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Genome-wide association study of language performance in Alzheimer's disease

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Abstract

Language impairment is common in prodromal stages of Alzheimer's disease (AD) and progresses over time. However, the genetic architecture underlying language performance is poorly understood. To identify novel genetic variants associated with language performance, we analyzed brain MRI and performed a genome-wide association study (GWAS) using a composite measure of language performance from the Alzheimer's Disease Neuroimaging Initiative (ADNI; n=1,560). The language composite score was associated with brain atrophy on MRI in language and semantic areas. GWAS identified *GLI3* (GLI family zinc finger 3) as significantly associated with language performance ($p < 5 \times 10^{-8}$). Enrichment of GWAS association was identified in pathways related to nervous system development and glutamate receptor function and trafficking. Our results, which warrant further investigation in independent and larger cohorts, implicate *GLI3*, a developmental transcription factor involved in patterning brain structures, as a putative gene associated with language dysfunction in AD.

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Keywords

Alzheimer’s disease; language performance; GWAS; *GLI3*; neuroimaging

1. Introduction

Alzheimer’s disease (AD), the most common neurodegenerative disorder, is clinically characterized by progressive cognitive impairment primarily in memory in the earliest stages. However, as the disease progresses, deficits can also be observed in other cognitive domains such as judgment, orientation, executive function, visuospatial ability, and language. Language impairment is a prevalent clinical feature that often occurs early on – sometimes even before AD is diagnosed, and progresses during the course of the disease (Ahmed, Haigh, de Jager, & Garrard, 2013). Several language tests have been shown to discriminate between cognitively normal (CN) and those with AD (Bertola et al., 2014; Clark et al., 2014; Gomez & White, 2006; Henry, Crawford, & Phillips, 2004). Hence, language impairment is one of several areas of cognitive decline that can aid in the clinical diagnosis of AD dementia according to the most recent criteria (McKhann et al., 2011). Language deficits during prodromal AD include impairments in word finding (Bayles, Tomoeda, & Trosset, 1990), naming (Hodges, Salmon, & Butters, 1991), and verbal fluency (Monsch et al., 1992; Nutter-Upham et al., 2008).

A large portion of the neuropsychological tests used to assess language impairment, such as the Boston Naming Test (BNT) and Animal Fluency, are dependent on semantic memory (Verma & Howard, 2012). It has been suggested that the language impairment observed in AD is primarily the result of a decline in semantic processing due to structural and organizational deterioration of semantic memory (Chertkow & Bub, 1990). Other memory systems affected include episodic memory, one of the earliest and most severely affected systems in AD (Hodges & Graham, 2001). Language impairments in prodromal and clinical AD have been previously associated with cortical atrophy predominantly in left temporal and parietal lobe regions, in addition to several other brain regions (Ahn et al., 2011; Apostolova et al., 2008; Domoto-Reilly, Sapolsky, Brickhouse, Dickerson, & Alzheimer's Disease Neuroimaging, 2012; Dos Santos et al., 2011). Similarly, there are functional changes on semantic fMRI tasks manifest early stage AD (Saykin et al., 1999). The genetic factors that contribute to different modalities of language function have also been investigated in adolescents and young adults with and without language disorders. For example, genome-wide association studies (GWAS) have identified candidate genes related to reading and language in adolescents to young adults (Gialluisi et al., 2014; Luciano et al., 2013) and the morphology of Heschl’s gyrus in young adults (Cai et al., 2014). However, the genetic architecture underlying language performance in older adults with and at risk for AD has not been previously studied. To identify novel genetic variants specifically associated with language performance, we conducted a GWAS as well as a pathway-based analysis using the Alzheimer’s Disease Neuroimaging Initiative (ADNI) cohort.

2. Material and methods

2.1 Alzheimer's disease neuroimaging initiative (ADNI)

The ADNI was launched in 2003 to help researchers and clinicians develop new treatments for mild cognitive impairment (MCI) and early AD dementia, monitor their effectiveness, and decrease the time and cost of clinical trials. One of ADNI's major goals is to test whether serial magnetic resonance imaging (MRI) (Grundman et al.), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and AD. This multi-year multi-site longitudinal study was started by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies, and non-profit organizations as a $60 million, 5-year public-private partnership. It was then extended for an additional 7 years through two additional phases of funding. The ADNI participants consist of AD dementia, MCI, and healthy elderly individuals with and without significant memory concern (i.e., SMC and CN, respectively). They were aged 55–90 years and recruited from 59 sites across the U.S. and Canada including medical and academic institutions. Further information can be found at http://www.adni-info.org/ and see (Aisen et al., 2010). The current study involved 1,575 subjects with genotyping: 370 CN, 94 SMC, 283 early MCI (EMCI), 515 late MCI (LMCI), and 313 AD.

2.2 Phenotypes

A composite measure of language was generated as the target phenotype for this study using the following language measures from the ADNI neuropsychological test battery - the BNT, the Animal Fluency test, and the naming portion of AD Assessment Schedule-Cog (ADAS-COG). Given the invariable presence of episodic memory deficits in the cognitively impaired ADNI population, we sought to capture language performance independent of episodic memory performance (Supplementary Table 1). Therefore, each language test (BNT, Animal Fluency, and ADAS-COG naming) was individually adjusted with a linear regression model for concomitant episodic memory deficits using a composite score for episodic memory (Crane et al., 2012) in IBM SPSS (version 22, Armonk, NY). The residuals were then used to generate a composite score using principal component analysis taking the resulting first component, which accounted for 66% of the variance, with the following loadings: animal fluency = 0.62, BNT = 0.84, and ADAS-COG naming = 0.76 (Supplementary Fig. 1). For each diagnostic group, a positive linear relationship was observed between each language measure with respect to episodic memory (Supplementary Table 2). Thus, diagnostic status was not considered in the derivation of the language composite score. This summary measure of language performance was used as the primary phenotype for GWAS. A total of 1,560 subjects (365 CN, 94 SMC, 280 EMCI, 511 LMCI, 310 AD) had all three language test scores available for analyses. Age and sex were significantly associated ($p<0.001$; age, $r = 0.15$; sex, $r = 0.11$) with the generated language composite score pre-adjusted for episodic memory and were used as covariates in the following analyses.

2.3 Structural MRI scans

Baseline 1.5T (ADNI-1) and 3T (ADNI-GO/2) magnetization-prepared rapid gradient-echo (MPRAGE) images were downloaded from the ADNI LONI site for all participants (http://adni.loni.usc.edu/). Scan processing with voxel-based morphometry (VBM) in Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and quality control were done as previously described (Risacher et al., 2013). Overall, 71 subjects (17 CN, 5 SMC, 12 EMCI, 21 LMCI, 16 AD) were excluded due to missing MRI scan data or failed processing, leaving 1,489 subjects available for analyses. Analyses were performed separately for each magnetic field strength.

2.4 Genotyping and imputation

The Illumina610-Quad BeadChip was used for genotyping all ADNI-1 participants and the Illumina HumanOmni Express BeadChip or the Illumina Omni 2.5M was used for participants enrolled in ADNI-GO or ADNI-2 (Saykin et al., 2015). Un-genotyped single nucleotide polymorphisms (SNPs) were imputed as previously described (Nho et al., 2015). Since population stratification is known to cause spurious association in disease studies, from the ADNI participants, we restricted our analyses to only non-Hispanic Caucasian subjects that clustered with CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) + TSI (Toscani in Italia) populations using HapMap 3 genotype data and the multidimensional scaling (MDS) analysis (www.hapmap.org). Standard sample and SNP quality control procedures were then implemented; SNPs were excluded if the genotyping call rate was < 95%, Hardy-Weinberg equilibrium test $p < 1 \times 10^{-6}$, sample call rate < 95%, or the minor allele frequency < 1%. In addition, sample sex marker mismatch check and DNA sample SNP fingerprint to microarray identity checks were also performed. MACH and 1000 Genomes Project data (build 37, hg19) as a reference panel were used for imputation. After quality control steps including filtering for MAF ≥ 5% on the imputed SNPs were applied, 1,575 of 1,716 ADNI participants and 5,574,300 SNPs remained for subsequent analyses.

2.5 Statistical analysis

A linear regression model in SPM8 using MRI scans was performed across voxels to evaluate the relationship of the language composite scores (unadjusted and pre-adjusted for episodic memory performance) with grey matter (GM) density. This enabled delineation of the regional associations between the language composite score and structural brain changes. Voxel-wise analysis was also used to determine the anatomical distribution of the association of the most significant SNP identified in the GWAS (*see section below*) with GM density. Voxel-wise analyses included age, sex, years of education, intracranial volume, and apolipoprotein E (*APOE*) ε4 status as covariates. Language abilities are typically lateralized to the left hemisphere, however some left handed individuals show right hemisphere or mixed dominance and thus handedness was also included as a covariate (Knecht et al., 2000). Significant results were displayed with a minimum cluster size (k;(Friston, Holmes, Worsley, Poline, & Frackowiak, 1995) of 100 contiguous voxels and a voxel-wise threshold of $p < 0.001$ (both uncorrected and corrected for multiple comparisons). Anatomical regions were defined using the x-y-z coordinates for the most significant voxel within each cluster. These coordinates were entered into the Talairach daemon (http://www.talairach.org/daemon.html) to receive the anatomical names for the GM regions closest to that coordinate (Lancaster et al., 2000).

A GWAS across the whole sample (n=1560) was performed using a linear regression under an additive genetic model in PLINK (Purcell et al., 2007) with the language composite pre-adjusted for episodic memory performance as the endophenotype to specifically target language-specific cognitive function in a population with known memory impairment (Rabin et al., 2009). Age, sex, handedness, years of education, *APOE* ε4 status, and genotyping platform were used as covariates. Bonferroni method was applied for correcting for multiple comparisons and SNPs with $p < 5 \times 10^{-8}$ were considered genome-wide significant (Pe'er, Yelensky, Altshuler, & Daly, 2008). Permutation testing (10^9 permutations) of the most significant SNP was implemented in PLINK to ensure that the language composite measure had did not deviate from normal distribution. Manhattan and Quantile-Quantile plots were generated in R using the 'qqman' package and regional association plots were generated with LocusZoom (Pruim et al., 2010). Linkage disequilibrium (LD) was estimated and visualized with D' using haploview (http://www.broad.mit.edu/mpg/haploview). The p-values obtained from the GWAS results and the GSA-SNP software (Nam, Kim, Kim, & Kim, 2010) were then utilized to identify pathways enriched with SNPs showing association to language performance (Ramanan, Kim, et al., 2012). We restricted downstream analysis to pathways containing 5–100 genes and used false discovery rate (FDR) to correct for pathway-level multiple comparisons. Pathway definitions were downloaded from the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp; canonical pathways set, version 4.0).

3. Results

Sample characteristics of the participants used in this study are presented in Table 1. To assess brain regions in which grey matter (GM) density is associated with the generated language composite score pre-adjusted for episodic memory, we used VBM. A significant association between poorer language performance, both adjusted and unadjusted for episodic memory performance, and reduced GM density throughout the brain was observed (Fig. 1 and supplemental Fig. 2–3). Specifically, worse performance on the unadjusted score was associated with reduced GM density in nearly the entire cortex (Supplemental Fig. 2). However, performance on the language composite pre-adjusted for episodic memory performance showed a more localized pattern of association, with poorer performance associated with reduced GM density in the middle and superior temporal gyrus (Brodmann area (BA) 21 and BA22), predominantly lateralized to the left hemisphere (Fig. 1 and supplemental Fig. 3). We observed a larger region significantly associated with language performance in ADNI-GO/2, likely due to the increased sensitivity of the 3T scans to degeneration.

A GWAS was performed to identify genetic variants that might influence language performance using the language composite score pre-adjusted for episodic memory as the phenotype. GWAS identified four SNPs (rs3801203, $p = 3.21 \times 10^{-9}$; rs3779159, $p = 7.12 \times$

10^{-9}; rs3801206, $p = 6.13 \times 10^{-9}$; and rs74745318, $p = 7.12 \times 10^{-9}$; supplemental Table 1) in the region of *GLI3* (GLI family zinc finger 3) that displayed genome-wide significant association with language function (Fig. 2a–b). The genomic inflation factor (λ) was 1.026 indicating no evidence for bias or inflation of our test statistics due to population stratification (Fig. 2c). These SNPs are intronic and demonstrated strong LD (Fig. 3a). To ensure our composite score had a normal distribution, permutation testing of the most significant SNP, rs3801203, resulted in the same p-value of 3.21×10^{-9} suggesting our phenotype had a normal distribution. Each SNP was also tested with BNT, ADAS-COG naming, and animal fluency, individually. All four *GLI3* SNPs were significantly associated with BNT and ADAS-COG naming ($p< 1 \times 10^{-5}$). The minor allele of each SNP (data not shown) including rs3801203, was associated with worse language performance across all subjects (Fig. 3b) and diagnostic groups with the exception of the smallest group, SMC (Fig. 3c). An additional 31 SNPs in five additional genes displayed suggestive association ($p < 5 \times 10^{-6}$) for language performance (Supplemental Table 3).

The four SNPs identified in the GWAS are in high LD thus we chose the most significant SNP, rs3801203, to determine if the genetic variants associated with language performance were also associated with GM density. *GLI3* SNP rs3801203 was associated with lower GM density in both the ADNI-1 and ADNI-GO/2 cohorts (Fig. 4). Specifically, in the ADNI-1 cohort, the minor allele of rs3801203 was associated with atrophy in the middle and superior temporal gyrus (BA 21 and BA22, respectively; Fig. 4a), amongst other regions such as the fusiform, cingulate gyrus, and precuneus (Supplemental Table 4). However, in the ADNI-GO/2 cohort, the minor allele of rs3801203 was only found to be associated with atrophy in the inferior frontal gyrus (orbitofrontal cortex; Fig. 4b).

Pathway- enrichment analysis identified 24 pathways associated (FDR $p < 0.01$) with language performance. The top 20 pathways are presented in Table 2 and include pathways related to nervous system development, such as neuronal migration, axon guidance, and cell differentiation. Additional pathways included glutamate receptor trafficking and function, immune activation, apoptosis, and others.

4. Discussion

We generated a language composite score using measures from animal fluency, BNT, and ADAS-COG naming. In the ADNI sample, MRI analysis identified significant GM atrophy across the whole-brain in relation to the language composite score unadjusted for episodic memory. However, GM atrophy in the left temporal, parietal, and frontal lobes were significantly associated with language performance pre-adjusted for episodic memory. These brain regions have previously been implicated in language and semantic memory processes (Apostolova et al., 2008; Domoto-Reilly et al., 2012; Joubert et al., 2010; McDonald, Bean, & Saykin, 2011; Saykin et al., 1999). We then investigated the effect of genetic variations on this language composite score using GWAS and pathway-enrichment analysis techniques in individuals at risk for and with AD from the ADNI cohort. GWAS identified variants in *GLI3* significantly associated with language impairment. Genetic variation in *GLI3* was also associated with lower GM density predominantly in the temporal lobes. Finally, neuronal development, glutamate receptor trafficking, immune function, and apoptotic pathways showed enriched associated with language performance in this sample. To our knowledge, this study is the first to identify *GLI3* variation as associated with language performance.

GLI3 encodes one of three GLI zinc finger transcription factors expressed early in development that is normally involved in patterning brain structures (Blaess, Corrales, & Joyner, 2006; Ruppert, Vogelstein, Arheden, & Kinzler, 1990). The GLI3 protein is an important downstream mediator of the Sonic Hedgehog pathway and can act as an activator or repressor in the presence or absence of Sonic Hedgehog, respectively (Wang, Fallon, & Beachy, 2000). Mutations to this pathway are causative for developmental disorders which affect the limbs, head, and face (Kalff-Suske et al., 1999; Kang, Graham, Olney, & Biesecker, 1997).

GLI3 may also have a more direct role in development of the corpus callosum (Amaniti et al., 2013; Magnani et al., 2014) and hippocampus (Hasenpusch-Theil et al., 2012; Palma & Ruiz i Altaba, 2004). One study showed *GLI3* expression was downregulated in the presence of Presenilin 1 (*PSEN1*), a protein which forms part of the γ-secretase complex involved in amyloid-beta production, ultimately leading to decreased neuronal differentiation (Paganelli et al., 2001). Notably, mutations in the *PSEN1* gene are causative for some forms of early-onset autosomal dominant AD, including mutations with an aphasic phenotype (Denvir et al., 2015). Another study showed *GLI3* expression is capable of repressing *Pitrm1* (pitrilysin metallopeptidase) using limb tissue from *GLI3* mutant mice (Town et al., 2009). The Pitrm1 protein is a metalloendopeptidase which is able to degrade amyloid-beta when it accumulates in mitochondria (Falkevall et al., 2006). Further Pitrm1 has been shown to have decreased expression in the temporal lobe of AD subjects (Alikhani et al., 2011), as well as decreased antisense expression in AD subjects compared to controls (Sekar et al., 2015). In light of these studies, our observed findings may suggest that this system could be dysregulated in older adults at risk for AD. Future studies exploring the relationships between *GLI3*, *PSEN1*, and *Pitrm1* within the brain and in the context of AD would help to elucidate any potential role of this system in AD etiology.

To date, the four *GLI3* SNPs identified in this study have not been linked to any other biological pathway or pathology to our knowledge. We found that these genetic variants were independently associated with BNT and ADAS-COG naming, but not with animal fluency, suggesting the language composite score was more reflective of naming rather than fluency. Furthermore, the minor allele of rs3801203 in the ADNI-1 cohort was associated with atrophy in brain regions associated with auditory processing. However, this finding did not replicate in the ADNI-GO/2 cohort which could be due to differences in scanner strength. This could also be due to the sample characteristics of these two cohorts. The ADNI-1 cohort includes mainly subjects who are later in the disease course and ADNI-GO/2 includes predominantly subjects that are earlier in the disease course. This could account for the larger variation in atrophy in relation to the language composite score seen in ADNI-GO/2. Moreover, the effect of rs3801203 may occur later in the disease course which could account for why more atrophy is observed in the ADNI-1 cohort.

Pathway-based analysis has provided useful insights into the underlying biological processes of complex genetic diseases by integrating and assessing the contribution of multiple genes and proteins across an intricate network of pathways and subnetworks (Ramanan, Shen, Moore, & Saykin, 2012). This pathway analysis identified several associations between nervous system development pathways and language performance. Specifically, one domain identified in the pathway analysis was glutamate receptor function and trafficking pathways. Glutamate is the primary excitatory neurotransmitter in the brain and is implicated in AD pathophysiology and neuronal cell death. Glutamate receptors have been under investigation as a therapeutic target for AD and other neurological disorders (Caraci et al., 2011; Morin et al., 2013; Olivares et al., 2012; Rosini, Simoni, Minarini, & Melchiorre, 2014). Memantine, a weak N-Methyl-D-aspartate channel blocker, has shown efficacy in reducing clinical decline in moderate AD and is widely used clinically (Reisberg et al., 2003; Tariot et al., 2004). Our results suggest that alterations in glutamate neurotransmission or receptor trafficking may play a critical role in language performance and functional semantic memory in AD.

One notable limitation of this study is the lack of a similar cohort to replicate our findings. Future studies investigating *GLI3* in independent cohorts with similar neuroimaging and cognitive assessments should be done to confirm our results. In addition, further investigations about the functional impact of the observed significant *GLI3* SNPs on a cellular or molecular level are warranted. Causal directions are yet to be determined but future studies may be able to capture the complicated relationship between the genetic, neuroimaging, and language domains using advanced statistical modeling such as mediation analysis or structural equation modeling. In view of the previously identified association of *GLI3* with an amyloid-related protein (e.g.,*PSEN1*) as described above, future studies should also focus on evaluating the impact of *GLI3* variation on amyloid phenotypes in older adults at risk for AD.

To our knowledge, this is the first reported GWAS of language performance in older adults at risk for or with AD. Our results identified novel associations of *GLI3*, a developmental transcription factor involved in patterning brain structures, with language dysfunction. Further, pathway analysis identified neuronal development and glutamate receptor pathways as enriched. Future studies will help to fully elucidate the underlying biology and importance of *GLI3* and identified pathways in AD etiology and/or their potential as therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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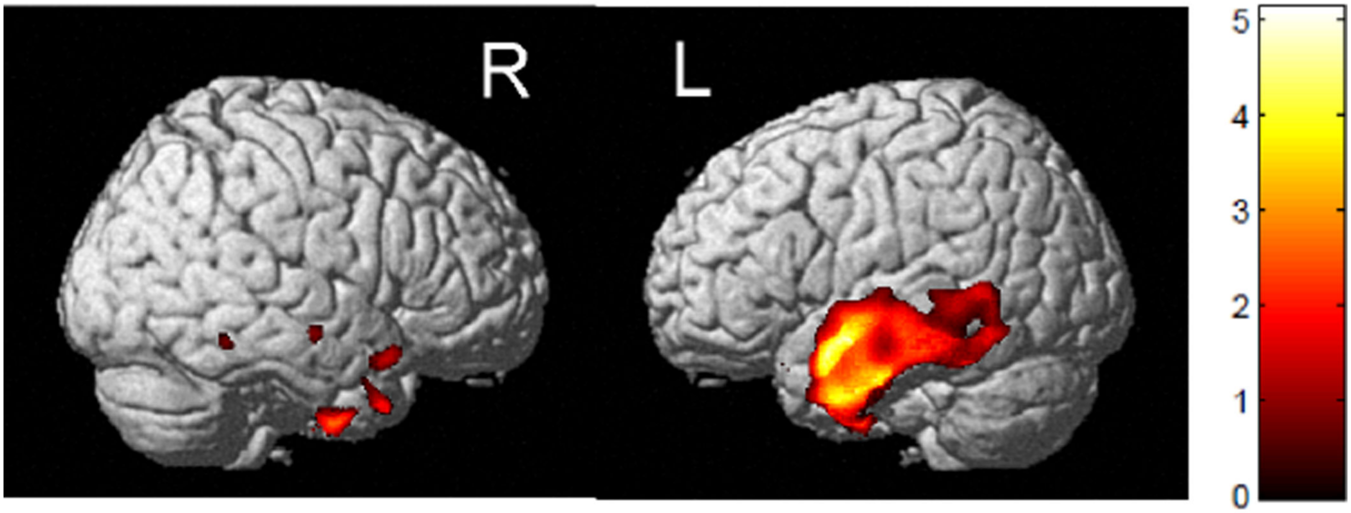
Highlights

- We identified novel genetic variants associated with language performance.
- Minor allele variants in *GLI3* are associated with worse language performance.
- Anatomical changes in language regions associated with language composite score.
- Developmental and glutamate pathways were related to lower language performance.

(a) ADNI-1



(b) ADNI-GO/2

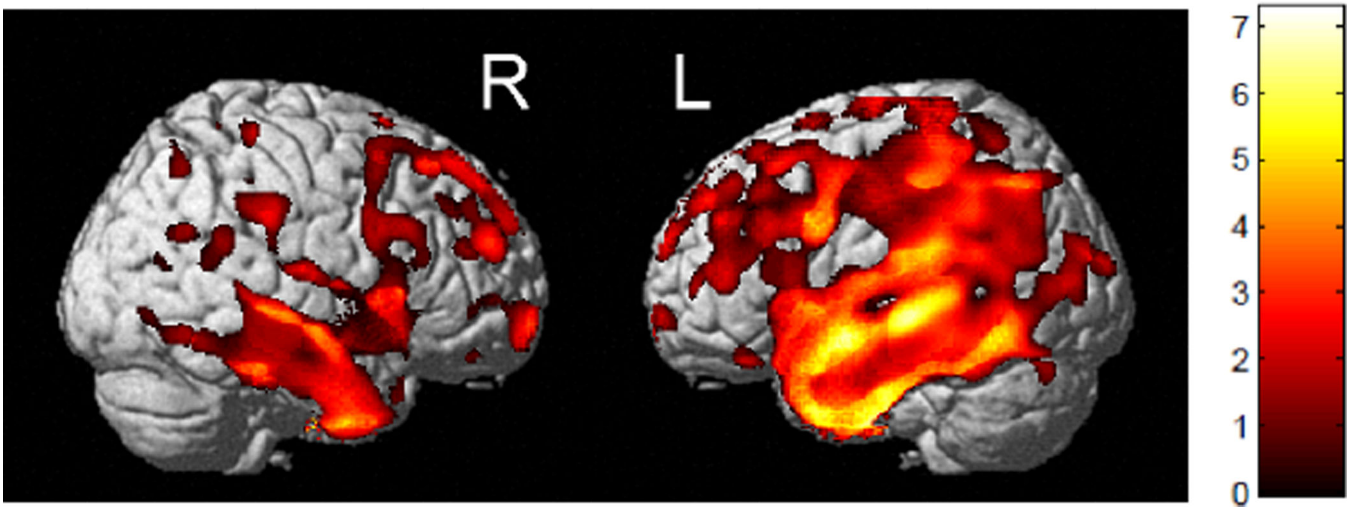


Figure 1.

Global regions associated with the language composite score pre-adjusted for episodic memory performance in ADNI participants. Grey matter density was positively correlated with the language composite score in the temporal lobe and language areas for both ADNI-1 (a) and ADNI-GO/2 (b). Covariates included age, sex, intracranial volume, *APOE* ε4 status, and handedness. Results are displayed at p<0.001 (uncorrected) and at a threshold (k) of 100 voxels.

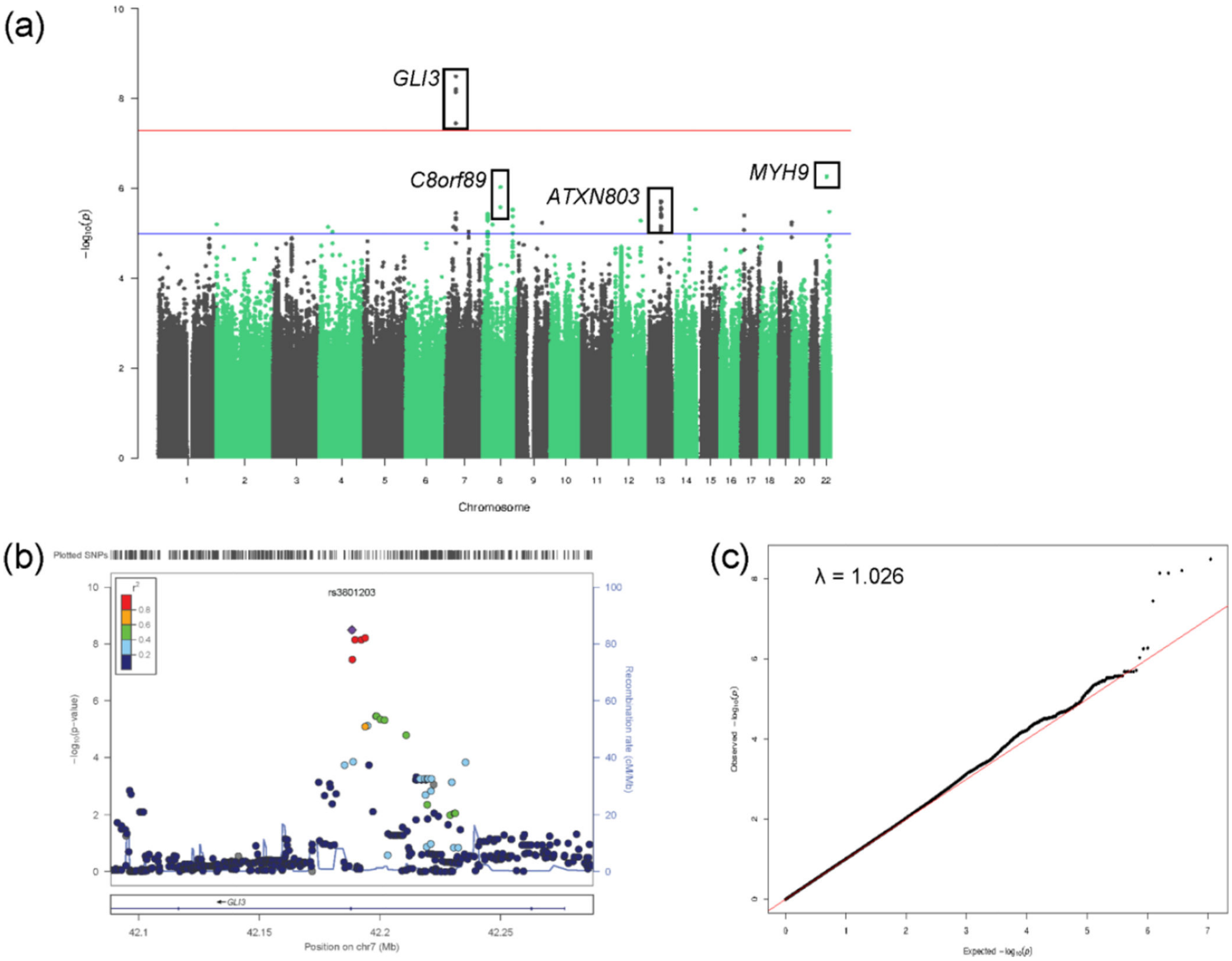


Figure 2.

Manhattan, regional association, and quantile-quantile plots for GWAS of language performance. (a) Observed –log^{10} p-values (y-axis) are displayed for all tested SNPs on each chromosome (x-axis). A SNP was considered genome-wide significant if $p < 5 \times 10^{-8}$ (above red line). Suggestive SNP associations were identified as those reaching genome-wide significance of $p < 1 \times 10^{-5}$ (above blue line). (b) Regional association plot showing the region around the most significant SNP in the GWAS. The SNPs within 500 kb of rs3801203 are plotted as their GWAS −log^{10} P-values against their NCBI 37 genomic position. The blue line indicates recombination rates estimated from the 1000 Genomes Project reference data. The color scale of r^2 values is used to label SNPs based on their correlation with rs3801203. (c) The genomic inflation factor (λ) was 1.026 suggesting no population stratification effect.

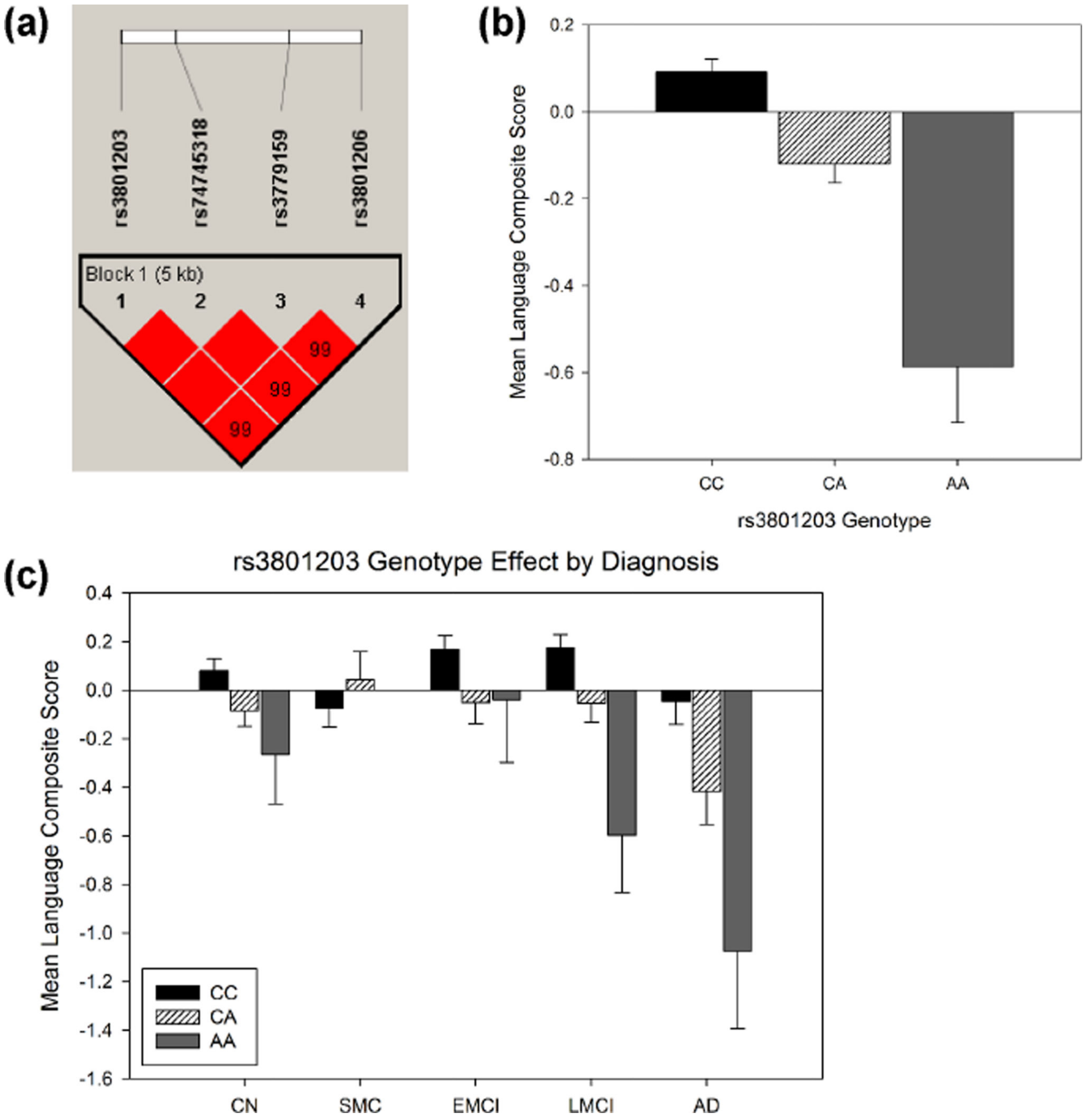
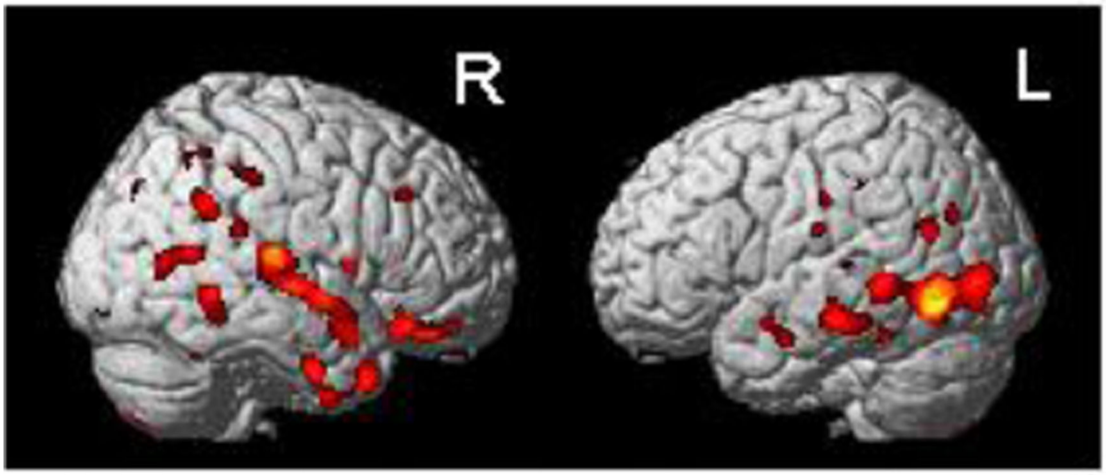


Figure 3.

GLI3 SNPs are associated with lower language performance. (a) Linkage disequilibrium map of the four *GLI3* SNPs significantly associated with language performance. The minor allele of rs3801203 (b-c, $p = 3.21 \times 10^{-9}$) was associated with lower language composite score in older adults at risk for or with AD, with the exception of the SMC group. Means adjusted for age, sex, years of education, genotyping platform, handedness, and *APOE* ε4 status. Standard errors are displayed.

CN = cognitively normal; SMC = significant memory concern; EMCI = early mild cognitive impairment; LMCI = late mild cognitive impairment; AD = Alzheimer’s disease

(a) ADNI-1



(b) ADNI-GO/2

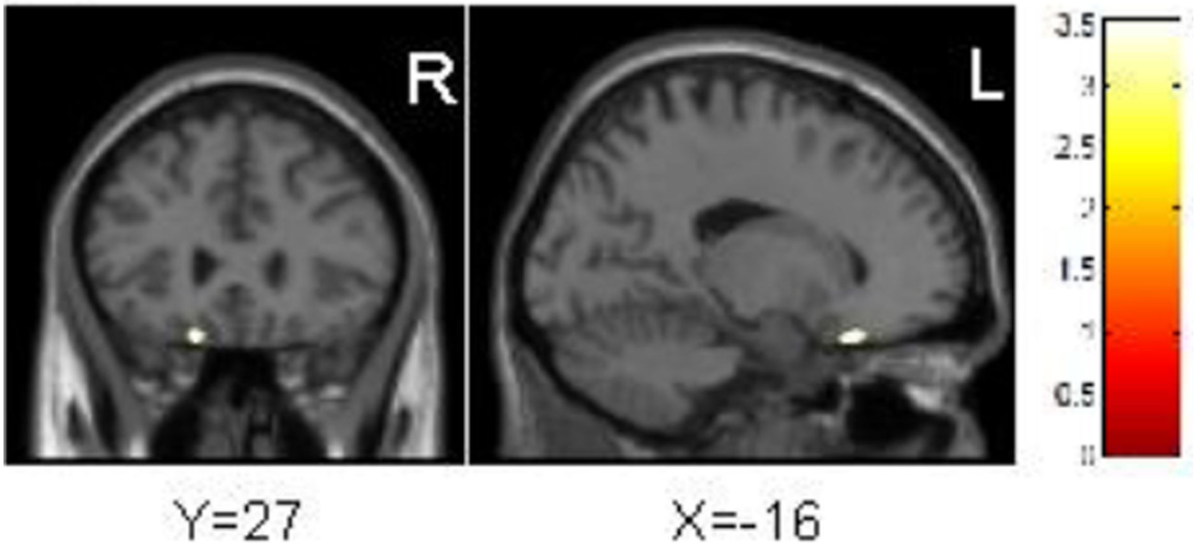


Figure 4.

The minor allele of *GLI3* SNP rs3801203 is associated with lower grey matter density in the temporal cortex of ADNI-1 participants (a) and the orbitofrontal cortex for ADNI-GO/2 participants (b). Covariates included age, sex, intracranial volume, *APOE* ε4 status, and handedness. Results are displayed at $p<0.001$ (uncorrected) and at a threshold (k) of 100 voxels.

Table 1

Baseline demographic characteristics.

Baseline Diagnosis	CN	SMC	EMCI	LMCI	AD
No. of Subjects	365	94	280	511	310
Male/Female	192/173	40/54	160/120	317/194	175/135
Baseline Years of Age (SD)	74.6 (5.6)	71.8 (5.7)	71.1 (7.4)	73.5 (7.7)	74.7 (7.8)
Baseline Years of Education (SD)	16.3 (2.6)	16.8 (2.6)	16.1 (2.7)	16 (2.9)	15.2 (3)
Right/Left Handed	338/27	82/12	251/29	457/54	286/24
***APOE* (ε4−/ε4+)**	266/99	62/32	160/120	231/280	105/205
Lang. Comp. Mean (SD) *	0.6 (0.6)	0.56 (0.5)	0.32 (0.7)	−0.14 (0.9)	−0.94 (1.2)

CN = cognitively normal; SMC = significant memory concern; EMCI = early mild cognitive impairment; LMCI = late mild cognitive impairment; AD = Alzheimer’s disease; SD = standard deviation

* Not adjusted for memory composite score.

Table 2

Top 20 pathways associated with language performance.

Pathway description (Source database)	Size[a]	Uncorrected p[b]
Netrin-1 signaling (Reactome)	36	2.38E-07
L1CAM interactions (Reactome)	76	9.39E-07
Arrythmogenic right ventricular cardiomyopathy (KEGG)	71	9.78E-07
Ion channel transport (Reactome)	48	5.33E-06
SHH pathway (Biocarta)	15	6.81E-06
Phosphatidylinositol signaling system (KEGG)	75	9.84E-06
Trafficking of AMPA receptors (Reactome)	26	1.17E-05
NRAGE signals death through JNK (Reactome)	37	1.39E-05
Adherens junction (KEGG)	71	1.39E-05
Synthesis of PIPs at the plasma membrane (Reactome)	28	1.59E-05
Integrin cell surface reactions (Reactome)	74	1.72E-05
FC gamma R-mediated phagocytosis (KEGG)	90	4.91E-05
ECM-receptor interaction (KEGG)	81	5.64E-05
Cell death signaling via NGRAGE, NRIF, and NADE (Reactome)	51	7.69E-05
Semaphorin interactions (Reactome)	61	9.89E-05
Hypertrophic cardiomyopathy-HCM (KEGG)	79	1.15E-04
Unblocking of NMDA receptor glutamate binding and activation (Reactome)	14	1.53E-04
Interaction between L1 and Ankyrins (Reactome)	20	1.57E-04
PI metabolism (Reactome)	44	1.59E-04
Glioma (KEGG)	63	1.73E-04

[a] Number of genes in the pathway.

[b] All pathway displayed are significant at false discovery rate (FDR)-corrected $p < 0.01$.