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Full-length Article

A genetic association study of *CSMD1* and *CSMD2* with cognitive function

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ABSTRACT

The complement cascade plays a role in synaptic pruning and synaptic plasticity, which seem to be involved in cognitive functions and psychiatric disorders. Genetic variants in the closely related *CSMD1* and *CSMD2* genes, which are implicated in complement regulation, are associated with schizophrenia. Since patients with schizophrenia often show cognitive impairments, we tested whether variants in *CSMD1* and *CSMD2* are also associated with cognitive functions *per se*. We took a discovery-replication approach, using well-characterized Scandinavian cohorts. A total of 1637 SNPs in *CSMD1* and 206 SNPs in *CSMD2* were tested for association with cognitive functions in the NCNG sample (Norwegian Cognitive NeuroGenetics; $n = 670$). Replication testing of SNPs with p -value < 0.001 (7 in *CSMD1* and 3 in *CSMD2*) was carried out in the TOP sample (Thematically Organized Psychosis; $n = 1025$) and the BETULA sample (Betula Longitudinal Study on aging, memory and dementia; $n = 1742$). Finally, we conducted a meta-analysis of these SNPs using all three samples. The previously identified schizophrenia marker in *CSMD1* (SNP rs10503253) was also included. The strongest association was observed between the *CSMD1* SNP rs2740931 and performance in immediate episodic memory (p -value = 5×10^{-6} , minor allele A, MAF 0.48–0.49, negative direction of effect). This association reached the study-wide significance level ($p \leq 1.2 \times 10^{-5}$). SNP rs10503253 was not significantly associated with cognitive functions in our samples. In conclusion, we studied $n = 3437$ individuals and found evidence that a variant in *CSMD1* is associated with cognitive function. Additional studies of larger samples with cognitive phenotypes will be needed to further clarify the role of *CSMD1* in cognitive phenotypes in health and disease.

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1. Introduction

Schizophrenia is a severe mental disorder which creates a considerable burden for the patients, their families and society (Robinson, 2011; Wittchen et al., 2011). In many patients, deficits in certain aspects of cognitive function have been observed (Kahn and Keefe, 2013). The heritability of schizophrenia is high

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(up to 75% Polderman et al., 2015). In the last decade many genetic variants implicated in schizophrenia have been identified (Purcell et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stefansson et al., 2008; International Schizophrenia Consortium, 2008), leading to a better understanding of the complexity of its genetic architecture. Recent studies have also shown that the genetic variants that are implicated in schizophrenia can influence cognitive abilities (Bulik-Sullivan et al., 2015; Lencz et al., 2014; McIntosh et al., 2013; Hill et al., 2014; Le Hellard et al., in press). Follow-up studies to determine the effect of these genes on cognitive functions may help us to understand the effect of genetic factors on cognitive dysfunction in schizophrenia, and vice versa. Recent genetic findings have implicated several biological pathways in schizophrenia, e.g. synaptic plasticity, the immune system and histone modifications (Network and Pathway Analysis Subgroup of the Psychiatric Genomics Consortium, 2015).

Synaptic plasticity is the ability of synapses to change over time. Several mechanisms are involved in synaptic plasticity, such as long-term potentiation, long-term depression and synaptic pruning. Synaptic pruning is the removal of unused synapses and is thought to be especially important for cognitive processes in the developing brain (Chung et al., 2015). The complement system, which plays a crucial role in the defense against pathogens in the immune system, is also involved in synaptic pruning (Stephan et al., 2012). It has been demonstrated that the complement cascade directs the tagging of obsolete synapses and their subsequent removal by microglial cells (Schafer et al., 2012). Pruning is essential for the development of the brain and of complex cognitive processes such as learning. In a rat model of synaptic plasticity, we observed up-regulation of several genes of the complement cascade following induction of long-term potentiation in the hippocampus (Havik et al., 2007). We subsequently performed a case-control association study to test genes of the complement cascade for their potential effect as genetic factors in schizophrenia. The strongest associations were observed for genetic variants located in the *CSMD1* and *CSMD2* genes (CUB and Sushi multiple domains 1, and CUB and Sushi multiple domains 2) (Havik et al., 2011). In a complementary hypothesis-free approach, the Psychiatric Genomics Consortium tested the whole genome for association (i.e. in a genome-wide association study, GWAS) with schizophrenia and identified, among other loci, additional variants located in *CSMD1* that were associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Ripke et al., 2011).

CSMD1 (OMIM ID 608397; 8p23.2) and *CSMD2* (OMIM ID 608398; 1p35.1) are described as regulators of complement activation and inflammation in the developing central nervous system (Kraus et al., 2006). Complement activation is gaining much attention because it is essential for synaptic plasticity and has recently been implicated in several brain-related conditions like schizophrenia (Sekar et al., 2016) and Alzheimer's disease (Hong et al., 2016). These findings have prompted further investigations of the *CSMD1* variant rs10503253, which is the SNP that showed the strongest association with schizophrenia in the PGC GWAS. Indeed, association has been reported between rs10503253 and several cognitive variables (Donohoe et al., 2013; Koiliari et al., 2014). Here, we tested genetic variants covering the whole *CSMD1* and *CSMD2* genes for association with cognitive abilities in three independent samples.

2. Methods and materials

The study utilizes a discovery-replication design. Data are first extracted from the discovery sample (NCNG, N = 670) by genome

wide genotyping, then tested for replication in two independent Scandinavian samples (BETULA, N = 1700 and TOP, N = 1025). A summary of the cohorts, the markers typed and the phenotypes analyzed is provided in Table 1. Informed consent was obtained from all participants.

2.1. NCNG sample

The NCNG (Norwegian Cognitive NeuroGenetics) sample, described fully in Espeseth et al. (2012), consists of 670 healthy individuals from whom genetic and cognitive data were collected. Participants aged 20–80 were recruited through advertisements in local newspapers in the Oslo and Bergen areas. Candidates with past or present neurological or psychiatric diseases or a history of substance abuse, learning deficits, or depression were excluded. Participants were required to be native speakers of Norwegian and to have completed basic education (mean education level is 14.2, SD 2.6). All participants gave their informed consent for participation, which included donation of a blood sample, DNA extraction and genotyping, and storage of the remaining blood sample in a biobank. All participants signed a statement of informed consent approved by the regional committee for Medical and Health Research Ethics (Southeast Norway; Project ID: S-03116).

For the present study, we selected six cognitive tests from the battery administered that would represent different domains of cognition. The six tests were chosen because we considered that they best represented each one of the different cognitive domains

Table 1

Details of the discovery sample (NCNG) and the replication samples (BETULA and TOP) used in this study. WASI: Wechsler Abbreviated Scale of Intelligence; CWIT, color-word interference test; D-KEFS, Delis-Kaplan Executive Function System.

	NCNG	BETULA	TOP
<i>Demographics</i>			
Sample size	670	1742	1025
% male	31.9	35.9	49.2
Mean age ± SD	47.6 ± 18.26	62.3 ± 13.3	36.2 ± 11.9
<i>Genotyping</i>			
Technology	Genome wide genotyping	Illumina iSelect INFINIUM	Genome wide genotyping and imputation
No. of <i>CSMD1</i> markers	1637	8	8
No. of <i>CSMD2</i> markers	206	3	3
<i>Cognitive abilities tested</i>			
Immediate episodic memory	California Verbal Learning Test-II: Total learning	Encoding and free recall of short sentences	California Verbal Learning Test-II: Total learning
Delayed episodic memory	California Verbal Learning Test-II: Recall	Delayed cued recall of nouns in sentences	California Verbal Learning Test-II: Recall
Processing speed	Third condition of CWIT from D-KEFS	Letter-digit substitution	Third condition of CWIT from D-KEFS
Semantic	knowledge	WASI-Vocabulary	Vocabulary
WASI-Visuospatial ability	Vocabulary	Block design test	WASI-Matrix Reasoning
Estimated IQ	WASI-Matrix Reasoning Vocabulary and Matrix Reasoning (WASI (32))	Estimated IQ from Vocabulary and the Block design test	Estimated from Block Design, Matrix Reasoning, Similarities and Vocabulary components of the WASI (32)

relevant to psychiatric disorders as reported in the literature (Toulopoulou et al., 2007; Bora et al., 2010; Simonsen et al., 2011), and because we could use a related test from the BETULA battery and the same test from the TOP sample. We analyzed: a) *immediate episodic memory*, measured with the California Verbal Learning Test II (CVLT II) total learning score; b) *delayed episodic memory*, measured with CVLT II – free recall after 30 min (Delis et al., 2000); c) *processing speed*, measured with the color-word inhibition condition of the Color-Word Interference Test (CWIT) which is part of the Delis-Kaplan Executive Function System (DKEFS) (Delis et al., 2001); d) *semantic knowledge* and e) *visuospatial ability* with the Vocabulary and the Matrix Reasoning subtests, respectively, from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999); and f) *estimated IQ*, with a general cognitive ability (IQ) score, derived from c) and d). For a more thorough description of the NCNG sample and tests, see Espeseth et al. (2012).

The whole sample underwent genome-wide genotyping using the Illumina Human610-Quad Beadchip (<http://www.illumina.com>). The quality control procedure is provided in Espeseth et al. (2012). Briefly, SNPs were filtered and excluded from the analysis if they had a call rate <0.95, minor allele frequency (MAF) <0.01 and Hardy-Weinberg Equilibrium (HWE) exact test $P < 0.001$.

Genotyped markers that covered the genes *CSMD1* and *CSMD2* were extracted from the genome-wide genotyping data (chr8: 2,792,875–4,852,328 and chr1: 33,979,599–34,631,443, respectively, in the hg19 build). *CSMD1* was covered by 1637 SNPs and *CSMD2* by 206 SNPs.

2.2. BETULA sample

The Betula Project is a longitudinal study, started in 1988, on aging, memory, and dementia (Nilsson et al., 1997). This sample consists of individuals that have been assessed for cognitive functions of memory, speed of processing, and attention. All participants signed informed consent, in accordance with the guidelines of the Swedish Council for Research in Humanities and Social Sciences. The measures of cognitive function used here were selected to allow comparison with the NCNG sample (see Table 1). The following tests were included: a) *immediate episodic memory*, measured by encoding and free recall of short sentences; b) *delayed episodic memory*, measured by delayed cued recall of nouns in sentences; c) *processing speed*, measured with a letter-digit substitution task; d) *semantic knowledge*, measured with a vocabulary word comprehension task; e) *visuospatial ability*, measured with a block design task where black and white cubes were to be arranged according to a given pattern; and f) *estimated IQ*; the IQ variable was estimated from the vocabulary and the block design tasks. All tasks are described in full detail in Nilsson et al. (1997). The mean education level for the BETULA sample is 10.2 years (SD 4.3).

We used a subset of 1800 BETULA samples for which DNA was available. Samples were genotyped for 3 markers in the *CSMD2* gene, and 8 markers in the *CSMD1* gene, which were selected due to their association in the NCNG sample. Genotyping was done with a custom Illumina Infinium iSelect custom design array (<http://www.illumina.com>). Samples were subjected to stringent quality control in PLINK (Purcell et al., 2007). SNPs were excluded from the analysis if they had a genotype success rate <0.95, MAF <0.01, or HWE exact test $P < 0.001$. After quality control of the iSelect genotyping data, 1742 individuals remained.

It should be noted that while the NCNG and TOP samples were filtered for cryptic relatedness and population structure, the BETULA sample used in this study was genotyped for fewer markers and thus could not be filtered for these parameters.

2.3. TOP sample

Participants were recruited as part of a large ongoing study on schizophrenia and bipolar disorder, the Thematic Organized Psychosis Research (TOP) Study, which is run by Oslo University Hospital, Norway (Athanasiu et al., 2010; Djurovic et al., 2010). The healthy participants were randomly selected from national statistical records from the same catchment area and contacted by letter inviting them to participate. The healthy sample was screened by interview for symptoms of severe mental illness and with the Primary Care Evaluation of Mental Disorders (PRIME-MD) (Spitzer et al., 1994), and subjects were excluded if they or any close relatives had a history of a severe psychiatric disorder (schizophrenia, bipolar disorder and major depression), or substance abuse or dependency in the last three months. Exclusion criteria for all groups were: IQ score below 70, hospitalized head injury, neurological disorder, unstable or uncontrolled medical condition that interferes with brain function (including hypothyroidism, uncontrolled hypertension and diabetes), and/or outside the age range 17–65 years. All participants gave written informed consent, and the study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, and the Biobank was approved by the Health Department.

A 3-h test battery (including measures of estimated premorbid IQ and adequate test effort) was administered in a fixed order with two breaks for refreshments. The battery of tests administered to the TOP participants is very similar to the battery administered to the NCNG participants. Therefore we selected the same measures: a) *immediate episodic memory* and b) *delayed episodic memory*, measured with the learning phase and the long delay recall, respectively, of the California Verbal Learning Test Second Edition (CVLT-II) (Delis et al., 2004); c) *processing speed*, measured with the 3rd condition of the DKEFS Color-Word Interference Test (Delis et al., 2005); d) *semantic knowledge*, measured with the vocabulary components of the WASI (Wechsler, 1999); e) *visuospatial ability*, measured with the Matrix Reasoning test of the WASI (Wechsler, 2007); and f) *estimated IQ*, derived from the Block Design, Matrix Reasoning, Similarities and Vocabulary components of the WASI (Wechsler, 1999, 2007). The mean education level for the TOP sample is 13.8 years (SD 2.9). Subjects from the TOP study included 312 schizophrenia patients, 240 patients diagnosed with bipolar disorder, 102 diagnosed with other psychosis and 323 healthy controls.

DNA samples collected from TOP participants were genotyped on the Affymetrix 6.0 array as previously reported (Athanasiu et al., 2010; Djurovic et al., 2010). 597,198 SNPs passed quality control filters. For SNPs that did not overlap with those on the Illumina array, or those genotyped in the other two samples, genotypes were further imputed with MACH (Li et al., 2010) (<http://www.sph.umich.edu/csg/abecasis/MACH/download/100G-PhaseI-Interim.html>) using the European sample in the Phase I release of the 1000 Genomes Project. Genotyping and imputation protocols are described in detail elsewhere (Finseth et al., 2014). Genotypes for the 11 markers (8 in *CSMD1* and 3 in *CSMD2*) selected from the association data in the NCNG sample, were extracted from the imputed TOP dataset.

2.4. Association analyses

The association of the SNPs with cognitive phenotypes was tested using linear regression performed in PLINK (Purcell et al., 2007). Age and sex were set as covariates except for the IQ measure, which had already been adjusted for age. In the TOP sample, since we used cases and controls, the diagnosis was also included as a covariate. In addition, all the markers were tested for associa-

tion in cases versus controls, but none showed any significance (min p -value 0.41).

The meta-analyses were performed for each trait using the inverse variance weighted model from the METAL software package (Willer et al., 2010), and overall measures of association were obtained.

We calculated the study-wide significance levels using PLINK to produce the correlation matrices and Nyholt's MatSpDLite (<http://neurogenetics.qimrberghofer.edu.au/matSpDLite/>) to determine the effective number of SNPs (Nyholt, 2004), and thus the study-wide correction threshold with the method of Li and Ji (2005). For the overall study (CSMD1 and CSMD2), the correlation matrix estimated that the total number of independent tests was 853, meaning a significance threshold at 6×10^{-5} . Using the same method to calculate the matrix correlation between the traits tested, we estimated that the effective Number of Independent Variables (VeffLi) was five, thus leading to a study-wide threshold value of 1.2×10^{-5} .

3. Results

The genomic organization of CSMD1 and CSMD2 is shown in Supplementary Fig. 1. In the NCNG sample, 1637 and 206 SNP variants located in CSMD1 and CSMD2, respectively, were tested for association with 6 cognitive measures using linear regression corrected for age and gender (full results are given in Supplementary Table 1). No marker reached genome-wide significance (p -value $< 5.0 \times 10^{-8}$). We selected markers with p -value < 0.001 for replication in the two independent samples (BETULA and TOP). Three markers in CSMD2 and 7 markers in CSMD1 met this criterion (see Supplementary Table 2). We also included the CSMD1 marker rs10503253, which was associated with schizophrenia in the PGC study (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), to test it for association in a meta-analysis of all three samples. In the BETULA sample, one marker (rs756094 in CSMD1) failed genotyping in an Illumina Infinium iSelect assay, while for the TOP sample, the genotypes for all the markers were used (see Supplementary Tables 3 and 4 for results in the BETULA and TOP samples respectively).

Tables 2–4 show the meta-analysis results for the selected markers across the 6 traits. The strongest association was observed for rs2740931 in CSMD1 (p -value = 5×10^{-6} , minor allele A, MAF 0.48–0.49) with immediate episodic memory (Table 2 and Supplementary Table 5). This passed the study-wide significance threshold of $p \leq 1.2 \times 10^{-5}$, as calculated using a correction based on the effective number of markers and traits tested (Nyholt, 2004; Li and

Ji, 2005). The association of rs2740931 with immediate episodic memory was further strengthened by the observation that all 3 samples show association with the same direction of effect, and the association was nominally significant in 2 of the 3 samples (NCNG $p = 0.0007$; BETULA $p = 0.0008$). Other nominally significant associations were observed but did not reach study-wide significance.

For the schizophrenia-associated marker rs10503253 in CSMD1, we did not observe any significant association across samples at the genome-wide or study-wide level. There was nominally significant association (p -value = 0.007; $\beta = 0.67$ for A allele; see Supplementary Tables 1 and 2) between this variant and matrix reasoning in the NCNG sample.

4. Discussion

Here we have tested genetic variants located in the CSMD1 and CSMD2 genes for their association with tasks representing several cognitive function domains. We found study-wide significant association between a genetic variant in the CSMD1 gene (rs2740931) and immediate episodic memory. We did not find significant association between cognitive abilities and rs10503253, which was the main signal of association between CSMD1 and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

In our study, we investigated the entire CSMD1 and CSMD2 genes, an approach that has advantages and disadvantages. The main disadvantage was that by testing more markers, we increased the risk of type 1 errors. This is especially true for larger genes, such as CSMD1 (2.0 MB) and CSMD2 (0.7 MB), which are more prone to variation than small genes. By the same token, however, larger genes are also more susceptible to functional variation. Thus, this gene-based approach has the advantage of allowing for allelic heterogeneity within and between the traits to be tested (Neale and Sham, 2004). We can therefore test if different markers in CSMD1 or CSMD2 have an effect on cognitive traits whether or not they have been identified as having an effect on schizophrenia. The principle of allelic heterogeneity is well documented in monogenic diseases where it has been shown that different independent variants within a gene can lead to similar phenotypic effects, but can also give rise to very different phenotypes. In brain-related traits, allelic heterogeneity is documented as having effects on gene expression (Ben-David et al., 2014), and on disorders such as Alzheimer disease (Ringman et al., 2014) and autism (Chen et al., 2015). Thus, it is important when considering a candidate gene for a phenotype that is related to the primary phenotype

Table 2

Meta-analysis of the association of CSMD1 and CSMD2 SNPs with immediate episodic memory and delayed episodic memory. For each SNP, the table shows the ID and the genomic location (hg19 build). For each trait, the table shows the number of individuals phenotyped (n), the z-score, the p -value and the direction of effect in the three samples (NCNG/BETULA/TOP). Bold text indicates SNPs with meta p -value < 0.05 . The asterisk indicates the CSMD1 SNP that reached study-wide significance (p -value $\leq 5.10^{-6}$). For all the markers described in this table, the alleles and allele frequencies in the different samples are reported in Supplementary Table 5. In the BETULA sample, marker rs756094 failed genotyping.

Gene	Marker	Chr	Position	Immediate episodic memory				Delayed episodic memory			
				n	z-score	p-value	Direction	n	z-score	p-value	Direction
CSMD2	rs12567192	1	34,270,994	3409	2.48	0.013	+++	3406	2.08	0.037	+++
	rs10798993	1	34,276,880	3409	2.54	0.011	+++	3406	2.15	0.031	+++
	rs1321623	1	34,306,705	3393	3.02	0.0025	+++	3390	1.83	0.067	+–+
CSMD1	rs7012625	8	3,717,935	3409	1.50	0.13	+++	3406	1.98	0.048	+–+
	rs756094	8	3,795,413	1673	–1.36	0.18	–?–	1673	0.03	0.97	–?+
	rs2740931	8	3,879,543	3408	–4.56	5×10^{-6}*	– – –	3405	–2.89	0.0039	– – –
	rs2740885	8	3,911,277	3408	–2.06	0.0399	– – –	3405	–1.15	0.25	– – –
	rs2740878	8	3,918,118	3409	1.56	0.12	+++	3406	0.65	0.52	+–+
	rs4304342	8	4,138,886	3409	0.79	0.43	+–+	3406	0.68	0.50	+–+
	rs10503253	8	4,180,844	3409	0.47	0.64	+–+	3406	–0.13	0.89	+–+
	rs2407592	8	4,610,345	3409	–0.57	0.57	+––	3406	–0.02	0.99	+––

Table 3Meta-analysis of the association of *CSMD1* and *CSMD2* SNPs with processing speed and visuospatial ability. For details, see Table 2.

Gene	Marker	Chr	Position	Processing speed				Visuospatial ability			
				n	z-score	p-value	Direction	n	z-score	p-value	Direction
CSMD2	rs12567192	1	34,270,994	2543	1.37	0.17	–++	3404	–0.41	0.68	++–
	rs10798993	1	34,276,880	2543	1.31	0.19	–++	3404	–0.36	0.72	++–
	rs1321623	1	34,306,705	2537	0.81	0.42	–++	3388	–0.06	0.96	++–
CSMD1	rs7012625	8	3,717,935	2543	–1.00	0.32	+––	3404	1.94	0.052	+++
	rs756094	8	3,795,413	1669	0.05	0.96	–?+	1671	–2.07	0.039	–?–
	rs2740931	8	3,879,543	2543	–0.68	0.50	+––	3403	–0.93	0.35	– – –
	rs2740885	8	3,911,277	2543	0.94	0.35	++–	3403	–2.37	0.018	– – –
	rs2740878	8	3,918,118	2543	–0.62	0.54	– – +	3404	2.42	0.015	+++
	rs4304342	8	4,138,886	2543	0.52	0.60	+++	3404	–0.22	0.82	+––
	rs10503253	8	4,180,844	2543	0.89	0.38	– – +	3404	0.67	0.50	++–
	rs2407592	8	4,610,345	2543	–0.14	0.89	–++	3404	0.43	0.67	++–

Table 4Meta-analysis of the association of *CSMD1* and *CSMD2* SNPs with semantic knowledge and IQ. For details, see Table 2.

Gene	Marker	Chr	Position	Semantic knowledge				Estimated IQ			
				n	z-score	p-value	Direction	n	z-score	p-value	Direction
CSMD2	rs12567192	1	34,270,994	3410	0.20	0.84	+––	3401	0.74	0.46	+–+
	rs10798993	1	34,276,880	3410	0.28	0.78	+––	3401	0.84	0.40	+–+
	rs1321623	1	34,306,705	3394	–1.10	0.27	+––	3385	–0.13	0.90	+––
CSMD1	rs7012625	8	3,717,935	3410	–0.68	0.50	+–+	3401	0.32	0.75	+–+
	rs756094	8	3,795,413	1668	–1.97	0.049	–?–	1670	–2.92	0.0036	–?–
	rs2740931	8	3,879,543	3409	–1.87	0.06	– – –	3400	–2.05	0.040	– – –
	rs2740885	8	3,911,277	3409	–1.67	0.10	– – +	3400	–2.57	0.010	– – –
	rs2740878	8	3,918,118	3410	1.54	0.12	++–	3401	2.22	0.026	+++
	rs4304342	8	4,138,886	3410	0.46	0.64	+++	3401	0.19	0.85	+–+
	rs10503253	8	4,180,844	3410	–0.06	0.95	+–+	3401	0.49	0.62	+–+
	rs2407592	8	4,610,345	3410	–0.39	0.70	+––	3401	–0.63	0.53	+–+

to include the possibility that the secondary phenotype could be influenced by other variants in the gene. Here, we observed an association between rs2740931 in *CSMD1* with the learning phase of the verbal memory test, and this SNP is independent of (i.e. not in linkage disequilibrium with) the schizophrenia-associated variant. Other studies have also reported association between *CSMD1* genetic variants, which are not correlated with rs10503253, and other traits that are relevant to a role of this gene in brain function and dysfunction. For example, at the brain imaging level, associations have been reported between genetic variants in *CSMD1* and brain fiber tracts (Giddaluru et al., 2016) or the default mode network (Meda et al., 2014). For psychiatric disorders, associations of *CSMD1* with bipolar disorder (Xu et al., 2014) and cognitive decline in schizophrenia (Hashimoto et al., 2013) have also been reported independent of rs10503253 in other studies. Genetic variants of *CSMD1* have also been reported to be associated with cannabis dependence (Sherva et al., 2016). Finally, rare variants in *CSMD1* have been implicated in autism (Cukier et al., 2014). Taken together, these observations and the results of our study do suggest that several independent variants in the *CSMD1* gene might be implicated in different brain-related phenotypes. It is however noteworthy that the same SNP, rs2740931, showed an association with autism (p -value = 5.38×10^{-5}), which did not reach genome-wide significance in the GWAS on autism reported by Anney et al. (2010).

Previous studies have reported association between rs10503253 in *CSMD1* and cognitive traits. Koiliari et al. (2014) found association with IQ, strategy formation, planning and set shifting in their sample ($N = 1149$). Donohoe et al. (2013) found association with Verbal IQ and logical memory in patients, and performance and full scale IQ in a cognitive trait x psychiatric trait interaction model ($N = 1296$ in total). Since both studies looked at only one SNP, the significance threshold criterion that was used

in those studies is not as stringent as the one we have applied here. At the nominal significance level, rs10503253 did show association with matrix reasoning in one of our samples (NCNG, $N = 670$), but this effect was not replicated and validated in the other samples. The studies of both Koiliari et al. (2014) and Donohoe et al. (2013) looked at samples smaller than the ones we analyzed here, which might explain why we did not replicate their findings in our study. Another possible explanation for these inconsistencies is that the phenotypes we tested here do not fully overlap with those tested by Koiliari et al. and Donohoe et al. Therefore further investigations in bigger samples will be required to resolve whether the schizophrenia-associated SNP rs10503253 is also associated with cognitive abilities, and, if so, which ones. It will also be valuable to test our markers in other samples to see if our results are replicated in other cohorts.

CSMD1 is a highly complex gene, which is composed of 48 exons and covers 2 Mb of DNA (see Supplementary Fig. 1). At least 8 isoforms have been reported. It is ubiquitously expressed in the body and has been associated with a wide range of diseases such as colon cancer (Farrell et al., 2008), Kawasaki disease (Burgner et al., 2009) and asthma (Hardin et al., 2014), reflecting its probable ubiquitous function. The association of this gene across disorders might be partially due to its large size which makes it prone to type I errors in genetic studies but also more prone to genetic variation of functional relevance. The function of *CSMD1* in the brain is not yet clear but there is some evidence that, as part of the brain complement cascade, it is implicated in the pruning of synapses. *CSMD1* encodes a transmembrane protein containing 15 consecutive complement control protein (CCP) domains, which are characteristic of complement inhibitors. The *CSMD1* protein has been shown to inhibit deposition of C3b (Escudero-Esparza et al., 2013). In mouse models of Alzheimer's disease, it has been shown that the complement-mediated clearing of synapses by microglia might be defective

(Hong et al., 2016). It was shown recently that the strongest association with schizophrenia observed by the PGC GWAS could be attributed to a variation in the number of copies of the complement C4 gene, which mediates synapse elimination during postnatal development (Sekar et al., 2016). CSMD1 promotes the degradation of the activated complement proteins C3b and C4b (Escudero-Esparza et al., 2013), although further work is needed to demonstrate how this affects synaptic pruning. It is now clear that the complement cascade plays an important role in synaptic pruning and is implicated in many traits that are dependent on synaptic plasticity, including cognitive functions and psychiatric and neurodegenerative disorders. As neuroimmunological studies are beginning to implicate the immune system in cognitive functions and dysfunctions, it will be interesting in the future to test whether immune markers and biomarkers may be related to cognition, we could not perform such an analysis with the data available to us here.

The SNP associated in our study, rs2740931, is located in intron 4 of the long transcript of CSMD1 near a region that is transcribed but not translated in the brain, as shown by RNAseq data from Maunakea et al. (2010) displayed in the UCSC genome browser (<https://genome-euro.ucsc.edu/>). This region was also sequenced by Maunakea et al. (2010) after chromatin immunoprecipitation (ChIP-seq) for the H3K4me2 modification, which is usually associated with gene activation. Thus it is possible that rs2740931 is in a brain-specific region of transcription but not translation. However, the sequencing was performed in one individual and further work will be needed to characterize this potential functional effect. Further investigation of this SNP and SNPs in LD with it, using Haploreg (http://archive.broadinstitute.org/mammals/haploreg/haploreg_v4.php) (Ward and Kellis, 2016), Braineac for brain eQTLs (<http://www.braineac.org>) (Ramasamy et al., 2014) or GTEx for other eQTLs (<http://www.gtexportal.org/home/>) (Consortium GT, 2015), did not provide further information on potential eQTLs in this region or other functional genetic variants.

An advantage of our study is that it contains only Scandinavian samples, which increases the homogeneity and thus the chance to identify genetic association (Pei et al., 2014); however, our study is still limited compared to the large sample sizes that genetic consortia can obtain. Until now, large multi-center meta-analyses of GWAS have been reported only for general cognitive abilities (e.g. from the CHARGE (Davies et al., 2015) or the COGENT (Lencz et al., 2014) consortia), verbal declarative memory delayed recall (Debette et al., 2015) and executive functioning – speed of processing (Ibrahim-Verbaas et al., 2016). We will probably have to wait until other consortia gather large samples phenotyped for additional cognitive abilities to cover a larger range of domains. However, another major challenge when collecting large samples to identify genes implicated in cognitive abilities, which is also a limitation of our study, is the reliability with which the phenotype can be compared across samples. Many different batteries of tests are used by different groups, and only a few studies have compared the test-to-test correlation of the different measures in one sample. However, several studies have shown that at a higher level, when common domains or general factors are derived from different batteries, their correlation to the domain and general factors is high (Johnson et al., 2004, 2008). Genetic variants in CSMD1 were not associated with the general factor of cognition in our study or in any of the larger studies published for general cognition (i.e. CHARGE Davies et al., 2015 and COGENT (Lencz et al., 2014)) even at more relaxed levels of significance as reported in the Supplementary tables. In our study, the genetic variant associated with immediate episodic memory shows some association with delayed recall, but not at the study-wide significance level. This variant was not associated with delayed recall performance in the GWAS of Debette et al. (2015). The observation of association with the

immediate or learning phase of a memory task and not with the delayed phase is in line with the observation by Luksys et al. that distinct genetic profiles might underlie specific processes of human episodic memory (Luksys et al., 2015). This supports the current efforts of consortia to perform large GWAS on a wider range of cognitive phenotypes.

5. Conclusion

We have presented evidence that a variant in CSMD1 is associated with cognitive function. It is an exciting time for genetic studies of cognition and mental disorders, because biological mechanisms are being revealed which will help to prove or refute old biological hypotheses. However, it is also a challenging time, because we are only beginning to understand the breadth of the multi-dimensional complexity of the genetic (and epigenetic) mechanisms that account for inter-individual variability in brain function. In due course, larger-scale studies will help solve these problems.

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Conflict of interest statement

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.bbi.2016.11.026>.

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