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The *CADM2* gene is associated with processing speed performance – evidence among elderly with Type 2 Diabetes

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Abstract:

Objectives: Recent large scale meta-analysis of genome wide association studies (GWAS) from multiple cohorts, demonstrated the association of the single nucleotide polymorphism (SNP) rs17518584, with processing speed (measured by the Digit Symbol Substitution Task (DSST) or the Letter Digit Substitution Test (LDST)), at GWAS significance level. This SNP is located within the cell adhesion molecule 2 (*CADM2*) gene. We aimed to validate this finding in our sample of 944 cognitively normal Jewish elderly individuals with type 2 diabetes (T2D), a population which is at risk for cognitive decline and dementia. **Methods:** Using linear regression, we studied the association of rs17518584 with DSST performance, adjusting for demographic, T2D-related characteristics and cardiovascular factors. In secondary analyses, associations with performance in four cognitive domains (episodic memory, language/semantic categorization, attention/working memory, executive function) and overall cognition were examined. **Results:** Controlling for sex, age at cognitive evaluation, years of education and ancestry, we found a significant association of this SNP with DSST performance (p=0.013), consistent with the expected effect direction. Results remained similar even when the additional covariates (T2D-related and cardiovascular factors) were included in the analysis (p=0.034). Moreover, this SNP was significantly associated with performance in the cognitive domains of language/semantic categorization and executive function, as well as overall cognition. **Conclusions:** Taken together, irrespective of T2D and cardiovascular factors, our findings provide independent support for the association of this genetic variant with processing speed (as well as with additional cognitive phenotypes), across cognitively normal elderly individuals.

Keywords: CADM2; Digit Symbol Substitution Test**;** processing speed; Cognition; Type 2 diabetes.

Introduction:

Type 2 diabetes (T2D) is a well-established risk factor for cognitive decline and dementia (both vascular dementia and Alzheimer's disease) (Ahtiluoto et al., 2010; Beeri et al., 2009; Luchsinger, 2010; Schnaider Beeri et al., 2004). The pathophysiology of this link is not clear, and probably involves processes such as insulin resistance, cerebrovascular mechanisms and advanced glycation end products (AGEs) accumulation (Beeri et al., 2009; Luchsinger, 2012). Genetic factors may contribute to this susceptibility, and several genes were associated with cognitive phenotypes among T2D patients, among them *TOMM40* (Greenbaum et al., 2014), *BIN1* (Greenbaum et al., 2016*)* and *HP* (Haptoglobin) (Ravona-Springer et al., 2013).

Information processing speed is a cognitive domain that reflects the speed at which cognitive operations are executed, mainly elementary cognitive tasks (Salthouse, 1996; Takeuchi and Kawashima, 2012). The heritability of performance in cognitive tests related to processing speed ranges from 12% to 68%, in different studies (Cirulli et al., 2010; Lee et al., 2012; Tucker-Drob et al., 2014; Wright et al., 2001). One of the most widely used measures for processing speed is the Digit Symbol Substitution Test/Task (DSST), a task in which a subject transcribes numbers into symbols according to a key as fast as possible and within a limited time (Wechsler, 1981).

Recently, a large scale meta-analysis of genome wide association studies (GWAS) data from multiple cohorts for executive function and processing speed was published (Ibrahim-Verbaas et al., 2016). The discovery phase included 20 cohorts with GWAS data of European ancestry non-demented older individuals (≥45 years) from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium. Performance on several cognitive tests was investigated, including DSST (for some cohorts, the LDST (Letter Digit Substitution Test) or other similar tests were used instead of DSST). Meta-analysis demonstrated only one association at GWAS significant levels (P value=3.12×10^{-8}, adjusted for age, sex and education), of the single nucleotide polymorphism (SNP) rs17518584 with processing speed performance (measured by the LDST/DSST tests, N=32,070 subjects for this test). In silico replication in independent cohorts provided nominal significance (N=1311 subjects). In the combined discovery and replication meta-analysis, the association of rs17518584 with LDST/DSST performance also reached GWAS significance (P=3.28×10^{-9}; positive effect of the T allele). This SNP is located 170 kb upstream of the transcription start site of the cell adhesion molecule 2 (*CADM2*) gene major transcript. However, rs17518584 is located within an intron of a variant transcript which includes an alternative first exon (Ibrahim-Verbaas et al., 2016). No further significant associations at GWAS level with performance in the other

cognitive tests (Trail Making Test parts A and B, semantic and phonemic fluency tests, and the Stroop Color and Word Test) were found.

Our aim in this study was to replicate the association of rs17518584 with DSST performance among our sample of cognitively normal T2D patients, who are at high risk for cognitive decline.

Methods:

Sample: The current study is based on the Israel Diabetes and Cognitive Decline (IDCD) study (Beeri et al., 2014), which consists of elderly Israeli Jewish (≥ 65 years old) T2D subjects, randomly recruited from the Maccabi Health system (MHS) diabetes registry. The aim of the IDCD project is to evaluate the relationship of cognitive decline and long-term T2D characteristics. All IDCD's subjects are cognitively normal at entry to the study, fluent in Hebrew (to ensure valid cognitive testing), living in central Israel, had an informant with substantial contact and gave informed consent to participate. They were free of major medical, psychiatric, or neurological conditions that may affect their cognitive status. Additional information regarding the IDCD study, entry criteria and recruitment process is given by Beeri et al. (2014)

Cognitive assessment: A comprehensive cognitive battery was administrated to all subjects by a neuropsychologist, including DSST (the primary outcome of this study). In this test, nine digit-symbol pairs are followed by a list of digits, that subject have to transcribe as fast as possible to the corresponding symbol within 90 seconds, and scored according to correct matches (Wechsler, 1981). The current study presents results based on a single (baseline) cognitive assessment only— Longitudinal IDCD assessments are ongoing.

In addition, as in our previous studies, raw scores of all administrated cognitive tests were transformed into *z* scores, and factor analysis of all tests revealed four cognitive domains, which were then scored as sums of z scores: episodic memory factor (included word list immediate and delayed recall, and recognition from the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) neuropsychological battery (Beeri et al., 2006; Welsh et al., 1994)); language/semantic categorization factor (letter and category fluency (Newcombe, 1969; Spreen and Benton, 1977), and similarities (Wechsler, 1981)); attention/working memory factor (diamond cancellation test and digit span (forward and backward) (Wechsler 1987)), and an executive factor (included the trails making A and B (Reitan, 1955), CERAD-constructional praxis and the DSST (Wechsler, 1981)). In addition, an overall cognition measure was created by summarizing the four domains. These were used as secondary outcomes.

The cognitive assessment was performed at the subject's residence (a handful of participants preferred to be tested at the Sheba Medical Center Memory Clinic instead) during morning time, and lasted approximately 2 hours. The neuropsychologist who administrated the assessment was blind to the T2D-related data. Additional questionnaires on cognitive and functional impairments were administrated to the subject and informant, including Clinical Dementia Rating (CDR) scale (Fillenbaum et al., 1996), Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and Geriatric Depression scale (GDS) (Yesavage, 1988). Importantly, a multidisciplinary team discussed and defined participants′ cognitive status (normal, mild cognitive impairment (MCI) or dementia), and only cognitively normal participants were included in the study (Beeri et al, 2014).

Genotyping and statistical analysis: SNP rs17518584 was genotyped in the IDCD sample (among subjects with available DNA), using Kompetitive Allele Specific PCR (KASP) genotyping technology (He et al., 2014; Semagn, 2014) by LGC genomics (Teddington, UK; www.lgcgenomics.com). Genotyping success rate was 98.5%. Quality control measures were implemented; 10% of the sample was blindly double-genotyped, and concordance rate was above 99%. For statistical analysis, we used PLINK (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007) and IBM SPSS Statistics for Windows, version 19.0 (Armonk, NY: IBM Copr.). ANOVA and chi square tests were used to evaluate the differences in sample's characteristics by the three rs17518584 genotypes.

We employed hierarchical linear regression under an additive model (same as in the original report (Ibrahim-Verbaas et al., 2016)) to study the association of SNPrs17518584 with DSST performance, while controlling for demographic covariates (sex, age at cognitive evaluation, years of education and ancestry (Ashkenazi vs. Non-Ashkenazi, based on self-report and land of birth data)). In the second step, we included in the statistical model a set of T2D-related characteristics (number of follow up years in the MHS diabetes registry, (surrogate of disease duration), mean Hemoglobin A1c (HbA1c) level and consumption of T2D medication). Data concerning consumption of T2D medication was classified into three categories: (a) no medication, (b) hypoglycemic medication only and (c) insulin or insulin plus hypoglycemic medication. Subjects were categorized by having ever/never taken the medication. In the last step, a third set of cardiovascular factors was added to the model (systolic and diastolic blood pressure, body mass index (BMI), creatinine, total cholesterol and triglycerides levels). The HbA1c and cardiovascular risk factors were calculated for each subject as the means of all assessments in the diabetes registry. A p-value of 0.05 (two sided) was considered statistically significant. Statistical power for rs17518584 association with DSST performance was calculated using Quanto version 1.2.4 software (http://hydra.usc.edu/gxe).

For secondary analysis, we also studied in a similar way the association of SNP rs17518584 with cognitive performance in the four cognitive domains measured in the IDCD study (episodic memory, language/semantic categorization, attention/working memory and executive factor), as well as overall cognition.

To evaluate the correlation between DSST performance and mean HbA1c level, we used partial correlation tests (controlling for the demographic variables). We repeated this analysis by stratifying the sample according to rs17518584 genotypes. Fisher's z-transformation was used to compare these partial correlations.

Results

The final analysis included 944 subjects, and demographic and clinical description of the sample is provided in Table 1, as well as comparison of the three genotypes on these characteristics. The groups did not differ on any of them. The SNP rs17518584 did not show deviation from Hardy-Weinberg equilibrium (p=0.58). The minor allele C frequency was 0.45. In linear regression (additive model- genotype coded as 0, 1 or 2 according to T allele count), we found a significant association of SNP rs17518584 with performance in DSST (β=0.07; p=0.013), when controlling for demographic covariates (sex, age at cognitive assessment, years of education and ancestry). Consistent with the original report (Ibrahim-Verbaas et al., 2016), the T allele was associated with better performance on the DSST (Tables 2A and 2B).

The results remained essentially unchanged when including additional set of T2D-related characteristics (follow up years in the diabetes registry, mean HbA1c level and T2D medication) (β=0.065; p=0.02), and following addition of a set of cardiovascular factors (systolic and diastolic blood pressure, BMI, creatinine, total cholesterol and triglycerides levels) to the model (β=0.059; p=0.034). Interestingly, the significance level was even stronger if, following the original report (Ibrahim-Verbaas et al., 2016), we controlled only for age and sex (β=0.095; p=0.003), or for age, sex and education (β=0.079; p=0.006) (see Supplement Table 1).

In the secondary analysis, we studied the association of rs17518584 with performance in four cognitive domains (episodic memory, language/semantic categorization, attention/working memory, executive function) as well as overall cognition. Controlling for demographic covariates, the association was significant for language/semantic categorization (β=0.069, p=0.015; when including T2D-related characteristics β=0.067, p=0.019; when adding also the cardiovascular covariates β=0.063, p=0.027), but not for attention/working memory and episodic memory (Table 2B).

Since the executive function factor and overall cognition measurement score in our sample included the DSST, we analyzed the association with and without the DSST contribution. When DSST was included, the association (controlling for demographic covariates) was significant for executive function (β=0.066, p=0.015) and overall cognition (β=0.065, p=0.014). The results remained significant even when the DSST score was excluded from the executive function factor (β=0.057; p=0.042) and overall cognition (β=0.061; p=0.021). Again, similar results were found when controlling also for T2D and cardiovascular covariates, although only at trend level for executive function (Table 2B). These results suggest that SNP rs17518584 involvement in executive function and overall cognition is not explained merely by DSST performance.

A posteriori power estimates for SNP rs17518584 association with DSST performance in our sample (944 participants, additive model, minor allele frequency of 0.45, required α=0.05), ranged from 82% (adjusting for only age and sex) to 44% (adjusting for all 13 covariates).

In the overall sample, DSST performance was significantly correlated with mean HbA1c (r-0.105; p=0.001) when controlling for demographic variables. However. the partial correlations of DSST with HbA1c did not significantly differ in the comparison of TC+CC genotypes carriers (N=663) vs. TT genotype (N=281), nor in the comparison of TC+TT (N=742) genotypes carriers vs. CC genotype (N=202) (Fisher Z transformation, p=0.45 and p=0.93 respectively, Supplement Table 2).

Discussion

This study demonstrates significant association of the SNP rs17518584 with performance in DSST, a measure for processing speed, in our sample of T2D patients, when controlling for relevant demographic variables, T2D-related characteristics and cardiovascular factors. This supports previous report of this SNP association with processing speed at GWAS significance level in a large-scale study (Ibrahim-Verbaas et al., 2016). Moreover, we show that this SNP is associated with language/semantic categorization performance, as well as executive function and overall cognition. The association with the cognitive domain of executive functions and with overall cognition does not seems to be dependent on rs17518584 involvement in DSST performance, as when the DSST is excluded from the analyses, the relationship is still significant for overall cognition, and at trend level for executive function.

Interestingly, in a meta-analysis of GWAS for general cognitive functioning in the CHARGE consortium, SNP rs17518584 did not reach GWAS significance level, but was the 95th SNP in order of significance ($p=1.34x10^{-6}$,defined as suggestive significance; approximately 53,900 participant) (Davies et al., 2015). However, the LDST/DSST were a component of the general

cognitive phenotype in some of the cohorts included this study (Davies et al., 2015). In addition, there was also partial overlap between the cohorts included in that study and the Ibrahim-Verbaas et al. (2016) study.

Taken together, our findings independently validate previous findings (Ibrahim-Verbaas et al., 2016), and expand the spectrum of cognitive domains associated with rs17518584. The results suggest that this association is relevant to the general elderly population, since adjusting for a plethora of T2D-related characteristics and cardiovascular factors in the analysis, did not affect the results.

Although the level of significance in our sample is modest (possibly due to the sample size limitation), the consistency of the same SNP and effect allele direction as in the original report (T allele is associated with better performance) and the use of the same cognitive test for processing speed (DSST)– strengthens our confidence in the true positive nature of our results.

This study has unique advantages such as the homogeneity of subject (Israelis from Jewish ancestry), and availability of data regarding possible demographic and clinical (based on multiple measurements) confounder variables, including data concerning glycemic control (mean HbA1c level). Among the limitations we should acknowledge the cross-sectional design of the study, and the lack of T2D free healthy controls, thus not enabling to study the modulating effect of T2D on the relationship of rs17518584 with processing speed.

Interestingly, although several GWAS for DSST were performed and published before the Ibrahim-Verbaas and collaborators study (2016), including one performed in a sample enriched with T2D patients, no clear associations at conventional GWAS level of significant ($p<5x10^{-8}$) was found (Cirulli et al., 2010; Cox et al., 2014; LeBlanc et al., 2012; Luciano et al., 2011). This may be due to the smaller sample size and probably limited statistical power, compared to the described GWAS meta-analysis (see introduction).

SNP rs17518584 is located upstream the *CADM2* gene (chromosome 3), but within an intron of a variant transcript that contains a different first exon. In bioinformatics analysis conducted as part of the original GWAS, this SNP was associated with *CADM2* RNA expression level in several brain regions, such as cingulate cortex, frontal cortex, general hemispheral cortex and more (Ibrahim-Verbaas et al., 2016). *CADM2* (also known as *SYNCAM2*) encodes synaptic cell adhesion molecule, and belongs to the immunoglobulin superfamily (Frei et al., 2014). The gene is widely expressed in developing and adult brain, and plays a role in maintaining synaptic circuitry (Thomas et al., 2008). Gene networks analysis (Ibrahim-Verbaas et al.,

2016) suggested involvement of CADM2 protein in glutamate signaling, gamma-aminobutyric acid (GABA) transport and neuronal cell-cell adhesion.

Recent GWAS showed association of this gene with attainment of a college or a university degree among 111,114 subjects (Davies et al., 2016). In addition, variants within *CADM2* were previously associated with several phenotypes, including BMI (Speliotes et al., 2010), risk of pediatric onset T2D (Miranda-Lora et al., 2017), lifetime cannabis use (Stringer et al., 2016), age at first sexual intercourse and risk taking propensity (Day et al., 2016). *CADM2* was suggested as a candidate gene to autism spectrum disorder (Casey et al., 2012). Therefore, this gene may be relevant to a broad range of phenotypes, including cognitive, metabolic and behavioral. Future studies at the neurobiological level are required to better understand *CADM2* involvement in processing speed, as well as other brain related phenotypes.

To conclude, our study supports previous findings regarding association of a genetic variant in *CADM2* with processing speed performance (as well as performance in additional cognitive phenotypes), and demonstrates this association among T2D patients, a population at high risk for cognitive decline.

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Table 1: Demographic and clinical description of the analyzed sample (overall and according to rs17518584 three genotype groups).

	Overall (N=944)			CC (N=202)		TC (N=461)		TT (N=281)		P value
	Mean	SD / %	range	Mean	SD / %	Mean	SD / %	Mean	SD / %	
Males	569	60.3%		115	56.9%	283	61.4%	171	60.9%	0.54
Age at cognitive evaluation (years)	71.7	4.6	65-85	71.7	4.5	71.6	4.7	71.9	4.5	0.67
Number of follow up years in the registry	9.7	4.4	0.33-19.9	10.2	4.2	9.7	4.5	9.4	4.4	0.17
Years of Education	13.2	3.5	0-26	12.9	3.5	13.2	3.6	13.3	3.4	0.42
Ashkenazi origin (N)	485	51.3%		95	47.0%	232	50.3%	158	56.2%	0.11
HbA1c (%, mean)	6.8	0.8	3.93-10.03	6.8	0.8	6.8	0.7	6.7	0.8	0.56
Total cholesterol (mg/dl, mean)	177.8	25.2	93.5-278.2	178.3	24.9	178.9	25.7	175.7	24.4	0.23
Creatinine (mg/dl, mean)	1.0	0.2	0.28-3.2	1.0	0.2	1.0	0.2	1.0	0.3	0.91
Triglyceride (mg/dl, mean)	156.8	64.2	41.4-914.2	160.5	62.4	157.2	62.4	153.6	68.4	0.50
BMI (kg/m2, mean)	28.3	4.2	18.1-50	28.9	4.3	28.1	4.2	28.2	4.3	0.077
Systolic BP (mmHg, mean)	134.4	9.8	99.9-171.4	134.8	9.3	134.4	10.0	134.1	9.7	0.74
Diastolic BP (mmHg, mean)	76.9	4.9	58.7-95.7	76.2	4.7	77.1	5.1	76.9	4.9	0.074
T2D medication										
No T2D medication (N)	52	5.5%		9	4.5%	29	6.3%	14	5.0%	
Hypoglycemic medication only (N)	749	79.3%		160	79.2%	357	77.4%	232	82.6%	
Insulin or Insulin plus hypoglycemic medication (N)	143	15.2%		33	16.3%	75	16.3%	35	12.4%	0.47

Values denote mean ± standard deviation, or number of subjects and percentage; P-values refer to one-way ANOVAs (parametric data) and Chi square tests (categorical data).

Abbreviations: BMI=Body mass index; BP=Blood pressure; HbA1c=Hemoglobin A1c; T2D=Type 2 diabetes; N=Number; SD=Standard deviation.

Table 2:

A. Performance in the DSST, cognitive domains and overall cognition, according to rs17518584 genotypes. For DSST, data is given as test score, while for all other cognitive measures, data is provided in summing of z scores.

	Overall (N=944)		CC (N=202)		TC (N=461)		TT (N=281)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DSST	34.27	10.6	32.81	11.0	34.14	10.6	35.54	10.3
Language/Semantic categorization	-0.04	2.4	-0.27	2.3	-0.14	2.4	0.30	2.4
Executive function	-0.02	3.0	-0.48	3.1	-0.01	3.0	0.30	3.0
Attention	0.00	2.2	-0.19	2.2	0.02	2.1	0.10	2.2
Episodic memory	-0.02	2.2	-0.07	2.3	-0.07	2.3	0.10	2.1
Overall cognition	-0.08	7.5	-1.02	7.5	-0.21	7.6	0.80	7.2
Executive function (without DSST)	-0.01	2.3	-0.33	2.3	0.01	2.3	0.19	2.3
Overall cognition (without DSST)	-0.07	6.8	-0.87	6.7	-0.18	6.9	0.69	6.5

B. Linear regression coefficients and P values for rs17518584 association with DSST, cognitive domains and overall cognition, adjusting for demographic covariates (sex, age at cognitive assessment, years of education and ancestry); additionally adjusted for T2D related characteristics (number of follow up years in the diabetes registry, mean HbA1c level and consumption of T2D medication); and additionally adjusted also for cardiovascular factors (systolic and diastolic blood pressure, BMI, creatinine, total cholesterol and triglycerides levels). B-unstandardized beta; SE-standard error for B; β-standardized beta.

	Adjusting for demographic variables			Additionally adjusting for T2D related characteristics			Additionally adjusting also for cardiovascular factors		
	B (SE)	β	P value	B (SE)	β	P value	B (SE)	β	P value
DSST	1.045 (0.419)	0.070	0.013	0.967 (0.416)	0.065	0.020	0.886 (0.418)	.059	.034
Language/Semantic categorization	0.234 (0.096)	0.069	0.015	0.226 (0.096)	0.067	0.019	0.214 (0.097)	0.063	0.027
Executive function	0.281 (0.116)	0.066	0.015	0.259 (0.115)	0.061	0.024	0.252 (0.116)	0.059	0.030
Attention	0.063 (0.090)	0.021	0.481	0.050 (0.090)	0.016	0.579	0.050 (0.091)	0.016	0.584
Episodic memory	0.104 (0.096)	0.033	0.277	0.097 (0.096)	0.031	0.311	0.101 (0.096)	0.032	0.297
Overall cognition	0.683 (0.277)	0.065	0.014	0.634 (0.275)	0.060	0.022	0.618 (0.278)	0.059	0.026
Executive function (without DSST)	0.184 (0.090)	0.057	0.042	0.169 (0.089)	0.053	0.059	0.170 (0.090)	0.053	0.060
Overall cognition (without DSST)	0.585 (0.254)	0.061	0.021	0.543 (0.253)	0.057	0.032	0.535 (0.255)	0.056	0.036