Alzheimer's & Dementia: Translational Research & Clinical Interventions 3 (2017) 254-261

Alzheimer's & Dementia

Featured Article

A dopamine receptor genetic variant enhances perceptual speed in cognitive healthy subjects

Sandra Barral[a,*], Christian G. Habeck[b], Elaine Gazes[b], Philip L. De Jager[c], David A. Bennett[d,e], Yaakov Stern[a,b]

[a]*Department of Neurology, G.H. Sergievsky Center, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY, USA*

[b]*Cognitive Neuroscience Division, Department of Neurology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY, USA*

[c]*Program in Translational Neuropsychiatric Genomics, Department of Neurology, Brigham and Women's Hospital Harvard Medical School, Boston, MA, USA*

[d]*Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA*

[e]*Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA*

Abstract

Introduction: Cognition is under strong genetic control, yet the specific genes are unknown.

Methods: One hundred and fifty-three cognitive healthy European subjects from the Reference Abilities Study (RANN) were genotyped for 1,160 variants within 446 neuropsychiatric genes. Adjusted linear regression models evaluated the association between the genetic variants and four reference abilities (Vocabulary, Episodic Memory, Perceptual Speed, and Reasoning).

Results: One hundred and fifty-nine variants nominally were found significant in the RANN cohort and re-evaluated in an independent cohort of 868 cognitive healthy subjects from the Religious Orders Study and Rush Memory Aging Project. Meta-analysis yielded a Bonferroni adjusted statistically significant association between perceptual speed and a variant located in the promoter of the dopamine receptor D4 gene, rs3756450 ($\beta = 0.23$, standard error $= 0.05$, $P_{meta} = 2.3 \times 10^{-5}$).

Discussion: Our data suggest that genetic variation in a dopamine pathway gene influences perceptual speed performance in cognitively healthy individuals.

Keywords: Cognitive performance; Cognitive healthy subjects; Candidate genes SNP association; Meta-analysis; Dopamine pathway

1. Introduction

A significant proportion of the differences observed in cognitive performance is attributable to genetic variability. Cross-sectional and longitudinal twin studies have consistently shown strong genetic influences on cognitive performance, in both normal variation and in the extremes of the normal distribution [1,2]. Results from a meta-analysis of 23 independent twin studies also showed that heritability estimates vary across the different specific domains [3].

Several studies have reported genetic associations with a priori biological relevant genes for cognition; however, results have not been consistently replicated [4–13]. Genetic-agnostic approaches through genome-wide association analysis (GWAS) have also been reported for different cognitive tasks [14–21]. GWAS studies have examined cognition in the context of pathologic cognitive variation, that is, Alzheimer's disease (AD) [22–28], and also in normal variation in healthy adults. Some of the loci reported in recent GWAS of cognitive function in middle and older nondemented subjects have been previously associated with AD [15–17], suggesting a possible genetic overlap between normal and

*Corresponding author. Tel.: 212-305-5139; Fax: 212-305-2518.
E-mail address: smb2174@cumc.columbia.edu

http://dx.doi.org/10.1016/j.trci.2017.03.004

pathologic cognitive variation in older age. However, some other studies have reported that many loci previously implicated in Late Onset Alzheimer's Disease (LOAD) were not associated with any cognitive domain [28,29] or AD pathology [30]. To better understand the natural disease resistance to brain neurodegeneration, increasing number of genetic studies are focusing on cognitive healthy individuals. The identification of genetic variants influencing cognitive function in nondemented cohorts can elucidate molecular mechanisms for preventing or delaying cognitive decline.

Among the main limitations of GWAS are the small genetic effects of the identified variants and the very stringent multiple testing correction needed to achieve genome-wide significance ($P \leq 6 \times 10^{-8}$) [31]. Alternative gene identification approaches include hypothesis-driven gene-based analysis, that is, candidate gene(s), which rely on already available experimental data that support the involvement of the genes being tested. The use of smaller focused single-nucleotide polymorphism (SNP) arrays represents a practical approach where SNPs cover a limited number of biological candidate genes. Such focused SNP arrays offer the advantages of lower cost and lower false discovery rate, especially in situations where a data set may have inadequate power for GWAS because of either size or other reasons [32].

The candidate gene approach has been questioned because of nonreplication of results and limits on its ability to include all possible causative polymorphisms [33]. However, rigorous epidemiologic principles, as previously described [33], may considerably improve its success. In addition, efforts from different human genome sequencing initiatives, such as 1000 genome sequencing project, provide extensive high coverage data information (genomic, transcriptomic, epigenomic, and proteomics) that will helpfully contribute to overcoming the shortcomings of the candidate gene approach [34].

Our candidate gene approach focused on genes previously investigated [32] based on their roles as functional domains important in psychiatric neurogenetics. Neurobiological studies of addiction, mood disorders, and psychoses have established the importance of mechanisms such as reward, stress resiliency, and executive cognitive control [35]. Among the implicated molecular networks and genes integral to those processes are signaling networks, stress/endocrine genes, and key neurotransmitter systems including dopamine (DA), serotonin, glutamate, γ-amino butyric acid (GABA), and acetylcholine.

We investigated whether SNPs tagging genes that are key players in different neurologic molecular networks (pharmacogenomics, pharmacodynamics, and behavioral) may influence individual performance on four previously reported reference abilities (RAs): episodic memory, fluid reasoning, perceptual speed, and vocabulary.

2. Methods

2.1. Study samples

Subjects were recruited from two different cohorts. Three hundred twenty-nine participants were recruited from the community for the Reference Ability Neural Network (RANN) study (referred to as RANN sample), of which only those with Caucasian ancestry ($n = 153$) were considered for analysis purposes, and 868 Caucasian participants from the Religious Orders Study and Rush Memory and Aging Project (ROSMAP sample).

2.2. RANN sample

2.2.1. Study participants

The RANN study includes healthy adults for whom cognitive assessment and MRI imaging are available. Subjects were free of medical or psychiatric conditions that could affect cognition. Detailed description of the cohorts can be found elsewhere [36].

2.2.2. Computation of cognitive phenotypes from neuropsychology data

A battery of 12 neuropsychological tests was selected to assess cognitive functioning in four cognitive domains: episodic memory, reasoning ability, perceptual speed, and vocabulary. Previous analyses demonstrated that the included tasks described latent unique latent variables for the four cognitive domains [37,38]. There were some missing data for the neuropsychological measures, but we decided to be as inclusive as possible. We therefore calculated average z-scores within each domain over all the three measures that were available; a missing value for the domain z-score was assigned only when all three measures were missing. All measures were adjusted such that a larger value indicated better performance, that is, completion times were flipped in sign. The measures that made up the domain z-scores all showed high correlation, lending good support for internal consistency, as can seen subsequently.

Three *memory* measures were based on subscores of the Selective Reminding Task (SRT) [39]. Participants in this task were initially asked to read a list of 12 words and then asked to recall as many as they could. For the following five trials they were reminded of the words that they did not report and were asked to again recall all the words in the list. Words are considered to enter long-term storage from the point when they are recalled twice in a row without reminders. The long-term storage subscore (SRT_LTS) is the sum over all words of the number of trials when each word was in long-term storage. Continuous long-term retrieval (SRT_CLRT) is the sum over all words of the number of trials for which the word was continuously recalled. The third memory measure was the number of words recalled on the last trial (SRTLast).

Reasoning ability was assessed with scores on three different tests. One test was the Wechsler Adult Intelligence Scale (WAIS) III Block Design test [40], in which participants were asked to reproduce a series of increasingly complex geometrical shapes using four or nine identical blocks with red, white, or split red and white sides. A second test was the WAIS III Letter-Number Sequencing test [40] in which participants were asked to recall progressively longer lists of intermixed letters and numbers in alphabetical and then numerical order. The third reasoning test was the Matrix Reasoning subtest from WAIS III [41] in which participants were asked to select which pattern in a set of eight possible patterns best completes a missing cell in a matrix. The two pairwise correlations between all three measures yielded a minimum value of $R = 0.28$, $P = 10^{-5}$.

Three measures were selected to assess perceptual speed. One was the score on the Digit Symbol subtest from the WAIS III [40]. Participants in this test were instructed to write the symbol corresponding to specific numbers as quickly as possible based on a key specifying the appropriate symbol for each digit. The score is the number of correctly produced symbols in 90 seconds. A second measure was the score on Part A of the Trail Making Test [42], in which participants were instructed to connect circles numbered from 1 to 24 as rapidly as possible and performance is assessed as the time to connect all 24 circles. The third speed measure was the number of colored ink patches named in 45 seconds in the Stroop Color Naming test.

Vocabulary was assessed with scores on the vocabulary subtest from the WAIS III [41], the Wechsler Test of Adult Reading [40], and the error score of the American National Adult Reading Test [43]. The vocabulary subtest asks participants to provide definitions for a series of increasingly advanced words, and the Wechsler Test of Adult Reading and National Adult Reading Test both involve participants correctly pronouncing irregularly spelled English words.

2.2.3. SNP genotyping array

A total of 329 study participants were genotyped for 1160 SNP arrays. Most variants (1129 SNPs from 415 candidate genes) were selected from a previously published array aimed to interrogate candidate genes in alcoholism, other addictions, and disorders of mood and anxiety [32].

Most genes represent the domains of vulnerability to drug use and pharmacodynamics response (DA, serotonin, glutamine, *GABA*, and opioid neurotransmitter genes), signaling genes, and genes modulating stress resiliency and behavioral dyscontrol domains. Candidate genes involved in pharmacokinetic domains (acohol dehydrogenase [*ADH*] gene cluster, and aldehyde dehydrogenases [*ALDH*] genes) are also represented. There is a high degree of overlap between functional gene categories because of pleiotropic actions of molecules on behavior. The array was subsequently complemented with the addition of variants in candidate genes (24 SNPs) that have been previously associated with increased risk of LOAD [44] and variants in candidate genes (seven SNPs) reported as associated with episodic memory performance [11–13,19].

2.2.4. Population stratification analysis

Because of the heterogeneous ethnic background of the RANN participants, we evaluated whether distribution of SNP frequencies differ among the different ethnic groups. Population stratification analysis were conducted using STRUCTURE software [45], which uses a systematic Bayesian clustering approach applying Markov Chain Monte Carlo estimation to place subjects into groups whose members share similar patterns of genetic variation. To maximize genotype information in the estimation of the different population clusters, RANN genetic data were complemented with publicly available SNP data from HapMap European, African, and Native populations (http://www.sanger.ac.uk/resources/downloads/human/hapmap3.html). SNPs in the RANN cohort were pruned based on the linkage disequilibrium pattern, and only SNPs in high linkage disequilibrium ($r^2 \geq 0.8$) were retained.

2.2.5. Single-marker SNP tests of association

Quality control of the SNP genotype data excluded SNPs with nonbiallelic distribution, minimum allele frequencies lower than 10% and with genotype frequencies significantly deviated from Hardy-Weinberg equilibrium ($P < .001$). SNP association with the four different RAs was assessed with linear regression assuming an additive model using PLINK [46]. All analyses were adjusted for sex, age, and education. Adjustment for multiple testing using Bonferroni correction (787 SNPs, four RAs tested) establishes the threshold for experimentwise significant as $P \leq 1.6 \times 10^{-5}$. However, because of the limited sample size of the RANN cohort, SNPs achieving nominal significance ($P \leq .05$) were selected for replication purposes.

2.3. ROSMAP sample

2.3.1. Study participants

The ROS participants are older Catholic nuns, priests, and brothers from groups across the United States. MAP includes older individuals from the metropolitan Chicago area. At the time of enrollment, participants were at least 50-year-old nondemented subjects. Detailed description of the cohorts can be found elsewhere [47,48]. The present analysis was restricted to cognitively healthy Caucasian participants.

2.3.2. Cognitive phenotypes

Nineteen tasks, from a battery of 21 cognitive performance tests, were chosen to assess four domains of cognitive function: episodic memory, perceptual speed, perceptual orientation, and vocabulary. The individual cognitive tests included in each of the cognitive domains are briefly listed subsequently. Detailed description of the individual can be found elsewhere [49]. Composite measures of the specific

cognitive domains were created by converting each test within each domain into a *z*-score and averaging the z-scores as previously described [50].

Episodic memory domain included seven measures of memory: immediate and delayed recall of story A from Logical Memory and of the East Boston Story and Word List Memory, Word List Recall, and Word List Recognition.

The four measures selected to assess *perceptual speed* included Symbol Digit Modalities Test, Number Comparison, and two indices from a modified version of the Stroop Neuropsychological Screening Test.

Perceptual orientation was assessed with scores on two different tests, 15-item version of Judgment of Line Orientation and a 16-item version of Standard Progressive Matrices.

Scores on three tests were included to assess vocabulary: a 15-item version of the Boston Naming Test, Verbal Fluency, and a 15-item reading test.

2.3.3. Imputation of the genome-wide genotype data

SNP genotyping was done on the Affymetrix Genechip 6.0 or Illumina OmniQuad Express platform as previously described [51]. Genotype imputation was performed with BEAGLE [52] (version 3.3.2) and generated dosage data on >35 million SNPs for each individual using the 1000 Genomes Project (2011 Phase 1b data freeze) as a reference. Analyses were limited to SNPs with minor allele frequencies ≥0.01 and imputation quality scores >0.3. For the current analysis, two different and independent imputed genotype batches were used (ROSMAP1 and ROSMAP2).

2.3.4. Single-marker SNP tests of association

Similarly to the RANN cohort analyses, SNP association with the four different cognitive phenotypes was assessed with linear regression assuming an additive model using PLINK [46]. All analyses were adjusted for sex, age, and education.

2.3.5. Meta-analysis of RANN and ROSMAP cohorts

The results from the individual data sets were then meta-analyzed using METAL [53] by weighting the effect size estimates (β-coefficients), by their estimated standard errors. The meta-analysis was also conducted in conjunction with Cochran's Q-test for heterogeneity [54] to investigate whether observed effect sizes were homogeneous across cohorts. Adjustment for multiple testing was carried out using Bonferroni correction, SNPs achieving $P \leq 3.1 \times 10^{-4}$ (159 SNPs were tested in the meta-analysis) were declared as statistically significant associated with the corresponding RA.

3. Results

Population stratification analyses clustered the total of 329 RANN participants into three ethnic subgroups: (1) 153 subjects of Caucasian ancestry, (2) 85 subjects with African-American ancestry, and (3) the remainder 91 subjects with mixed ethnicity. To minimize the risk of false-positive associations, analyses were limited to the Caucasian ancestry RANN participants.

The characteristics of the individual study cohorts are shown in Table 1. Participants from ROSMAP were older (85 ± 7 vs. 54 ± 17) and had a higher proportion of women (70% vs. 50%) when compared with RANN participants. We did not find significant differences in the average years of education between RANN and ROSMAP's participants.

After quality control analyses in the RANN cohort, 787 SNPs were considered for association analyses (SNP characteristics are shown in Supplementary Table 1). Evaluation of the genetic association between SNPs and each of the RAs yielded 159 SNPs that achieved nominal significant associations ($P \leq .05$) across the four RAs (28 SNPs nominally associated with reasoning, 45 SNPs nominally associated with episodic memory, 38 SNPs nominally associated with speed, and 48 SNPs nominally associated with vocabulary). The strongest SNP association was observed between vocabulary and an intronic variant in the corticotropin-releasing hormone receptor 1 gene (*CRHR1* rs110402 β = 0.31, standard error = 0.07, $P = 6.1 \times 10^{-5}$), a gene that encodes a receptor that binds neuropeptides of the corticotropin hormone family, major regulators of the hypothalamic-pituitary-adrenal pathway. Although the association achieved experiment-wise statistical significance after Bonferroni correction ($P \leq 1.6 \times 10^{-5}$), the SNP was not found to be significantly associated in the ROSMAP replication cohorts (data not shown).

The subset of 159 SNPs nominally associated variants in the RANN cohort was tested for association in the ROSMAP cohorts. SNP association results from RANN and ROSMAP cohorts were then meta-analyzed. SNPs that achieved $P < .05$ in the meta-analysis are shown in Table 2. Meta-analysis results identified variant rs3758653 located in the promoter region of the dopamine receptor D4 (*DRD4*) gene strongly associated with perceptual speed, reaching statistical significance after adjusting for multiple testing ($P_{meta} = 2.3 \times 10^{-5}$). Compared with carriers of the C allele at SNP rs3758653, carriers of one or two copies of the T allele demonstrated better performance on processing speed, that is, in the RANN cohort, average speed performance (and standard deviation) was 0.03 (0.84) for T allele carriers versus −0.16 (0.74) for C allele carriers. The heterogeneity test (Supplementary Table 1) demonstrated that SNP effect was homogenous across the

Table 1
Characteristics of the study cohorts

Variable	RANN	ROSMAP
Number of individuals	153	939
% Female	50	70
Age, average ± SD	54 ± 17	85 ± 7
Education, average ± SD	17 ± 2	17 ± 4

Abbreviations: RANN, Reference Ability Neural Network Study, Columbia University; ROSMAP, Religious Order and Rush Aging Project cohorts; SD, standard deviations.

Table 2
Meta-analysis of RANN and ROSMAP cohorts

			RANN				ROSMAP_b1				ROSMAP_b2				Meta-analysis			
Reference abilities	Gene	SNP	A1	β	SE	*P*	A1	β	SE	*P*	A1	β	SE	*P*	β	SE	P_{meta}	Dir.
Episodic memory	**GABRA4**	**rs4695183**	**C**	**−0.42**	**0.17**	**.014**	**T**	**0.07**	**0.05**	**.163**	**T**	**0.14**	**0.10**	**.172**	**0.11**	**0.04**	**.014**	**+++**
Episodic memory	GRIN2B	rs2192977	A	0.29	0.10	.004	T	−0.07	0.03	.027	T	0.02	0.05	.696	0.06	0.02	.014	++−
Episodic memory	GRIN2B	rs12829455	A	−0.23	0.10	.029	G	0.03	0.03	.445	G	0.06	0.05	.210	−0.05	0.02	.045	−−−
Reasoning	SLC6A11	rs2581206	C	−0.17	0.08	.032	A	0.07	0.08	.352	A	0.03	0.15	.833	0.11	0.05	.040	+++
Reasoning	SLC6A11	rs1881354	A	−0.16	0.08	.045	G	0.09	0.08	.271	G	0.03	0.15	.861	−0.11	0.05	.033	−−−
Reasoning	CDKL3	rs326626	G	−0.23	0.11	.043	C	0.10	0.13	.447	C	0.38	0.25	.136	0.20	0.08	.014	+++
Reasoning	NR3C1	rs6877893	G	−0.18	0.07	.017	G	−0.10	0.07	.155	G	0.03	0.15	.831	0.12	0.05	.009	++−
Reasoning	**EPHA1**	rs11767557	**C**	**−0.23**	**0.10**	**.024**	**T**	**0.15**	**0.09**	**.107**	**T**	**0.27**	**0.19**	**.165**	**0.20**	**0.06**	**.002**	**+++**
Reasoning	ADRA1A	rs2644627	C	−0.19	0.08	.016	G	0.07	0.08	.361	G	0.02	0.15	.886	−0.12	0.05	.028	−−−
Reasoning	NTRK2	rs11795386	T	−0.24	0.09	.013	C	0.11	0.10	.267	C	−0.18	0.20	.376	−0.15	0.06	.022	−−+
Reasoning	CH25H	rs11203006	G	0.23	0.12	.046	A	−0.10	0.11	.398	A	−0.09	0.19	.634	−0.15	0.07	.046	−−−
Reasoning	MAPT	rs8079215	C	0.19	0.09	.045	T	−0.11	0.08	.212	T	0.14	0.18	.454	−0.12	0.06	.039	−−+
Reasoning	GALR1	rs2717164	G	−0.39	0.16	.020	T	0.18	0.30	.553	T	1.04	0.68	.126	0.37	0.14	.007	+++
Speed	GABRA4	rs1398176	T	−0.33	0.10	.002	C	0.05	0.06	.433	C	−0.01	0.12	.905	−0.10	0.05	.029	−−+
Speed	GABRB1	rs971353	C	−0.19	0.09	.035	T	0.13	0.05	.008	T	0.03	0.10	.765	0.13	0.04	.002	+++
Speed	RP1L1	rs4841401	C	−0.22	0.07	.004	G	0.02	0.05	.626	G	0.19	0.09	.034	−0.10	0.04	.005	−−−
Speed	**DRD4**	**rs3758653**	**C**	**−0.23**	**0.09**	**.010**	**T**	**0.15**	**0.09**	**.094**	**T**	**0.32**	**0.10**	**.002**	**0.23**	**0.05**	**2.3×10^{-5}**	**+++**
Speed	CHRNA5	rs7180002	T	0.17	0.07	.021	T	0.05	0.05	.262	T	0.19	0.08	.023	−0.11	0.04	.002	−−−
Speed	SLC6A2	rs36008	A	0.41	0.20	.044	T	−0.18	0.15	.234	T	−0.17	0.30	.565	0.25	0.11	.025	+++
Speed	GALR1	rs2717164	G	−0.33	0.15	.033	T	0.16	0.17	.341	T	0.63	0.38	.100	0.29	0.11	.008	+++
Speed	GRIK1	rs457474	C	−0.36	0.10	3.2×10^{-4}	G	0.05	0.06	.354	G	0.16	0.11	.162	−0.14	0.05	.003	−−−
Vocabulary	CREB1	rs2551640	G	−0.19	0.08	.013	G	−0.05	0.05	.298	G	−0.07	0.08	.396	0.09	0.04	.023	+++
Vocabulary	LPCAT1	rs3756450	G	−0.28	0.12	.021	A	0.10	0.07	.128	A	0.02	0.11	.871	0.12	0.05	.028	+++
Vocabulary	EPHA1	rs11767557	C	−0.20	0.10	.045	T	0.05	0.05	.314	T	0.31	0.10	.002	0.12	0.04	.004	+++
Vocabulary	**SLC18A1**	**rs2270641**	**G**	**−0.22**	**0.08**	**.004**	**G**	**−0.08**	**0.04**	**.062**	**G**	**−0.11**	**0.08**	**.174**	**0.11**	**0.03**	**.001**	**+++**
Vocabulary	TPH2	rs1352250	A	0.19	0.08	.017	G	−0.05	0.04	.242	G	−0.02	0.08	.802	0.07	0.03	.036	+++
Vocabulary	GABRB3	rs2114217	T	−0.21	0.10	.030	A	0.08	0.06	.196	A	0.19	0.12	.103	0.13	0.05	.008	+++
Vocabulary	CRHR1	rs12938031	G	−0.23	0.08	.006	G	−0.04	0.04	.337	G	−0.03	0.08	.695	0.07	0.03	.032	+++

Abbreviations: RANN, Reference Ability Neural Network; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; SE, standard error; SNP, single-nucleotide polymorphism.

Only SNPs reaching nominal significance ($P \leq .05$) are shown (ROSMAP_b1 and ROSMAP_b2 correspond to the two independent genotype batches for the cohort). Strongest SNP associations within each of the reference abilities are highlighted in bold.

different cohorts ($P = .450$), and in fact, the direction of the SNP effect was consistent across the three cohorts.

Although not reaching statistical significance after multiple testing correction ($P \leq 3.1 \times 10^{-4}$), we observed in the meta-analysis associations that reached nominal significance and had a consistent direction of the genetic effect across the three cohorts. For reasoning, the strongest association was observed for an intronic variant in the erythropoietin-producing hepatoma receptor A1 (*EPHA1*) gene ($P_{meta} = .002$), a gene that has been reported to increase the risk of developing LOAD. An intronic variant in the *GABA* type A receptor Alpha4 (*GABRA4*) gene, involved in the GABAergic neurotransmission of the central nervous system, appeared to be associated with better performance on episodic memory ($P_{meta} = .014$). Finally, a coding variant in the solute carrier family 18 member A1 (*SLC18A1*) gene, a vesicular monoamine transporter, was nominally significant associated with vocabulary ($P_{meta} = .001$).

4. Discussion

Our meta-analysis of genetic associations between SNPs within neuropsychiatric pathway genes and cognitive domains identified a variant in the promoter region of the *DRD4* gene strongly associated with performance on speed of processing ($P_{meta} = 2.3 \times 10^{-5}$) in cognitively healthy subjects.

Dopamine, a catecholamine neurotransmitter, influences cognition and motor/limbic functions [55]. Dysfunction of the dopaminergic system is involved in a variety of disorders, including schizophrenia, Parkinson's disease, and drug addiction.

Substantial evidence from animal studies has shown the key role of dopamine in regulating performance across a variety of cognitive domains. For example, in rodents and monkeys lesions of dopaminergic nerve terminals at different sites lead to impartment in memory and spatial attention functions [56]. Patients with Huntington's and Parkinson's disease have demonstrated deficits across multiple cognitive domains including episodic memory, verbal fluency, perceptual speed, and reasoning [57,58]. Pharmacologic studies have shown administration of psychostimulant drugs or antagonists of dopamine receptors are associated with enhancement/impairment of performance in tasks such as processing speed [59,60].

The *DRD4* gene is widely heavily expressed in the frontal cortex [61], where it appears to modulate GABAergic signaling [62], suggesting a potential mechanism underlying the involvement of D4 receptors in frontal cortex cognitive function.

However, the relationship between dopamine and cognitive performance is highly complex and the precise role of DA in cognition is not well understood. Pharmacologic studies have shown that in disorders such as schizophrenia, cognitive symptoms can be improved by specifically administrating blockers of dopamine D4 receptors [63]. Furthermore, the cognitive benefits derived from *DRD4* blocking seem to be only effective when other dopamine receptors are not blocked, suggesting a unique role for *DRD4* in inhibiting cognitive processes [64]. Current research suggests that manipulation of dopamine will have paradoxical cognitive consequences depending on the type of task under study, the brain region that is implicated, and the baseline levels of dopamine in that brain region [65].

Variants in *DRD4* gene have associated with schizophrenia [66] and bipolar disorder [67]. A 48 bp short tandem repeat polymorphism in the exon 3 is the most replicated genetic risk factor for attention-deficit/hyperactivity disorder (ADHD) [68]. In a sample of 245 healthy Caucasians adults, the same polymorphism appeared to be strongly associated with slower speed of performance on multiple cognitive tasks [69].

The *DRD4* promoter variant rs3758653 reported here has been previously associated with schizophrenia [66], heroin addiction [66], and AD [70]. To our knowledge, this is the first report implicating the same *DRD4* polymorphism in cognitively European healthy individuals with a sample size >1000. In a smaller sample ($n = \sim 500$ healthy Chinese adults), a correlation between this SNP and the speed of processing of the Tower of Hanoi task was reported [71]. Regarding the functional implications of the gene's promoter variants, it has been shown that the *DRD4* gene's polymorphisms lead to the difference in how well the receptors bind with dopamine and similar compounds [72], and therefore it has been assumed this basic difference leads to the differences observed in the phenotypes.

One unique feature of this study was the four selected cognitive domains capturing most variance in age-related cognitive function. Researchers often evaluate neural correlates of age-related cognitive changes using the performance of one specific task that purportedly taps a domain. In contrast, it has been repeatedly demonstrated that performance across the age span on large batteries of diverse cognitive tests can be parsimoniously represented by a set of four RAs: episodic memory, fluid ability, perceptual speed, and vocabulary [73,74]. On the basis of these findings, Salthouse and Ferrer-Caja [37] have argued that a productive and efficient approach to cognitive aging research is to try to understand how aging impacts performance of this small set of general RAs, rather than on specific tasks. Thus, we used three measures per domain that we found to be compatible with Salthouse' s latent variable measures of the four RAs [38]. We could therefore be confident that we were using comprehensive and accurate measures of cognitive aging.

There are some limitations of our study. First, the limited sample size of the discovery cohort may reduce statistical power to detect a true effect [75]. However, the fact that we replicated our finding in an independent cohort provides further support for the association. Second, it is possible that the list of target genes studied failed to include the genes that may play an important role in cognitive function. Third, we cannot discard the possibility that environmental factors may explain the differences observed in cognitive performance, in fact, due to the complex nature of cognition, is highly likely that there may be an interaction between genetic and environmental factors.

Acknowledgments

This work was supported by National Institute of Health grants: RF1 AG038465, R01 AG026158, P30AG10161, RF1AG15819, R01AG17917, and U01AG46152.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trci.2017.03.004.

RESEARCH IN CONTEXT

1. Systematic review: Genetic variants strongly influence cognition in both pathologic and normal variations. Candidate gene and genome-wide association study have reported potential loci, but no specific genes have been identified. We investigated whether genetic variants in genes in different neurologic molecular networks are associated with domains capturing variance in age-related cognition.
2. Interpretation: Our results identified a variant in the promoter region of the dopamine receptor D4 gene strongly associated with performance on speed of processing. To our knowledge, this is the first report implicating this gene in a large cohort ($n = 1021$) of cognitively healthy European subjects. The identification of genetic variants influencing healthy cognition is crucial to understand the natural disease resistance to brain neurodegeneration.
3. Future directions: The precise role of dopamine in cognition is still not well understood. Future studies will require whole genome gene sequencing in larger samples followed by functional genomic studies aimed to identify the causal variants.

References

[1] Haworth CM, Wright MJ, Martin NW, Martin NG, Boomsma DI, Bartels M, et al. A twin study of the genetics of high cognitive ability selected from 11,000 twin pairs in six studies from four countries. Behav Genet 2009;39:359–70.

[2] Plomin R, Haworth CM. Genetics of high cognitive abilities. Behav Genet 2009;39:347–9.

[3] Kan KJ, Wicherts JM, Dolan CV, van der Maas HL. On the nature and nurture of intelligence and specific cognitive abilities: the more heritable, the more culture dependent. Psychol Sci 2013;24:2420–8.

[4] Barral S, Reitz C, Small SA, Mayeux R. Genetic variants in a 'cAMP element binding protein' (CREB)-dependent histone acetylation pathway influence memory performance in cognitively healthy elderly individuals. Neurobiol Aging 2014;35:2881.e7–10.

[5] Deary IJ, Hamilton G, Hayward C, Whalley LJ, Powell J, Starr JM, et al. Nicastrin gene polymorphisms, cognitive ability level and cognitive ageing. Neurosci Lett 2005;373:110–4.

[6] Deary IJ, Harris SE, Fox HC, Hayward C, Wright AF, Starr JM, et al. KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. Neurosci Lett 2005;378:22–7.

[7] Deary IJ, Hayward C, Permana PA, Nair S, Whalley LJ, Starr JM, et al. Polymorphisms in the gene encoding 11B-hydroxysteroid dehydrogenase type 1 (HSD11B1) and lifetime cognitive change. Neurosci Lett 2006;393:74–7.

[8] Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, et al. The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. Mol Psychiatry 2006;11:505–13.

[9] Harris SE, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ. The functional COMT polymorphism, Val 158 Met, is associated with logical memory and the personality trait intellect/imagination in a cohort of healthy 79 year olds. Neurosci Lett 2005;385:1–6.

[10] Huentelman MJ, Papassotiropoulos A, Craig DW, Hoerndli FJ, Pearson JV, Huynh KD, et al. Calmodulin-binding transcription activator 1 (CAMTA1) alleles predispose human episodic memory performance. Hum Mol Genet 2007;16:1469–77.

[11] Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, et al. Common Kibra alleles are associated with human memory performance. Science 2006;314:475–8.

[12] Reynolds CA, Jansson M, Gatz M, Pedersen NL. Longitudinal change in memory performance associated with HTR2A polymorphism. Neurobiol Aging 2006;27:150–4.

[13] Vogler C, Spalek K, Aerni A, Demougin P, Muller A, Huynh KD, et al. CPEB3 is associated with human episodic memory. Front Behav Neurosci 2009;3:4.

[14] Davies G, Armstrong N, Bis JC, Bressler J, Chouraki V, Giddaluru S, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53949). Mol Psychiatry 2015;20:183–92.

[15] Davies G, Marioni RE, Liewald DC, Hill WD, Hagenaars SP, Harris SE, et al. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). Mol Psychiatry 2016;21:758–67.

[16] Debette S, Ibrahim Verbaas CA, Bressler J, Schuur M, Smith A, Bis JC, et al. Genome-wide studies of verbal declarative memory in nondemented older people: the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium. Biol Psychiatry 2015;77:749–63.

[17] Ibrahim-Verbaas CA, Bressler J, Debette S, Schuur M, Smith AV, Bis JC, et al. GWAS for executive function and processing speed suggests involvement of the CADM2 gene. Mol Psychiatry 2016;21:189–97.

[18] Kirkpatrick RM, McGue M, Iacono WG, Miller MB, Basu S. Results of a "GWAS plus:" general cognitive ability is substantially heritable and massively polygenic. PLoS One 2014;9:e112390.

[19] Papassotiropoulos A, Stefanova E, Vogler C, Gschwind L, Ackermann S, Spalek K, et al. A genome-wide survey and functional brain imaging study identify CTNNBL1 as a memory-related gene. Mol Psychiatry 2013;18:255–63.

[20] Pawlowski TL, Huentelman MJ. Identification of a common variant affecting human episodic memory performance using a pooled genome-wide association approach: a case study of disease gene identification. Methods Mol Biol 2011;700:261–9.

[21] Rietveld CA, Esko T, Davies G, Pers TH, Turley P, Benyamin B, et al. Common genetic variants associated with cognitive performance identified using the proxy-phenotype method. Proc Natl Acad Sci U S A 2014;111:13790–4.

[22] Carrasquillo MM, Crook JE, Pedraza O, Thomas CS, Pankratz VS, Allen M, et al. Late-onset Alzheimer's risk variants in memory decline, incident mild cognitive impairment, and Alzheimer's disease. Neurobiol Aging 2015;36:60–7.

[23] Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. N Engl J Med 2009;361:255–63.

[24] Chibnik LB, Shulman JM, Leurgans SE, Schneider JA, Wilson RS, Tran D, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. Ann Neurol 2011;69:560–9.

[25] Keenan BT, Shulman JM, Chibnik LB, Raj T, Tran D, Sabuncu MR, et al. A coding variant in CR1 interacts with APOE-epsilon4 to influence cognitive decline. Hum Mol Genet 2012;21:2377–88.

[26] Sweet RA, Seltman H, Emanuel JE, Lopez OL, Becker JT, Bis JC, et al. Effect of Alzheimer's disease risk genes on trajectories of cognitive function in the Cardiovascular Health Study. Am J Psychiatry 2012;169:954–62.

[27] Thambisetty M, Beason-Held LL, An Y, Kraut M, Nalls M, Hernandez DG, et al. Alzheimer risk variant CLU and brain function during aging. Biol Psychiatry 2013;73:399–405.

[28] Vivot A, Glymour MM, Tzourio C, Amouyel P, Chene G, Dufouil C. Association of Alzheimer's related genotypes with cognitive decline in multiple domains: results from the Three-City Dijon study. Mol Psychiatry 2015;20:1173–8.

[29] Harris SE, Davies G, Luciano M, Payton A, Fox HC, Haggarty P, et al. Polygenic risk for Alzheimer's disease is not associated with cognitive ability or cognitive aging in non-demented older people. J Alzheimers Dis 2014;39:565–74.

[30] Farfel JM, Yu L, Buchman AS, Schneider JA, De Jager PL, Bennett DA. Relation of genomic variants for Alzheimer disease dementia to common neuropathologies. Neurology 2016;87:489–96.

[31] Korte A, Farlow A. The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 2013;9:29.

[32] Hodgkinson CA, Yuan Q, Xu K, Shen PH, Heinz E, Lobos EA, et al. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. Alcohol Alcohol 2008;43:505–15.

[33] Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3:391–7.

[34] Patnala R, Clements J, Batra J. Candidate gene association studies: a comprehensive guide to useful in silico tools. BMC Genet 2013;14:39.

[35] Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. Nat Rev Genet 2005;6:521–32.

[36] Stern Y, Habeck C, Steffener J, Barulli D, Gazes Y, Razlighi Q, et al. The Reference Ability Neural Network Study: motivation, design, and initial feasibility analyses. Neuroimage 2014;103:139–51.

[37] Salthouse TA, Ferrer-Caja E. What needs to be explained to account for age-related effects on multiple cognitive variables? Psychol Aging 2003;18:91–110.

[38] Razlighi QR, Oh H, Habeck C, O'Shea D, Gazes E, Eich T, et al. Dynamic patterns of brain structure-behavior correlation across the lifespan. Cereb Cortex 2016:1–14 [E-pub ahead of print].

[39] Buschke H, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. Neurology 1974;24:1019–25.

[40] Wechsler DS. Adult Intelligence Scale—III. San Antonio, TX: The Psychological Corporation; 1997.
[41] Wechsler DS. Wechsler Memory Scale—III. San Antonio, TX: The Psychological Corporation; 1997.
[42] Reitan RM, Wolfson D. The Halstead-Reitan Neurophychological Test Battery. Tuscan, AZ: Neuropsychological Press; 1987.
[43] Grober E, Sliwinski M. Development and validation of a model for estimating premorbid verbal intelligence in the elderly. J Clin Exp Neuropsychol 1991;13:933–49.
[44] Shen L, Jia J. An overview of Genome-Wide Association Studies in Alzheimer's disease. Neurosci Bull 2016;32:183–90.
[45] Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–59.
[46] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559–75.
[47] Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the religious orders study. Curr Alzheimer Res 2012; 9:628–45.
[48] Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the rush Memory and Aging Project. Curr Alzheimer Res 2012;9:646–63.
[49] Wilson RS, Boyle PA, Yu L, Barnes LL, Sytsma J, Buchman AS, et al. Temporal course and pathologic basis of unawareness of memory loss in dementia. Neurology 2015;85:984–91.
[50] Wilson RS, Barnes LL, Krueger KR, Hoganson G, Bienias JL, Bennett DA. Early and late life cognitive activity and cognitive systems in old age. J Int Neuropsychol Soc 2005;11:400–7.
[51] Bennett DA, Yu L, De Jager PL. Building a pipeline to discover and validate novel therapeutic targets and lead compounds for Alzheimer's disease. Biochem Pharmacol 2014;88:617–30.
[52] Browning BL, Browning SR. Genotype imputation with millions of reference samples. Am J Hum Genet 2016;98:116–26.
[53] Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010; 26:2190–1.
[54] Cochran WG. The combination of estimates from different experiments. Biometrics 1954;10:101–29.
[55] McEntee WJ, Mair RG, Langlais PJ. Neurochemical specificity of learning: dopamine and motor learning. Yale J Biol Med 1987; 60:187–93.
[56] Simon H, Taghzouti K, Le Moal M. Deficits in spatial-memory tasks following lesions of septal dopaminergic terminals in the rat. Behav Brain Res 1986;19:7–16.
[57] Brown RG, Marsden CD. Cognitive function in Parkinson's disease: from description to theory. Trends Neurosci 1990;13:21–9.
[58] Weimer E, Ries S, Tost H, Braus DF. Frontal dysfunctions in Huntington's disease—neuropsychology and therapy. Psychiatr Prax 2003; 30:33–6.
[59] Cools R, Robbins TW. Chemistry of the adaptive mind. Philos Trans A Math Phys Eng Sci 2004;362:2871–88.
[60] Halliday R, Naylor H, Brandeis D, Callaway E, Yano L, Herzig K. The effect of D-amphetamine, clonidine, and yohimbine on human information processing. Psychophysiology 1994;31:331–7.
[61] Cadet JL, Jayanthi S, McCoy MT, Beauvais G, Cai NS. Dopamine D1 receptors, regulation of gene expression in the brain, and neurodegeneration. CNS Neurol Disord Drug Targets 2010;9:526–38.
[62] Wang X, Zhong P, Yan Z. Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. J Neurosci 2002;22:9185–93.
[63] Arnsten AF, Murphy B, Merchant K. The selective dopamine D4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. Neuropsychopharmacology 2000; 23:405–10.
[64] Wong AH, Van Tol HH. The dopamine D4 receptors and mechanisms of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1091–9.
[65] Cools R, D'Esposito M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol Psychiatry 2011; 69:e113–25.
[66] Lai JH, Zhu YS, Huo ZH, Sun RF, Yu B, Wang YP, et al. Association study of polymorphisms in the promoter region of DRD4 with schizophrenia, depression, and heroin addiction. Brain Res 2010; 1359:227–32.
[67] Seifuddin F, Mahon PB, Judy J, Pirooznia M, Jancic D, Taylor J, et al. Meta-analysis of genetic association studies on bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 2012;159B:508–18.
[68] Faraone SV, Doyle AE, Mick E, Biederman J. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 2001;158:1052–7.
[69] Szekely A, Balota DA, Duchek JM, Nemoda Z, Vereczkei A, Sasvari-Szekely M. Genetic factors of reaction time performance: DRD4 7-repeat allele associated with slower responses. Genes Brain Behav 2011;10:129–36.
[70] Lin WY, Wu BT, Lee CC, Sheu JJ, Liu SH, Wang WF, et al. Association analysis of dopaminergic gene variants (Comt, Drd4 And Dat1) with Alzheimer's disease. J Biol Regul Homeost Agents 2012; 26:401–10.
[71] Zhao L, Wang Y, Wei J, Yang X, Ni P, Gu X, et al. Association of gender, age, education and polymorphism of DRD4 gene with cognitive functions in adults. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2015; 32:391–4.
[72] Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, et al. Multiple dopamine D4 receptor variants in the human population. Nature 1992;358:149–52.
[73] Salthouse TA, Pink JE, Tucker-Drob EM. Contextual analysis of fluid intelligence. Intelligence 2008;36:464–86.
[74] Salthouse TA. Relations between cognitive abilities and measures of executive functioning. Neuropsychology 2005;19:532–45.
[75] Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013;14:365–76.