II recall total score, WAIS III letter-number sequencing and WAIS III digit-span backwards.[35] Outliers were removed before principal components analysis. The MHT1947 score at age 11 years was residualised for age. The sample size, the mean values and standard deviations of the cognitive variables have been published previously.[36] This preparation was performed using SPSS version 14.0.

For the replication cohorts, similar preparation of the cognitive phenotypes was performed. Outliers with Z scores greater than ± 3 were removed. Phenotypes were standardised to enable comparison. In LBC1921, a general cognitive ability factor was derived from principal components analysis of Raven's Progressive Matrices, verbal fluency and logical memory as described previously.[35] In ABC1936, a general cognitive ability factor was derived from principal components analysis of Raven's Progressive Matrices, Digit Symbol, AVLT, Uses of Common Objects as described previously as g_f.[26] In LBC1921 and ABC1936, the MHT score at age 11 years was residualised for age in days at age 11 years. The phenotypes in LBC1921 and ABC1936 were corrected for age and gender and the standardised scores were used for all subsequent analyses.

In Manchester and Newcastle samples, the individual cognitive tests have been described previously,[22] and the phenotypes for this study of cognition in old age were prepared together as follows. For a general cognitive ability factor, empirical Bayes' (EB) estimates for each individual were obtained from a random effects model fitted by maximum likelihood (ML) to the standardised age regressed residuals obtained for each sex from the Alice Heim 4 test (1970) and the Cattell (1960) 'Culture Fair' test scores.[26] A similar approach was taken for a speed factor based on the Visual Search for letters and Savage (1984) Alphabet Coding Task tests. With up to seven measures (Verbal Free Recall for 30 words, Verbal Free Recall for 10 words, Cumulative Verbal Learning, Pictorial Recognition Memory test, Memory for Shapes and Location, Propositions about people, Memory Circle) available to form a general memory factor, individual EB estimates were obtained from the standardised age regressed residuals from each test using a one-factor model fitted by ML.

Although different sets of tests were used to construct the general cognitive ability factor, it is well established that the general factors derived from different mental test batteries tend to rank people almost identically.[37]

Genotype data. All SNPs were in HWE as judged by the HW exact SNP tests (all P-values >0.001 (Haploview default) and are reported in Supplementary Table 1). The genotyping data were of good quality, as the mean genotyping rate in LBC1936 was 99% (range 92–100%) in 1038 samples. The MAF of all markers were >0.044. The genotype frequencies were similar to the HapMap CEPH population (mean difference in genotype frequencies=0.03, minimum 0.002, maximum 0.08). Further characteristics of the SNPs investigated are listed in Supplementary Table 1. SNPs in each replication sample were checked for MAF (all markers MAF >0.08), HWE (all P-values >0.001) and imputation quality (mean r^2=0.97 (0.053)).

Association analysis. In the discovery sample, LBC1936, genotype–phenotype analyses were performed using PLINK version 1.07.[38] Linear regression analysis under an additive genetic model was performed, including gender and age in days at testing at age 70 years as covariates. In a separate analysis, MHT score at age 11 years (age residualised) was included as a covariate to adjust for previous cognitive ability, thus allowing us to specifically identify associations with cognitive functions in old age, while adjusting for cognitive differences in youth (ie, cognitive ageing). This inclusion of a previous measurement as a covariate is a widely used 'measure' of change. Standardised β scores, the standard error (SE) and their corresponding P-values are reported.

In the replication sample, linear regression analysis for an additive genetic model was performed using MACH2QTL,[39] incorporating dosage information. For ABC1936, age and sex were included as covariates in the model. For Manchester and Newcastle replication cohorts, the analysis was performed separately by gender owing to the preparation of the phenotypes in a sex-specific manner. β Scores, the SE and the corresponding P-values are reported. Meta-analyses were performed in Manchester and Newcastle cohorts to combine results for men and women, and in all the cohorts on overlapping phenotypes (General Cognition Factor and Memory tests) in the replication samples using an inverse variance weighted model.[40]

Statistical significance and power. In the first instance, the significance threshold was determined by the Bonferroni method, which corrects the critical significance level by the number of tests (n) performed (α=0.05/n), and is commonly used in candidate gene studies.[41] It is recognized that the Bonferroni correction can be overly conservative for non-independent tests.[42] Therefore, we calculated the number of independent tests performed. Based on matrix spectral decomposition,[43] the 70 SNPs represent 46 independent variables. A principal component analysis of the specific cognitive test components of the five cognitive measures in the discovery sample of LBC1936 was performed. These are the Moray House test at age 11 years, Moray House test at age 70 years, logical memory I total recall score, logical memory II delayed recall total score, spatial span forward, spatial span backward, verbal paired associates I and verbal paired associates II, symbol search, digit symbol, simple RT mean (log-transformed), choice RT mean, inspection time, matrix reasoning, letter-number sequencing, digit-span backwards and block design. This resulted in three components with eigenvalues >1 (5.04, 1.47, 1.15). It should be noted, however, that the scree plot (available from the first author upon request) confirms the presence of one general factor of cognition, accounting for 36% of the total variance. The mean of the absolute factor loadings on the first unrotated component was 0.59 (range from 0.37 for simple RT mean to 0.80 for the Moray House Test at age 70 years). The adjusted Bonferroni significance threshold thus applied was $P<0.0004$ (0.05/(3 cognitive components$\times$46 independent SNPs)). Replication was sought for nominal significant associations ($P<0.05$). For evidence of replication, $P<0.05$ was taken as significant evidence.

The power to detect an additive effect of a causal variant, in linkage disequilibrium D'=1, of a marker with an allele frequency of 0.2, accounting for 1–2% of the variance, at type-1 error rate adjusted for multiple testing (P-value ≤ 0.0004) was 28–77% in LBC1936 (N=1038). This was estimated using the variance component quantitative trait loci association module in the genetic power calculator.[44]

RESULTS

In all, 70 haplotype-tagging SNPs in six genes (*AFG3L2*, *FRAP1*, *MAT1A*, *MAT2A*, *SYNJ1* and *SYNJ2*) were tested for association with cognitive abilities, including age and gender as covariates. The cognitive abilities tested were a verbal reasoning test at age 11 years (a version of the MHT), the same test again at age 70 years, a general cognitive ability factor, a general memory factor and a general mental speed factor, also at age 70 years. No SNP associations surpassed the Bonferroni level of correction for multiple testing ($P<0.0004$). Suggestive single SNP associations (unadjusted P-values <0.05) were detected in variants of four of these genes with cognitive abilities: *SYNJ2*, *MAT1A*, *AFG3L2* and *SYNJ1* (Table 1). No associations with cognition were detected in *FRAP1* and *MAT2A* (all P-values >0.1) (Table 1). Supplementary Figure 1 shows that 14 of the 44 SNPs tagging *SYNJ2* were suggestively associated with cognitive abilities. The strongest association was an intronic *SYNJ2* SNP rs10945973 with the general memory factor (P=0.004).

Variants in these six genes (*AFG3L2*, *FRAP1*, *MAT1A*, *MAT2A*, *SYNJ1* and *SYNJ2*) were further tested for association with cognitive ageing by including age 11 years cognitive ability (based on MHT scores), in addition to sex and age, as a covariate. Significant SNP associations were detected in four of the genes (13 SNPs): *SYNJ2*, *MAT1A*, *AFG3L2* and *SYNJ1*. No associations with cognitive ageing were detected in *FRAP1* and *MAT2A* (all P-values >0.1) (Supplementary Table 2).

Table 1 Significant associations of longevity gene variants to cognitive abilities in LBC1936

				MHT age 11 years			*MHT age 70 years*			*G cognition*			*G memory*			*G speed*		
Gene	*CHR*	*SNP*	*MA*	*β*	*SE*	*P*	*β*	*SE*	*P*	*β*	*SE*	*P*	*β*	*SE*	*P*	*β*	*SE*	*P*
SYNJ2	6	rs11961283	C	**−0.064**	**0.032**	***0.043***	−0.043	0.031	*0.158*	−0.022	0.030	*0.467*	**−0.071**	**0.031**	***0.022***	−0.023	0.032	*0.472*
	6	rs6455937	C	−0.043	0.032	*0.177*	−0.033	0.031	*0.290*	−0.037	0.031	*0.223*	**−0.063**	**0.031**	***0.044***	−0.031	0.032	*0.332*
	6	rs7772395	C	**−0.068**	**0.032**	***0.035***	−0.051	0.031	*0.103*	−0.041	0.031	*0.190*	−0.059	0.031	*0.059*	−0.053	0.032	*0.104*
	6	rs10455935	A	−0.037	0.032	*0.251*	−0.028	0.031	*0.378*	−0.017	0.031	*0.582*	**−0.063**	**0.031**	***0.043***	0.010	0.032	*0.752*
	6	rs10945973	A	0.016	0.032	*0.623*	0.036	0.031	*0.253*	0.036	0.031	*0.238*	**0.090**	**0.031**	***0.0037***	0.000	0.032	*0.998*
	6	rs6906464	T	−0.060	0.032	*0.062*	**−0.086**	**0.031**	***0.0057***	−0.044	0.031	*0.148*	**−0.067**	**0.031**	***0.029***	−0.057	0.032	*0.075*
	6	rs9356200	C	0.047	0.032	*0.139*	0.059	0.031	*0.058*	0.015	0.031	*0.625*	**0.074**	**0.031**	***0.017***	0.003	0.032	*0.915*
	6	rs9456954	A	−0.042	0.032	*0.187*	**−0.070**	**0.031**	***0.024***	0.010	0.031	*0.734*	−0.042	0.031	*0.169*	−0.026	0.032	*0.416*
	6	rs7758206	C	−0.050	0.032	*0.117*	**−0.063**	**0.031**	***0.040***	−0.008	0.030	*0.784*	−0.038	0.031	*0.213*	−0.047	0.032	*0.139*
	6	rs9459093	C	−0.034	0.032	*0.283*	−0.059	0.031	*0.058*	−0.043	0.031	*0.161*	**−0.079**	**0.031**	***0.011***	0.007	0.032	*0.822*
	6	rs751873	T	−0.030	0.033	*0.366*	−0.023	0.032	*0.475*	0.000	0.031	*0.995*	**−0.067**	**0.032**	***0.033***	0.033	0.033	*0.310*
	6	rs3818457	A	**0.067**	**0.033**	***0.043***	0.034	0.032	*0.288*	0.041	0.031	*0.193*	**0.063**	**0.032**	***0.047***	0.024	0.033	*0.460*
	6	rs1744169	T	**0.082**	**0.033**	***0.012***	0.059	0.032	*0.065*	0.054	0.031	*0.084*	0.051	0.032	*0.105*	0.040	0.033	*0.225*
	6	rs2502601	T	**0.067**	**0.032**	***0.036***	0.051	0.031	*0.099*	0.046	0.031	*0.129*	**0.078**	**0.031**	***0.011***	0.031	0.032	*0.331*
MAT1A	10	rs3851059	A	0.008	0.032	*0.809*	0.027	0.031	*0.387*	−0.053	0.031	*0.085*	**−0.076**	**0.031**	***0.014***	−0.022	0.032	*0.496*
	10	rs4933327	A	−0.013	0.032	*0.683*	0.037	0.031	*0.228*	−0.058	0.030	*0.057*	**−0.089**	**0.031**	***0.0039***	−0.031	0.032	*0.336*
AFG3L2	18	rs9964979	T	−0.048	0.032	*0.136*	**−0.074**	**0.031**	***0.019***	**−0.062**	**0.031**	***0.047***	**−0.102**	**0.031**	***0.0011***	−0.010	0.032	*0.750*
SYNJ1	21	rs845022	T	−0.058	0.032	*0.069*	−0.036	0.031	*0.250*	**−0.068**	**0.030**	***0.025***	−0.033	0.031	*0.285*	−0.047	0.032	*0.138*
	21	rs7279487	C	**0.090**	**0.032**	***0.0050***	**0.064**	**0.031**	***0.039***	0.060	0.031	*0.051*	0.059	0.031	*0.055*	0.064	**0.032**	***0.045***
	21	rs844996	G	**0.086**	**0.032**	***0.0076***	0.047	0.031	*0.135*	**0.067**	**0.031**	***0.031***	**0.063**	**0.031**	***0.046***	0.059	0.032	*0.067*

Abbreviations: MHT, Moray House Test, which is a verbal reasoning test taken at age 11 and 70 years; G, general ability factors; CHR, chromosome; MA, minor allele; *P*, *P*-value; *β*, the standardised regression coefficient, where a positive regression coefficient shows that the minor allele increases phenotype mean.
Associations that surpass the nominal significance level adjusted for the number of independent phenotypes tested are shown ($P<0.05$, in bold). The 50 SNPs that did not show association with cognitive abilities are not shown; these latter results are available from the authors.
The *P*-values are represented in italics.

Overall, 21 SNPs in four genes (*SYNJ2*, *MAT1A*, *AFG3L2* and *SYNJ1*) were suggestively associated (unadjusted $P<0.05$) with cognitive abilities in old age (not cognitive ageing) in our discovery cohort, LBC1936, shown in Table 1 and Supplementary Table 2. These 21 SNPs explain 0.36–0.8% (by squaring the standardised *β* values in Table 1) of the variance in the cognitive phenotypes investigated. Replication of the 21 SNPs associated with cognitive abilities was sought in four elderly independent cohorts from the CAGES consortium with relevant cognitive phenotypes and genotype data imputed from a genome-wide association study. Independent association analysis was performed in each replication cohort. In ABC1936, MHT at age 11 years, block design (to replicate MHT at age 70 years), general cognitive factor and AVLT (to replicate memory) were tested for association. In LBC1921, MHT at age 11 years, MHT at age 79 years, general cognitive factor and logical memory (to replicate memory) were tested for association. In Manchester and Newcastle, general cognitive, speed and memory factors were tested for association.

Replication of SNP associations with *SYNJ2* was present in the four cohorts: ABC1936, LBC1921, Manchester and Newcastle (Supplementary Tables 3–6). Some of the SNP associations were replicated in the targeted phenotype from LBC1936 (LBC1921: rs6455937 and rs9459093 for memory, and rs7772395 for MHT age 11 years; Newcastle: rs6455937 and rs10455935 for memory). Across all the replication samples, the effect was in the same direction for the significant SNPs, and it was the same direction as the discovery sample, LBC1936. A meta-analysis showed significant association of six *SYNJ2* SNPs (Table 2). Three SNPs were significant for both general cognitive ability and memory (rs6455937, rs7772395 and rs10455935). Three SNPs were significant for memory abilities only: rs11961283, rs10945973 and rs9459093. These six SNPs explain 0.45–1.4% of the variance in the meta-analysed general cognitive ability factor and memory ability.

DISCUSSION

This study proposed six novel candidate genes (*AFG3L2*, *FRAP1*, *MAT1A*, *MAT2A*, *SYNJ1* and *SYNJ2*) for cognition in old age and cognitive ageing. The novelty of the choice was to select evolutionary conserved genes for longevity, found through a comparative functional genomics approach and to test for association with human cognitive ageing. Initially, we investigated 70 haplotype-tagging SNPs in six genes for association with cognitive ability at age 11 and 70 years. We tested for association with cognitive ageing by including cognitive ability at age 11 years as a covariate. There were suggestive associations in four genes with cognitive ability, *SYNJ2*, *MAT1A*, *AFG3L2* and *SYNJ1*. The association of 13 out of the 20 suggestive SNPs for cognitive ability remained associated with cognitive ageing, with the same direction of effect, but to a lesser degree of effect and significance as shown in Table 1 and Supplementary Table 2. This could possibly be explained by the phenotype as any change variable is always less reliably measured than a trait, or that the genetic associations are not specific to cognitive ageing. SNP associations in *SYNJ2* were replicated in a meta-analysis of general cognitive ability and memory ability. Given the neuronal functionality of *SYNJ2*, this gene may be one of the many genes with a small effect influential in cognitive abilities.

SYNJ2 has biological plausibility to support its role in cognitive processes. *SYNJ2* is an ubiquitously expressed inositol polyphosphate 5-phosphatase, shown specifically to be expressed in nerve terminals[45] and differentially expressed in hippocampal subregions of the

Table 2 Meta-analysis of G factor (*N*=2401) and memory (*N*=2412) in the replication samples ABC1936, LBC1921, Manchester and Newcastle

				G cognition				*Memory*			
Gene	*EA*	*OA*	*MA*	*Effect*	*SE*	*P*	*Dir*	*Effect*	*SE*	*P*	*Dir*
SYNJ2											
rs11961283	T	C	C	0.067	0.035	0.056	+−−−++	**0.103**	**0.034**	**0.0025**	**++++++**
rs6455937	A	C	C	**0.078**	**0.030**	**0.0090**	**++++−+**	**0.089**	**0.029**	**0.0023**	**+++−++**
rs7772395	A	C	C	**0.098**	**0.036**	**0.0066**	**+++−++**	**0.116**	**0.035**	**0.0009**	**+++−++**
rs10455935	A	G	A	**−0.065**	**0.029**	**0.028**	−−−−−−	**−0.085**	**0.029**	**0.0032**	**−−−+−−**
rs10455936	T	C	T	0.001	0.034	0.977	+−−−++	−0.008	0.033	0.806	+−−+−+
rs10945973	A	G	A	0.042	0.030	0.159	++++++	**0.066**	**0.029**	**0.024**	**+++−++**
rs6906464	A	T	T	0.042	0.034	0.220	+−+−++	0.063	0.033	0.059	+++−++
rs9356200	T	C	C	−0.027	0.029	0.364	++−−+−	−0.051	0.029	0.078	−−−−−−
rs9456954	A	T	A	−0.006	0.032	0.854	−+−+−−	−0.054	0.032	0.093	−−−−−−
rs7758206	C	G	C	0.009	0.037	0.814	++−+−−	−0.051	0.036	0.158	+−−−++
rs9459093	T	C	C	0.028	0.029	0.332	+−++−+	**0.058**	**0.029**	**0.042**	**+−++−+**
rs751873	T	C	T	0.052	0.031	0.087	−+−+−+	−0.002	0.030	0.951	+−−−−+
rs3818457	T	C	A (T)	0.023	0.029	0.421	+−++−−	0.021	0.028	0.463	+−+−+−
rs1744169	A	G	T (A)	0.021	0.030	0.478	+−++−+	0.013	0.029	0.648	+++−++
rs2502601	A	G	T (A)	0.009	0.029	0.768	+−−++−	0.007	0.028	0.816	+−+−++
MAT1A	A	G	A	−0.025	0.031	0.430	−−−+−+	−0.013	0.031	0.679	+−++−−
rs3851059											
rs4933327	A	G	A	−0.023	0.035	0.511	−−++−−	−0.030	0.034	0.380	+−++−−
AFG3L2	T	C	T	−0.019	0.040	0.635	−−−+++	−0.032	0.039	0.407	+−−−+−
rs9964979											
SYNJ1											
rs845022	A	T	T	−0.027	0.029	0.351	++−−−−	0.008	0.028	0.771	+−+−+−
rs7279487	T	C	C	0.033	0.041	0.414	−+−+++	0.008	0.040	0.848	+++−−−
rs844996	T	C	G (C)	−0.001	0.049	0.977	+++−++	0.063	0.048	0.188	+++−++

Abbreviations: EA, effect allele; OA, other allele; MA, minor allele from Table 1 to enable comparison (alleles within parentheses are to clarify strand differences); SE, standard error.
P-values <0.05 are highlighted in bold. Dir is the direction of effect of LBC1921, ABC1936, Manchester males, Manchester females, Newcastle males and Newcastle females.

non-human primate marmoset.[46] *SYNJ2* is differentially expressed in major depressive disorder,[47] and is part of the neuronal processes implicated in cognitive function[48] and cognitive deficits,[49] is associated with age-related spatial learning impairments in rats,[50] and has been hypothesised to be involved in normal development of the brain.[49] *SYNJ2* is a target for the 'Genes to Cognition' project (http://www.genes2cognition.org). Alternative splicing of *SYNJ2* results in multiple transcript variants and is an important candidate considering its role in ubiquitous signal-transduction pathways.[51] Gene Ontology (GO) links five biological processes to *SYNJ2* through inferred electronic annotation (brain development (GO: 007420), dephosphorylation (GO: 0016311), inositol phosphate dephosphorylation (GO: 0046855), phosphoinositide dephosphorylation (GO: 0046856) and intracellular distribution of mitochondria (GO: 0048312)).

A limitation of our study is the lack of consistent association between cohorts, both at the SNP level, despite using the same SNPs, and at the phenotype level, despite each general factor and intelligence test capturing overlapping cognitive abilities. There are many reasons that may explain this common occurrence in replication studies. First, and most likely, is that the original finding in LBC1936 may be a false positive as none of the associations reported here surpassed the Bonferroni correction. Second, the result may be a true positive with overestimated effect sizes, which would reduce the power of detection in our replication cohorts.[52] Third, the original finding may be a true positive and the effect size unbiased, but sample size, selection bias, presence of allelic heterogeneity or hidden population sub-structure, and phenotypic heterogeneity might prevent replication. The cohorts differ in time of recruitment, age, location and cognitive tests as detailed in the Materials and Methods section. Generally, those samples that have a greater number of more varied cognitive tests will have a more reliable general cognitive ability phenotype, and the same principle applies within cognitive domains. These differences may have limited the degree to which the attempted replication was truly a replication. The lack of association in the Manchester sample may be due to subtle population substructure differences between North East and North West England; however, there is no strong evidence of population stratification, as reported previously.[26] However, the lack of association in the Manchester sample should not be due to phenotypic heterogeneity as the cognitive tasks were performed by the same test co-ordinators and the collection of tests were the same for both Newcastle and Manchester cohorts. Furthermore, the non-replication of specific SNPs could be explained by varying linkage disequilibrium in the different cohorts between the causative variant (presumably not genotyped) and the tagging SNPs. Another limitation of the study was that the SNP selection strategy was based on tagging the genes with HapMap SNPs. The coverage of two genes, *FRAP1* and *MAT2A*, with HapMap SNPs was small.

Therefore, these genes cannot be definitely excluded as associated with cognitive abilities.

This is the first survey of longevity genes, unveiled by a comparative genomics approach, for association with cognitive abilities across the life course. We are the first to report *SYNJ2*, as a preliminary candidate gene awaiting independent replication, influencing cognitive abilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the cohort participants and team members who contributed to these studies. Genotyping of the CAGES cohorts and the analyses conducted here were supported by the UK's Biotechnology and Biological Sciences Research Council (BBSRC). Phenotype collection in the Lothian Birth Cohort 1936 was supported by Research Into Ageing (continues as part of Age UK's The Disconnected Mind project). Phenotype collection in the Lothian Birth Cohort 1921 was supported by the BBSRC, The Royal Society and The Chief Scientist Office of the Scottish Government. Phenotype collection in the Aberdeen Birth Cohort 1936 was supported by BBSRC, the Welcome Trust and the Alzheimer's Research trust. Phenotype collection in the Manchester and Newcastle Longitudinal Studies of Cognitive Aging cohorts was supported by Social Science Research Council, Medical Research Council, Economic and Social Research Council, Research Into Ageing, Wellcome Trust and Unilever plc. LML is supported by AXA Research Fund during some of this work. ML is a Royal Society of Edinburgh/Lloyds TSB Foundation for Scotland Personal Research Fellow. The work was undertaken in The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the BBSRC, EPSRC, ESRC and MRC is gratefully acknowledged.

AUTHOR CONTRIBUTIONS

Data analysis: Lopez. Study design: Lopez and Deary. Manuscript Preparation: Lopez, Deary, Harris, Luciano, Gow, Starr and Pendleton. Individual study design/management: Tenesa, Payton, Ke, Whalley, Fox, Ollier, Pickles, Porteous, Horan, Pendleton, Starr and Deary. Genetic preparation: Lopez, Liewald, Davies, Harris, Luciano and Tenesa. Phenotype preparation: Lopez, Luciano, Gow, Payton, Ke, Whalley, Fox, Ollier, Pickles, Horan, Pendleton, Starr and Deary. Manuscript review: Lopez, Harris, Luciano, Liewald, Davies, Gow, Tenesa, Payton, Ke, Whalley, Fox, Ollier, Pickles, Porteous, Horan, Pendleton, Starr and Deary.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)