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Genome-wide association study identified INSC gene associated with Trail Making Test Part A and Alzheimer's disease related cognitive phenotypes

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ABSTRACT

Background: The Trail Making Test (TMT) Part A (TMT-A) is a good measure of performance on cognitive processing speed. This study aimed to perform a genome-wide association study of TMT-A in Alzheimer's disease (AD).

Methods: A total of 757 individuals with TMT-A phenotypes and 620,901 single nucleotide polymorphisms (SNPs) were extracted from the Alzheimer's Disease Neuroimaging Initiative 1 (ADNI-1) cohort. AD related cognitive phenotypes include TMT-A, TMT-B, Functional Activities Questionnaire (FAQ), Clinical Dementia Rating Sum of Boxes (CDR-SB), and Alzheimer's Disease Assessment Scale–Cognitive Subscale 13 (ADAS13). Multivariable linear regression analysis of TMT-A was conducted using PLINK software. The most TMT-A associated gene was tested with Color Trails Test 1 Form A (CTTA), a culturally fair analog of the TMT-A. Functional annotation of SNPs was performed using the RegulomeDB and Genotype-Tissue Expression (GTEx) databases.

Results: The best signal with TMT-A was rs1108010 ($p = 4.34 \times 10^{-8}$) at 11p15.2 within INSC gene, which was also associated with TMT-B, FAQ, CDR-SB, and ADAS13 ($p = 2.47 \times 10^{-4}$, 8.56×10^{-3}, 0.0127 and 0.0188, respectively). Furthermore, suggestive loci were identified such as FOXD2 and CLTA with TMT-A, GBP1/GBP3 with TMT-B, GRIK2 with FAQ, BAALC and CCDC146 with CDR-SB, BAALC and NKAIN2 with ADAS13. Additionally, the best SNP within INSC associated with CTTA was rs7931705 ($p = 6.15 \times 10^{-5}$). Several SNPs had significant eQTLs using GTEx.

Conclusions: We identified several genes/loci associated with TMT-A and AD related phenotypes. These findings offer the potential for new insights into the pathogenesis of cognitive function and Alzheimer's disease.

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[1] Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia that affects memory, thinking and behavior developed from multiple factors, such as genetics, lifestyle, and environment (Weller and Budson, 2018). Globally, over 50 million people are living with AD and other dementias (*Alzheimer's Association*, 2020). To date, there is no specific treatment to cure Alzheimer's disease. Thus, as the disease progresses, a person with AD will develop cognitive decline and lose the ability to maintain their activities of daily living (Graff-Radford and Lunde, 2020). Several tests have been developed to assess the progression of AD and identify pathways to slow the progress of the disease.

The Trail Making Test (TMT) is commonly used in neuropsychological assessments for cognitive function in link to visual information processing (Fellows et al., 2017; Nikolova et al., 2015; Reitan and Wolfson, 1985a; Sun et al., 2020). The first part A (TMT-A) tests perceptual motor skill of visual search speed and tracking (Fellows et al., 2017; Nikolova et al., 2015; Sun et al., 2020). The second part (TMT-B) assesses executive function including working memory and task-switching abilities (Fellows et al., 2017; Terada et al., 2013; Tombaugh, 2004). The TMT-A and TMT-B are correlated ($r = 0.66$) (Corrigan and Hinkeldey, 1987). Poor performance in both TMT-A and TMT-B are associated with impaired cognitive function in AD and aging (Knapstad et al., 2019; Nikolova et al., 2015; Sun et al., 2020), affecting mnemonic structures such as anterior cingulate, thalamus and striatum (e.g., Terada et al., 2013). The Color Trails Test (CTT) was considered as a test of intention of minimizing the cultural bias and provides a reliable cognitive measure (D'Elia et al., 1996). CTT tests effortful executive processing abilities relative to the TMT and there is no difference in performance on the CTTA and TMT-A, suggesting functionally equivalent performance across both tasks (Dugbartey et al., 2000).

Family and twin studies have indicated that genetic and environmental factors and their interactions contribute to processing speed and TMTs (Ibrahim-Verbaas et al., 2016; Ising et al., 2014), with a heritability of 0.24 to 0.90 for information processing speed (Vernon, 1989) and 0.42 for TMT (Lee et al., 2012). Furthermore, other studies estimated the heritability for TMT-A between 0.23 and 0.38 (Knowles et al., 2014; Vasilopoulos et al., 2012).

The genome-wide association study (GWAS) is a powerful tool for unlocking the genetic basis of complex diseases and over 30 genetic loci have been found to affect AD, its age of onset and disease severity and related phenotypes (Lambert et al., 2013; Neuner et al., 2020; Robinson et al., 2017). However, few GWAS have focused on TMT test. A GWAS in Germany population found a genetic variant in the DSG1 gene region and TMT-A performance predominantly reflecting visual processing speed (Ising et al., 2014). Another GWAS found 12 single nucleotide polymorphisms (SNPs) of several genes/loci (such as METAP1 and ADH5) associated with TMT-A (Ibrahim-Verbaas et al., 2016). Recently, a GWAS in UK Biobank identified three genes (CRNKL1, CASOP5 and NAA20) significantly associated with TMT-A (Hagenaars et al., 2018). However, no GWAS has been done on the genetic loci underlying TMT-A test performance in AD. In this study, we performed a GWAS for TMT-A in AD using the data of the Alzheimer's Disease Neuroimaging Initiative (ADNI) and identified a novel gene, inscuteable (INSC) that is associated with TMT-A and AD-related phenotypes. Moreover, we also validated the INSC gene in association with CTTA, which is similar to TMT-A, both test effortful executive processing abilities (Dugbartey et al., 2000) using the data from The Columbia University Study of Caribbean Hispanics with Familial and Sporadic Late Onset Alzheimer's disease-dbGaP Study Accession.

2. Materials and methods

2.1. The ADNI sample

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. The ADNI is an ongoing, longitudinal, multicenter study designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. There was an Institutional Review Board exemption for current study due to secondary data analysis.

2.2. Measurements

2.2.1. Subjects and GWAS genotyping

Data of 800 individuals, 200 patients with early AD, 400 with MCI, and 200 with cognitive normal as controls (CN) were extracted from the ADNI cohort 1. A total of 620,901 SNPs were genotyped using DNA samples obtained from the blood of studied subjects. APOE genotyping was performed using an APOE genotyping kit, as described in http://www.adni-info.org/Scientists/Pdfs/adniproceduresmanual12.pdf (also see http://www.adni-info.org for detailed information on blood sample collection, DNA preparation, and genotyping methods). APOE-ε4 carriers were defined as individuals with at least one ε4 allele while non-carriers were defined as individuals with no ε4 allele (APOE-ε4-0) (Table 1). Social-demographic factors included gender, age, race, and educational level. Gender was self-reported as either male or female. Age and years of education were considered as continuous variables. Race consisted of four subgroups: non-Hispanic White, non-Hispanic African American, Hispanic, and others.

2.2.2. Neuropsychological assessment

The TMT is a test of processing speed and executive function. The TMT-A is a test of psychomotor processing speed and visual scanning. An array of numbers on a page is presented to the subjects and they are

Table 1
Descriptive statistics.

	ADNI sample (N = 755)	Caribbean Hispanics sample (N = 1354)
Variable	N (%)/mean ± SD	N (%)/mean ± SD
Gender (%, female)	308 (40.8%)	912 (67.4%)
APOE-ε4 (%, ε4)	374 (49.5%)	–
Age (year)	75.20 ± 6.72	73.63 ± 8.45
Education (year)	15.55 ± 3.05	7.86 ± 5.04
Race		
White	684 (90.6)	–
Hispanic	17 (2.3%)	1354 (100%)
African American	36 (4.8%)	–
Other	18 (2.4%)	–
Diagnosis		
CN	214 (28.3%)	1050 (77.5%)
AD	173 (22.9%)	304 (22.5%)
MCI	368 (48.8%)	–
TMT-A	47.61 ± 27.08	–
CCTA	–	13.79 ± 21.68
TMT-B	133.90 ± 80.32	–
FAQ	4.90 ± 6.50	–
CDR-SB	1.77 ± 1.83	–
ADAS13	18.36 ± 9.19	–

Abbreviations: CN: Cognitive Normal; AD: Alzheimer Disease; MCI: Mild Cognitive Impairment; TMT-A: Trail Making Test Part A; CCTA: Color Trails Test 1 Form A; TMT-B: Trail Making Test Part B; FAQ: The Functional Activities Questionnaire; CDR-SB: The Clinical Dementia Rating Scale Sum of Boxes; ADAS13: The Assessment Scale-cognitive subscale 13.

instructed to draw lines connecting the numbers in sequential order within the time allowed. The TMT-B provides cognitive flexibility measures: psychomotor processing speed, visual scanning, and attentional set shifting. An array of numbers and letters is presented to the subjects and they are asked to draw connecting lines while alternating between numbers and letters in sequential order. Although both TMT-A and TMT-B depend on visuomotor and perceptual-scanning skills, TMT-B also requires considerable cognitive flexibility in shifting from number to letter sets under time pressure. The TMT-A score is a measure of cognitive processing speed and the TMT-B test score is a measure of executive functioning. The higher scores of TMT-B indicate greater impairment (Reitan and Wolfson, 1985b; Reitan and Wolfson, 1995). The Functional Activities Questionnaire (FAQ) measures activities of daily living and the higher total scores represent increased disability (Pfeffer et al., 1982). The Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) was utilized to examine the global cognition of participants. A higher CDR-SB score indicates a more severe degree of cognitive deficits (Morris, 1993). The Assessment Scale-cognitive subscale 13 (ADAS13) is a 13-item cognitive test and higher scores reflect poorer cognitive performance (Mohs et al., 1997).

2.3. Statistical analyses

2.3.1. Genome-wide association analysis

For the initial GWAS analysis, HelixTree Software (http://www.goldenhelix.com/SNP_Variation/HelixTree/index.html) was used to assess control genotype data for conformity with Hardy-Weinberg equilibrium (HWE). To deal with population stratification, the principal-component analysis (PCA) approach (Price et al., 2006) in HelixTree was used. Then, multivariate linear regression analysis of TMT-A as a continuous trait was conducted using PLINKv1.9 (www.cog-genomics.org/plink/1.9/), adjusted for gender, age, education, AD status, racial group, APOE genotype and first three principal components (PCs) (Chang et al., 2015). The same procedure was performed for TMT-B, FAQ, CDR-SB, and ADAS13.

2.3.2. Multiple testing

For statistical significance, we used a very conservative per test significance level of $\alpha = 5 \times 10^{-8}$ (Dudbridge and Gusnanto, 2008; Pe'er et al., 2008). At the same time, we also used a less stringent criterion of "suggestive association" with a cut-off of $\alpha = 10^{-4}$.

2.3.3. Haploblock and haplotype analysis

Pairwise linkage disequilibrium (LD) statistics (D′) were assessed for controls and haplotype blocks were constructed using HAPLOVIEW software (Barrett et al., 2005). Haplotype analysis of TMT-A was performed using the multivariate linear model using the PLINK v1.07 (Purcell et al., 2007). The asymptotic *p*-values for the linear regression models were observed while the regression coefficient (B) and its standard error were estimated using PLINK v1.07.

2.4. Association analysis of CTTA in Caribbean Hispanics sample

To validate the association of INSC gene, we used data from The Columbia University Study of Caribbean Hispanics with Familial and Sporadic Late Onset Alzheimer's disease-dbGaP Study Accession: phs000496.v1.p1 (Lee et al., 2011) to examine the association of INSC gene with CTTA. There are 326 SNPs within INSC gene available. There are 1354 individuals with CTTA. Multivariate linear regression analysis of CTTA as a continuous trait was conducted using PLINKv1.9 (www.cog-genomics.org/plink/1.9/), adjusted for gender, age, and education.

2.5. Functional analysis in silico

We evaluated whether these identified associated variants were located in regions of the genes that might have potential functional importance using three online functional prediction websites: SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), RegulomeDB (http://regulomedb.org/), and the Genotype-Tissue Expression (GTEx) (www.gtexportal.org). First, the sequences containing the associated SNPs were examined for microRNA binding sites, splicing sites, regulatory gene regions, and species-conserved regions using NIH-SNP Function Prediction (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi). Second, we examined the potential functional consequences of associated SNPs in RegulomeDB (Boyle et al., 2012), which has a self-developed score system with a score ranging from 1 to 7. A higher score indicates less functional significance. Third, the eQTLs of associated SNPs were assessed by the GTEx database.

3. Results

3.1. Genotype quality control and demographics

After removing SNPs with HWE $p < 0.0001$ or call rates <95% or minor allele frequency (MAF) <1%, 534,934 SNPs remained. After merging GWAS SNPs and phenotypes, 755 individuals with TMT-A phenotype and GWAS genotypes were left including 173 individuals with AD, 368 with MCI, and 214 with CN (Table 1). Among studied population, 40.8% were females, 49.5% had at least one APOE-ε4 allele, 90.6% are non-Hispanic White, 2.3% are Hispanic and 4.8% are non-Hispanic African American. Mean values and standard deviations for TMT-A, TMTB, FAQ, CDR-SB, and ADAS-13 are also presented in Table 1. Due to skewed distribution of TMT-A, log transformation for TMT-A was performed for further analysis (Fig. 1).

3.2. Genome-wide association analyses in the ADNI sample

Through GWAS we identified 66 SNPs associated with TMT-A with $p < 10^{-4}$ (Supplementary Table S1). Table 2 shows the top three findings for each of these phenotypes: TMT-A, TMT-B, FAQ, CDR-SB and ADAS13. A more comprehensive list of SNPs associated with TMT-A, TMT-B, FAQ, CDR-SB and ADAS13 with *p* values $<10^{-5}$ is presented in Supplementary Table S2. The best signal for TMT-A was found at rs1108010 ($p = 4.34 \times 10^{-8}$) within the INSC gene, which was associated with the performance scores for cognitive tests TMT-B, FAQ, CDR-SB, and ADAS13 ($p = 2.47 \times 10^{-4}$, 8.56×10^{-3}, 0.0127 and 0.0188, respectively). The second interesting locus for TMT-A was rs2165194 ($p = 1.71 \times 10^{-6}$) within the FOXD2 gene, which was also associated with the above tests TMT-B, FAQ, and CDR-SB ($p = 0.0137$, 0.0165 and 0.0172, respectively) except for ADAS13. In addition, rs2025879 within clathrin light chain (CLTA) gene were associated with TMT-A ($p = 2.87 \times 10^{-6}$). Regarding additional phenotypes, two SNPs (rs6675866 and rs6428496) within GBP1 gene were associated with TMT-B ($p = 2.33 \times 10^{-6}$ and 8.48×10^{-6}, respectively). The top SNP with FAQ was rs4840200 within GRIK2 ($p = 1.22 \times 10^{-6}$). Furthermore, two CRD-SB associated SNPs (rs10955311 and rs1779335) ($p = 9.40 \times 10^{-8}$ and 4.22×10^{-7}) within BAALC gene were also associated with TMT-A, TMT-B, FAQ and ADAS13 (Table 2). Additionally, the SNP rs6569364 within NKAIN2 was associated with ADAS13 ($p = 3.84 \times 10^{-6}$).

3.3. SNPs within INSC gene in the ADNI sample

There are 186 SNPs within INSC gene, of which 17 SNPs within INSC gene were associated with TMT-A ($p < 0.05$). A more comprehensive list of all 186 SNPs is presented in Supplementary Table S1. Table 3 lists the top 10 findings within INSC gene. In Table 3, there were three SNPs (rs1108010, rs1792549 and rs12800439), which were located within INSC gene with $p < 10^{-4}$. In addition, six SNPs of this gene were associated with TMT-B, six SNPs associated with FAQ, three SNPs associated with CRD-SB and three SNPs associated with ADAS13 ($p < 0.05$).

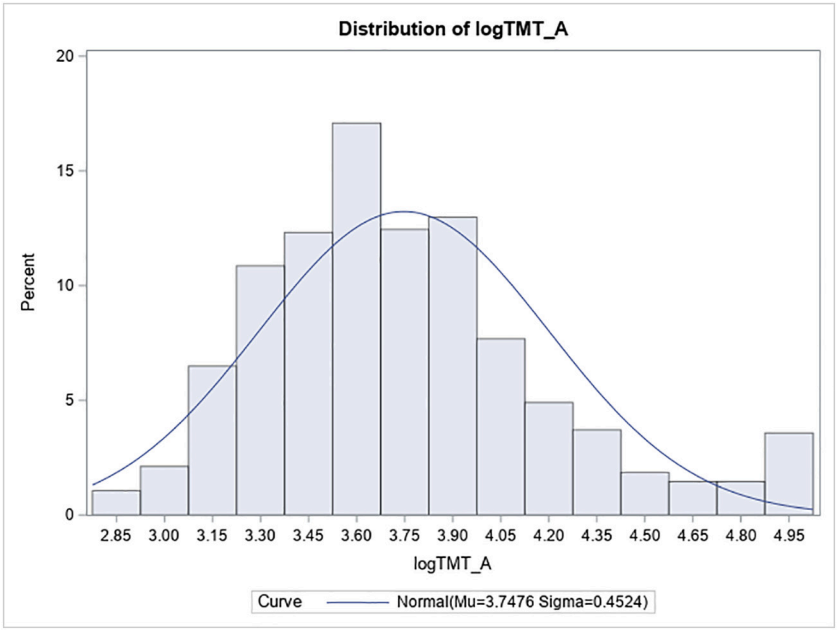

Fig. 1. Histogram of log transformed Trail Making Test (TMT) Part A.

3.4. Haploblock and haplotype analysis of INSC gene in the ADNI sample

Using HAPLOVIEW, we identified 3 haplotype blocks within INSC gene including the flanking SNPs of best hit (rs1108010). LD structure is illustrated in Fig. 2. Two-SNP haplotype analyses based on PLINK revealed that the A-T haplotype inferred from rs1629709 and rs11108010 ($D' = 1.0$) was associated with TMT-A ($p = 6.07 \times 10^{-8}$) and T-T haplotype inferred from rs1108010 and rs1540154 ($D' = 0.84$) was associated with TMT-A ($p = 1.53 \times 10^{-7}$) (Table 4).

3.5. Association analysis of INSC gene with CTTA in the Caribbean Hispanics sample

To validate above findings in an independent sample, we tested associated INSC gene with CTTA. Table 5 shows 6 SNPs within the INSC gene associated with CTTA in the Caribbean Hispanics sample. The best SNP associated with CTTA was rs7931705 ($p = 6.15 \times 10^{-5}$). A more comprehensive list of SNPs with $p < 0.05$ is presented in Supplementary Table S3.

3.6. In silico analysis

We evaluated whether these associated variants were located in regions of the gene that might have potential functional importance. The sequences containing the associated SNPs were examined for microRNA binding sites, splicing sites, regulatory gene regions, and species-conserved regions using NIH-SNP Function Prediction (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi). We found rs17793351 in BAALC gene (Table 2), rs1945628 in INSC gene (Table 3), rs6486235 and rs7931705 in INSC gene (Table 5) may have regulatory potential

Table 2
Top 14 associated SNPs in the genome-wide association studies in the ADNI sample. Bold signifies the P values of top 3 SNPs for each outcome.

CHR	SNP	Position	Gene	DB[a]	MA[b]	MAF[c]	HW[d]	P_TMT-A[e]	P_TMT-B[f]	P_FAQ[g]	P_CDR-SB[h]	P_ADAS13[i]
11	rs1108010	15,173,268	INSC	4	T	0.32	0.961	**4.34E-08**	2.47E-04	8.56E-03	1.27E-02	1.88E-02
1	rs2165194	47,989,563	FOXD2	4	G	0.41	0.355	**1.71E-06**	1.37E-02	1.65E-02	1.72E-02	0.076
9	rs2025879	36,194,455	CLTA	5	A	0.32	0.709	**2.87E-06**	4.63E-02	0.847	0.582	4.07E-02
1	rs6675866	89,290,398	GBP1/GBP3/GBP7	5	T	0.20	0.387	7.59E-05	**2.33E-06**	0.01871	0.009455	0.004223
1	rs11803889	170,010,198	DNM3/METTL3	7	A	0.21	0.188	0.006442	**3.98E-06**	0.05849	0.008232	0.04409
1	rs6428496	89,283,335	GBP1/GBP3/GBP7	5	T	0.19	0.511	0.0004804	**8.48E-06**	0.03583	0.02611	0.00581
6	rs4840200	102,433,996	GRIK2	5	C	0.03	0.006	0.7281	0.3146	**1.22E-06**	0.0183	0.2326
9	rs10114675	81,949,283	LOC347119	4	A	0.13	0.308	0.2083	0.03746	**1.40E-06**	0.002214	0.0024
18	rs1461705	38,149,629	LOC284260	6	T	0.39	0.207	0.005777	0.02413	**2.27E-06**	0.0004478	0.0002637
8	rs10955311	104,247,753	BAALC	5	C	0.12	0.061	0.01403	0.002304	1.09E-05	**9.40E-08**	**4.51E-06**
8	rs17793351	104,258,033	BAALC	5	A	0.12	0.092	0.0152	0.00379	2.75E-05	**4.22E-07**	4.38E-05
7	rs996251	76,682,808	CCDC146	7	G	0.02	0.023	0.01583	0.02898	6.36E-05	**2.32E-06**	4.98E-05
6	rs6569364	124,199,951	NKAIN2	5	A	0.22	0.230	0.1226	0.001507	0.0004875	0.0004764	**3.84E-06**
17	rs9890008	14,412,418	LOC388339	7	A	0.25	0.226	0.03024	0.0267	0.008303	0.0009398	**4.92E-06**

[a] RegulomeDB score;
[b] Minor allele;
[c] Minor allele frequency;
[d] Hardy-Weinberg equilibrium *p*-value;
[e] *p*-value based on linear regression for LogTMT-A;
[f] *p*-value based on linear regression for TMT-B;
[g] *p*-value based on linear regression for FAQ;
[h] *p*-value based on linear regression for CRD-SB;
[i] *p*-value based on linear regression for ADAS13.

Table 3
Top 10 SNPs associated with TMT-A within INSC gene in the ADNI sample.

CHR	SNP	Position	DB[a]	MA[b]	MAF[c]	HW[d]	P_logTMT-A[e]	P_TMT-B[f]	P_FAQ[g]	P_CDR-SB[h]	P_ADAS13[i]
11	rs1108010	15,173,268	4	T	0.32	0.961	4.34E-08	2.47E-04	8.56E-03	1.27E-02	1.88E-02
11	rs1792549	15,182,234	5	G	0.33	0.914	5.96E-05	4.29E-03	3.92E-02	0.088	0.129
11	rs12800439	15,155,861	5	G	0.33	0.167	6.44E-05	7.75E-03	1.86E-02	7.69E-03	0.200
11	rs4343008	15,184,630	5	T	0.36	0.342	2.09E-04	9.32E-03	0.082	0.161	0.353
11	rs1612783	15,142,305	7	A	0.37	0.285	4.85E-04	4.61E-02	0.072	0.069	0.246
11	rs1540154	15,177,668	4	C	0.29	0.134	9.45E-04	0.141	4.27E-02	0.114	0.113
11	rs3110501	15,156,988	5	G	0.49	0.786	4.75E-03	2.09E-02	4.29E-02	4.66E-02	0.274
11	rs4756800	15,180,311	4	A	0.27	0.407	9.84E-03	0.051	2.76E-02	0.120	2.06E-03
11	rs1573542	15,152,532	5	A	0.49	0.862	9.94E-03	0.052	0.094	0.118	0.439
11	rs1945628	15,184,565	5	T	0.21	0.29	1.295E-02	0.051	0.166	0.554	3.10E-02

[a] RegulomeDB score;
[b] Minor allele;
[c] Minor allele frequency;
[d] Hardy-Weinberg equilibrium *p*-value;
[e] *p*-value based on linear regression for LogTMT-A;
[f] *p*-value based on linear regression for TMT-B;
[g] *p*-value based on linear regression for FAQ;
[h] *p*-value based on linear regression for CRD-SB;
[i] *p*-value based on linear regression for ADAS13.

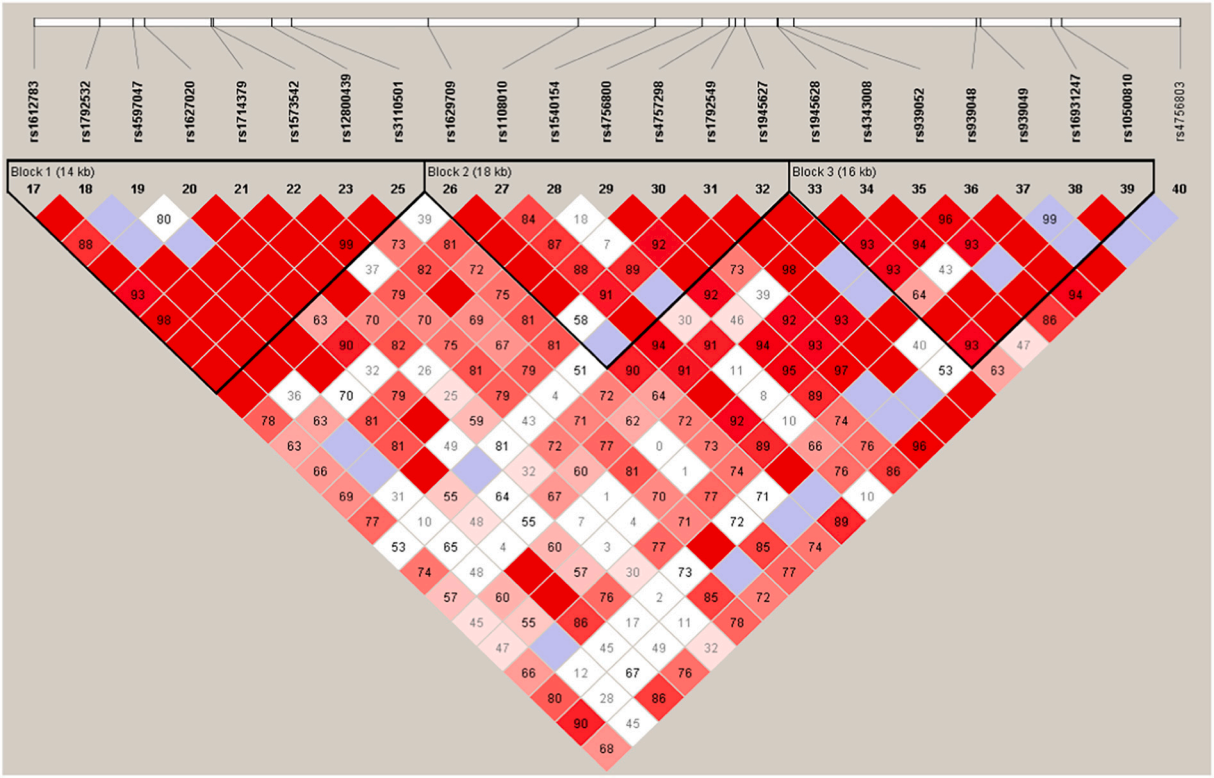

Fig. 2. Haplotype Structure (D′) of SNPs within INSC at 11p15.2 including the top SNP rs1108010.

since RegulomeDB returned scores of 5, which were medium score. RegulomeDB returned score of <3 is considered functional important. Furthermore, the SNPs in Tables 2, 3, and 5 had RegulomeDB scores from 4 to 7, which showed very limited functional consequences since lower the RegulomeDB score more evidence of functionality. In addition, eight SNPs in Table 2 had significant eQTLs in GTEx database: rs1108010 (INSC), rs2165194 (FOXD2), rs2025879 (CLTA), rs6675866 and rs6428496 (GBP1/GBP3/GBP7), rs11803889 (DNM3/METTL13), rs10955311 and rs17793351 (BAALC). A more comprehensive list of SNPs and eQTLs is presented in Supplementary Table S4.

4. Discussion

In this article we reported the first GWAS for TMT-A in AD using the ADNI cohort. We have identified 17 SNPs within the INSC gene associated with TMT-A ($p < 0.05$), especially the SNP (rs1108010) within the INSC gene reached genome-wide significant association ($p < 5 \times 10^{-8}$) and two additional SNPs (rs1792549 and rs12800439, $p < 10^{-4}$), which supported the association of INSC with cognitive measurement in TMT-A. Haplotype analysis further supports the single marker associations of INSC gene with TMT-A. Furthermore, two additional genes (FOXD2 and CLTA) have suggestive associations with TMT-A. Moreover, suggestive loci were identified, including GBP1/GBP3 with TMT-B, GRIK2 with

Table 4
Haplotypes associated with TMT-A in INSC gene in the ADNI sample.

SNPs	Haplotype[a]	Frequency[b]	B[c]	*t*[d]	P[e]
rs1629709 - rs1108010	A-T	0.32	0.06	29.9	6.07E-08
	G-C	0.25	−0.01	0.17	0.68
	A-C	0.43	−0.05	23.7	1.37E-06
rs1108010 – rs1540154	C-C	0.28	−0.04	12.7	3.85E-04
	T-T	0.32	0.06	28.1	1.53E-07
	C-T	0.40	−0.02	3.09	0.079

[a] Haplotype inferred from 2 SNPs;
[b] Haplotype frequency;
[c] Regression coefficient based on linear regression for LogTMT-A;
[d] t value based on linear regression for LogTMT-A;
[e] *p*-value based on linear regression for LogTMT-A.

FAQ, BAALC and CCDC146 with CDR-SB, BAALC and NKAIN2 with ADAS13. Additionally, the association of INSC with CTTA was validated with additional sample. Several SNPs associated with TMT-A and other AD-related phenotypes had significant eQTLs using GTEx. Novel genes/loci identified in association with TMT-A, in addition to the known genetic loci for AD (Robinson et al., 2017), suggesting that AD and related phenotypes are determined by the combinatorial effect of multiple genetic variants in the genome (Neuner et al., 2020).

The INSC gene that was first isolated and characterized in Drosophila embryo has an cytoskeleton-organizing role in cell shape control (Kraut and Campos-Ortega, 1996, Kraut et al., 1996). The inscuteable (Insc; mlnsc in mammals), a spindle orientation adaptor protein, is located at human chromosome 11p15.2 (Izaki et al., 2006; Katoh and Katoh, 2003). RT-PCR analysis showed its wide expression in human brain, lung, liver, kidney, pancreas, and small intestine, with expression of the short form in lung, pancreas, small intestine, and kidney only (Izaki et al., 2006). INSC protein binds to its partner proteins and forms a core spindle orientation complex machinery that regulate cytoskeleton rearrangement in specialized cells to recruit the mitotic spindle during cell division (Tadenev and Tarchini, 2017). Later studies support the role of the INSC gene in embryonic especially nervous system development, for example, regulating spindle orientation and basal protein localization during Neuroblasts (NB) asymmetric division and to secure the segregation of Numb towards the future ganglion mother cells (GMC) in Drosophila, determining the epithelial polarity and cells size (Cai et al., 2003; Culurgioni et al., 2011; Kraut et al., 1996; Schober et al., 1999). In the absence of INSC, supernumerary NBs are formed from a subpopulation of GMCs which has inherited insufficient Numb (An et al., 2017). INSC is also necessary for the correct asymmetric divisions of the larval brain neuroblasts (An et al., 2017), murine skin progenitors and neural stem cells (Culurgioni et al., 2018). At the synaptic level in the nervous system, INSC protein has been shown to regulate synapse structure and function (Tadenev and Tarchini, 2017), which may underlie the mechanism of its association with TMT-A and TMT-B tasks and other cognitive tasks such as FAQ, CDR-SB and ADAS13 performance. The present study provided evidence that the INSC gene product played an important role in the cognitive function and the best TMT-A associated SNP rs1108010 within INSC had significant eQTLs. AD is determined by the combinatorial effect of multiple genetic variants across the chromosomes in the genome (Neuner et al., 2020). Over 30 genetic loci have been found to affect risk, age of onset and disease severity involved in immune/inflammation function based on GWAS studies (Lambert et al., 2013; Neuner et al., 2020; Robinson et al., 2017). On chromosome 11, several genes are also found in association with AD, for example, SP11 associates with late onset of AD (LOAD), amyloid and family history (AD-by-proxy), and SORL1 associates with autosomal dominant AD, LOAD, brain volume and white matter phenotype which are reduced in AD. APP, PSEN1 and PSEN2 associate with early onset AD. Majority of the genetic loci are associated with LOAD, which are located on almost all chromosomes except the chromosome 12 (Lambert et al., 2013; Neuner et al., 2020), for example, APOE4, ABCA7, CR1, CLU, BIN1,PICALM, and SP11 and SOQL1 are found to be associated with LOAD. At least 8 genes MS4A4, TREM2, CR1, CD33, INPP5D, HLA-DRB5-DRB1, MEF2C and SORL1 are involved in immune, inflammation, and hippocampal synaptic function. At least 5 genes CD2AP/NME6, EPHA1 and SORL1/CASS4 are involved in cytoskeleton-membrane trafficking, intracellular signaling and/or Aβ (clearance) pathway. Two genes CASS4 and FERMT2 are in tau pathway, and other loci BIN1 and ZCWPW1 are associated with endocytosis and epigenetics, respectively. Three genes, EPHA2, CLU and PICALM, have overlapping effects in immune function and cholesterol metabolism.

The second-best SNP hit, associated with TMT-A, was rs2165194 within FOXD2 gene (Table 2), also known as FKHL17, FREAC-9, FREAC9 at 1p33 (Ernstsson et al., 1997). This SNP had significant eQTLs in artery – tibial and artery – aorta. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity, RNA polymerase II-specific. *FOXD2* is a candidate gene for meningioma (Ho et al., 2015; Sulman et al., 2004). The FOXD2 may be associated with cardiometabolic disease and play a role in gene regulation (Franzén et al., 2016).

The third locus was rs2025879 within CLTA gene, also known as LCA, at 9p13.3 (Gross, 2011; Ponnambalam et al., 1994). This SNP had significant eQTLs in esophagus - gastroesophageal junction and cells - cultured fibroblasts. rs2025879 was shown to be associated with DNA methylation and gene expression in adipose tissue (Grundberg et al., 2013). The CLTA gene may play a role in basolateral protein trafficking in epithelial cells (Deborde et al., 2008). SNP rs10972786 was associated with bladder cancer (Menashe et al., 2012). A recent study revealed the particular importance of the protein trafficking and clearance mechanisms, including CLTA and others in maintaining the homeostasis of the metastable subproteome associated with AD (Kundra et al., 2017). Based on databased of Online Mendelian Inheritance in Man (OMIM, htt

Table 5
Top SNPs within INSC gene associated with CTAA in the Caribbean Hispanics sample ($p < 0.01$).

Chr	SNP	Position	DB[a]	MA[b]	MAF[c]	HW[d]	B[e]	P_CTTA[f]
11	rs7931705	15,343,817	5	A	0.26	0.926	10.49	6.15E-05
11	rs6486235	15,331,505	4	C	0.31	0.213	8.39	2.54E-04
11	rs7932019	15,328,453	5	G	0.31	0.601	6.30	3.97E-04
11	rs7937161	15,448,583	6	G	0.05	0.477	2.48	5.15E-03
11	rs730993	15,985,264	5	G	0.31	0.601	6.30	7.70E-03
11	rs1065024	15,989,350	4	G	0.05	0.477	2.48	8.91E-03

[a] RegulomeDB;
[b] Minor allele;
[c] Minor allele frequency;
[d] Hardy-Weinberg equilibrium *p*-value;
[e] Regression coefficient based on linear regression for CTTA;
[f] *p*-value based on linear regression for CTTA.

ps://www.omim.org/), knockout of calcyon led to a significant deficit in clathrin-mediated endocytosis in mouse neocortical neurons. The neocortex is well known as the largest part of the mammalian brain and is the seat of our higher cognitive functions. Xiao et al. (2006) found that the heavy chain-binding region and C-terminal domain of CLTA interacted with the C-terminal domain of calcyon using yeast 2-hybrid analysis of a human brain cDNA library.

The top SNP associated with TMT-B is rs6675866 with GBP1 gene at 1p22.2 (Olszewski et al., 2006), which is also associated with TMT-A, FAQ, CDR-SB and ADAS13. This SNP had significant eQTLs in many tissues such as artery – aorta, cells - cultured fibroblasts, cells - EBV-transformed lymphocytes, heart, lung, nerve – tibial, and thyroid. Previous studies suggested that GBP1 may serve as a useful marker of uterine receptivity in the human (Kumar et al., 2001) and high expression of GBP1, GBP2, and GBP3 in endothelial cells (Tripal et al., 2007). A previous study showed that risk variants of GBP2, one of GBP genes were potentially associated with AD (Ertekin-Taner, 2010).

Regarding FAQ, the best SNP is rs4840200 within GRIK2 gene (also known as EAA4, GLR6, MRT6, GLUK6, GLUR6, and GluK2) at 6q16.3 (Paschen et al., 1994). Northern analysis showed that this gene expressed in both human cerebral and cerebellar cortices (Paschen et al., 1994). This gene is also shown highly expressed in other brain regions and its mutations have been reported to be associated with cortical development, autism, behavioral disorder, epilepsy, longevity, and schizophrenia (e.g., Jamain et al., 2002; Choi et al., 2009; Broer et al., 2015; Cordoba et al., 2015; Guzman et al., 2017).

Two SNPs (rs10955311 rs17793351) within BAALC gene at 8q22.3 (Tanner et al., 2001) were associated with CRD-SB and ADAS13. The gene was found to be expressed in brain and high expression in neural tissues (Tanner et al., 2001). GTEx database revealed several eQTLs in spleen and whole blood. This gene has been associated with acute myeloid leukemia (AML) (Damm et al., 2011, 2012; Eisfeld et al., 2012; Nadimi et al., 2016).

SNP rs6569364 within NKAIN2 (also known as TCBA; TCBA1; FAM77B; NKAIP2) 6q22.31 (Yue et al., 2006) was associated ADAS13. Low mRNA expression of NKAIN2 has been reported in castration-resistant prostate cancer (Sircar et al., 2012). It has been found under-expression of NKAIN2 in human brain and central nerve system (http://www.oncomine.org/resource/login). NKAIN2 may act as an oncogene in neuroblastoma (Romania et al., 2013) and play a role in prostate cancer development and progression and other human malignancies (Mao et al., 2016). This gene has been found to be associated with neuroticism (Calboli et al., 2010), alcohol dependence (Wang et al., 2011), and schizophrenia (Edwards et al., 2016). A recent study showed that the variants of NKAIN2 gene were associated with ventricular volume and ventricular enlargement occurred in several neurodegenerative and psychiatric diseases (Li et al., 2019).

This study has several strengths. First, we performed the first GWAS of TMT-A related to AD. Second, one SNP within INSC gene reached genome-wide significant level. Flanking SNPs and haplotype analyses further supported the association of INSC with TMT-A. Several SNPs within the INSC gene were associated with AD-related phenotypes such as TMT-B, FAQ, CRD-SB and ADAS13. Third, GWAS further identified several suggestive loci associated with TMT-A, TMT-B, FAQ, CDR-SB, and ADAS13. Fourth, the best TMT-A associate SNP were validated to be associated with CTTA. Additionally, several SNPs in associated candidate genes showed significant eQTLs using GTEx suggesting functionally important. These findings suggested that the INSC and other candidate genes (e.g., FOXD2, CTLA, GBP1, BAALC, and NKAIN2) may have pleiotropic effect on several cognitive phenotypes.

Several limitations need to be acknowledged. First, the sample size of GWAS is moderate. For the purpose of SNP-level power analysis, based on QUANTO 1.2.4 software (Gauderman and Morrison, 2009), the power for TMTA (assume 755 individuals with TMTA for gene discovery, mean ± SD = 47.61 ± 27.08, $\alpha = 5 \times 10^{-8}$), the population frequency of variant allele (qA) of 20%, and the true proportion of variation in trait phenotype explained by gene (Rg^2) of 3–6%, the power could reach 26–92%. Given $\alpha = 10^{-4}$ ad the true proportion of variation in trait phenotype explained by gene (Rg^2) of 3–6%, the power could reach 82–99%. Second, the SNP panel of the GWAS may have limited coverage of the genome, future deep sequences on these candidate gene regions are needed. Third, the INSC gene was validated in CTTA - a culturally fair analog of the TMT-A and replication of other AD-related phenotypes were not available. Therefore, associations with these markers and gene regions are required further replications in other study samples, targeted gene sequence, as well as more functional studies before any statement about causality is warranted.

5. Conclusions

We identified a novel locus (INSC) for TMT-A with genome-wide significance and several suggestive loci associated with TMT-A, TMT-B, FAQ, CDR-SB and ADAS13. These findings may offer new insights into the pathogenesis of cognitive impairment in AD. In addition, the findings will serve as a resource for replication in other populations to elucidate the potential role of these genetic variants in AD and AD-related phenotypes. Further identification of additional variants and disease-causing polymorphisms within these genes and their functions would help us better understand the pathogenesis of AD and AD-related phenotypes.

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Authors' contributions

KW and CX designed the study; KW, CX, DX, and CCX managed the literature searches, conducted data analyses, and drafted part of this the manuscript; AS, RON, YL, and UP interpreted the results and provided a substantive review of the manuscript. All authors read and approved the manuscript.

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Ethical statement

This multi-centered research project was approved by institutional review boards at each site and has obtained authorized written informed consent from participants (http://adni.loni.usc.edu/). There was an Institutional Review Board exemption for current study due to secondary data analysis.

Disclosure

All authors have reported no financial interests or potential conflicts of interest.

Data availability statement

The data were downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). Application for access to the ADNI data can be submitted by anyone at http://adni.loni.usc.edu/data-samples/access-data/. The process includes completion of an online application form and acceptance of Data Use Agreement. All data used in the study were downloaded from ADNI in May 2020.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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