

Featured Article

## Carriers of a common variant in the dopamine transporter gene have greater dementia risk, cognitive decline, and faster ventricular expansion

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**Abstract** Introduction: Genetic variants in DAT1, the gene encoding the dopamine transporter (DAT) protein, have been implicated in many brain disorders. In a recent case-control study of Alzheimer's disease (AD), a regulatory polymorphism in DAT1 showed a significant association with the clinical stages of dementia. Methods: We tested whether this variant was associated with increased AD risk, and with measures of cognitive decline and longitudinal ventricular expansion, in a large sample of elderly participants with genetic, neurocognitive, and neuroimaging data from the Alzheimer's Disease Neuroimaging Initiative. Results: The minor allele—previously linked with increased DAT expression in vitro—was more common in AD patients than in both individuals with mild cognitive impairment and healthy elderly controls. The same allele was also associated with poorer cognitive performance and faster ventricular expansion, independently of diagnosis. Discussion: These results may be due to reduced dopaminergic transmission in carriers of the DAT1 mutation. © 2015 The Alzheimer's Association. Published by Elsevier Inc. All rights reserved.

**Keywords:** Neuroimaging genetics; Ventricular expansion; Dopamine transporter; Dementia; DAT1

### 1. Introduction

Dopamine (DA) is a powerful regulator of many aspects of brain function, and altered DA transmission can contribute to cognitive impairment [1]. Common variants in DA-related genes have been implicated in cognitive

function, age-related cognitive decline, and dementia severity [2,3]. The dopamine transporter (DAT) protein regulates neurotransmission by terminating DA signaling at the synapse through high-affinity reuptake of DA into pre-synaptic terminals [4]. The DAT protein limits the activation

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<sup>y</sup>Denotes equal contribution.

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of DA receptors [5], and changes in DAT expression directly affect the concentration of synaptic DA and the kinetics of reuptake [6,7]. This protein is encoded by the DAT1 (or SLC6A3) gene [8], and DAT1 variants may be related to various brain disorders [9].

A recent Taiwanese study reported an association between the major T allele at rs6347 of DAT1 and moderate dementia [3]. In other words, among demented participants, the minor C allele was significantly more prevalent in patients with severe dementia than in individuals with moderate dementia [3]. Here, we sought to replicate this association in Caucasians, and hypothesized that the minor C allele at this locus would be more common in elderly individuals with Alzheimer's disease (AD) than in both subjects with mild cognitive impairment (MCI) and healthy elderly controls (CON).

The rs6347 single nucleotide polymorphism (SNP) is a common synonymous variant (T. C, Minor Allele Frequency 5 0.299) in exon 9 of DAT1 [10]. It does not affect the amino acid sequence, but may be a regulatory variant [8]. As DA has a crucial role in cognition [1], and age-related cognitive decline [2], we also predicted that the same allele would be associated with poorer cognitive performance, independently of disease status.

DA also regulates the formation of neurotoxic amyloid beta (A $\beta$ ) oligomers [11], and lateral ventricular enlargement indicates an accumulation of brain tissue loss [12]. We therefore hypothesized that carriers of the minor allele at rs6347 would show faster expansion of the lateral ventricles, independently of their dementia status. We tested these predictions in a large elderly cohort (N 5 738), with genetic, neurocognitive, and neuroimaging data.

## 2. Methods

### 2.1. Subjects

Data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Written informed consent was obtained from all participants. To avoid the known effects of population stratification on genetic analysis, we included only non-Hispanic Caucasian subjects [13]. Our final analysis comprised 738 individuals (average age 75.52  $\pm$  6.78 years; 438 men/300 women) including 173 AD, 359 MCI, and 206 CON at baseline.

### 2.2. Cognitive testing and genotyping

All subjects completed detailed cognitive assessments including the Mini-Mental State Examination (MMSE)

### 2.3. Statistical analyses of allele frequency and associations of rs6347 genotype with MMSE scores

The distributions of allele frequencies for rs6347 were evaluated by  $\chi^2$  tests using contingency tables in SPSS 21.0. Statistical analyses of the odds ratio and 95% confidence interval were conducted based on the presence of the minor C allele. We then used the number of minor C alleles at rs6347 to predict baseline MMSE scores, assuming an additive model for allele effects.

### 2.4. Image acquisition, correction, and preprocessing

Participants were scanned with a standardized magnetic resonance imaging (MRI) protocol developed for this cohort [15,16]. Briefly, high-resolution structural brain MRI scans were acquired at 58 sites across North America, using 1.5 Tesla MRI scanners. A sagittal 3D MP-RAGE sequence was used, and optimized for consistency across sites [16] (TR/TE 5 2400/1000 ms; flip angle 5 8 $^\circ$ ; FOV 5 24 cm; final reconstructed voxel resolution 5 0.9375  $\times$  0.9375  $\times$  1.2 mm $^3$ ). Image quality control procedures and postacquisition correction of various image artifacts were performed at a single site (Mayo Clinic) [16].

### 2.5. Segmentation of the lateral ventricles

Raw MRI scans were preprocessed to reduce signal inhomogeneity and linearly registered to a template (using a nine-parameter registration). Prior methods for ventricular segmentation have used semiautomated, automated [17], and single-atlas or multiatlas methods [18]. Here we segmented the ventricles with our modified multiatlas approach described previously [19]. An inverse-consistent fluid registration with a mutual information fidelity term aligned a set of hand-labeled ventricular templates to each scan [20]. The template surfaces were registered into homologous point-to-point correspondence as a group using medial-spherical registration [21]. This approach is very similar to that of [22], except ours is based on surface geometry rather than image voxels. One subject whose meshes deviated by several millimeters from the actual periventricular boundaries was excluded. Our final analysis included 737 ADNI subjects at baseline, 623 at 12-month follow-up, and 481 at 24-month follow-up.

### 2.6. Statistical associations of rs6347 genotype with ventricular volumes

We first determined if genotype at the rs6347 locus might be associated with baseline ventricular volumes, after adjust-

also testing both recessive and additive models of minor allele effects. We used changes in volume of the lateral ventricles (in cubic mm) as dependent variables, controlling for age, sex, diagnosis, and APOE genotype, with subjects included as a random factor and time point (i.e., change in volume at 12 months and 24 months) as a repeated measure.

### 3. Results

#### 3.1. Primary analyses

##### 3.1.1. Allele frequency

Allele frequency was computed from genotype frequency in baseline participants (N = 738) and significantly differed across the three diagnostic groups (P = .018, Table 1). The minor C allele was more prevalent in AD than in both CON (P = .006, Table 2) and MCI (P = .039, Table 3), but did not significantly differ between MCI and CON (Table 4).

##### 3.1.2. Associations of rs6347 genotype with MMSE scores

MMSE scores significantly differed between the three genotype groups (P = .006, Fig. 1). Carriers of two C alleles performed more poorly than both carriers of 0 (P = .004) and 1 (P = .026) C allele, but carriers of 0 and 1 C alleles did not differ, suggesting a recessive model of minor allele effects on cognitive decline (Fig. 1). These differences remained significant after controlling for sex, age, diagnosis, and APOE status (P = .030, F-ratio = 3.524).

##### 3.1.3. Associations of rs6347 genotype with ventricular expansion

Baseline ventricular volumes and volume loss over a period of 2 years (in cubic mm) are reported in Table 5, by diagnostic and genotype groups. Genotype at the rs6347 locus was not significantly related to total ventricular volume at baseline (P = .474, F-ratio = 0.514 and P = .612, F-ratio = 0.491 for the recessive and additive models, respectively), after controlling for sex, age, and diagnosis.

Table 1  
Genotype and allele frequency by diagnostic group (baseline subjects)

	CON	MCI	AD	Pearson Chi-square test
Total (N = 738)	206	359	173	
Genotype frequency				
TT	126 (61%)	203 (57%)	85 (49%)	P = .091
TC	68 (33%)	131 (36%)	68 (39%)	
CC	12 (6%)	25 (7%)	20 (12%)	

Table 2  
Allele frequency in the AD versus CON group

	Allele frequency		Pearson Chi-square test	Odds ratio (confidence interval)
	C	T		
AD	108	238	P = .006	1.578 (1.141–2.184)
CON	92	320		

Abbreviations: AD, Alzheimer's disease; CON, healthy elderly controls.

However, carrying the minor C allele was associated with greater overall ventricular expansion over a period of 2 years (P = .017 and P = .037 for the recessive and additive models, respectively), after controlling for sex, age, and dementia status (Table 6). These results remained significant after introducing APOE genotype as an additional covariate (Table 6).

#### 3.2. Post-hoc analyses

##### 3.2.1. Ventricular expansion within each hemisphere

Carrying the minor C allele was associated with greater expansion in the left (P = .013 and P = .035 for the recessive and additive models, respectively) and right ventricle (P = .035 and P = .034), after controlling for age, sex, and diagnosis (Table 6). These results remained significant after controlling for APOE genotype (Table 6).

##### 3.2.2. Genotype by diagnosis interaction

We found no significant genotype by diagnosis interaction in the analyses of total (P = .715 and P = .320 for the recessive and additive models, respectively), left (P = .784 and P = .677), or right ventricular expansion (P = .726 and P = .110, Table 6).

##### 3.2.3. Effects of genotype and diagnosis on participants' ages

Table 7 indicates mean baseline ages in participants stratified by diagnosis, and substratified by rs6347 genotype groups. Diagnosis was not significantly related to age after controlling for sex, rs6347 genotype, and APOE status (P = .310, F-ratio = 1.173). Likewise, genotype at rs6347 was not significantly related to age after controlling for sex, diagnosis, and APOE status, although there was a trend for more C alleles to be associated with younger age (P = .078, F-ratio = 2.557).

Table 3  
Allele frequency in the AD versus MCI group

	Allele frequency		Pearson Chi-square test	Odds ratio (confidence interval)
	C	T		
AD	108	238	P = .006	1.578 (1.141–2.184)
MCI	92	320		

Table 4  
Allele frequency in the MCI versus CON group

	Allele frequency		Pearson Chi-square test	Odds ratio (confidence interval)
	C	T		
MCI	181	537	P 5 .277	1.172 (0.880–1.561)
CON	92	320		

Abbreviations: MCI, mild cognitive impairment; CON, healthy elderly controls.

### 3.2.4. Ethno-racial differences in allele frequency, linkage disequilibrium, and predicted target genes

As shown in Fig. 2, the minor C allele at the rs6347 locus is more frequent in Europeans (CEU and TSI) than in Asians (CHD and CHB). In people of African ancestry (ASN and YRI), the minor allele at rs6347 is not the C but the T allele. Fig. 3 illustrates the pattern of linkage disequilibrium (LD) across the DAT1 (SLC6A3) gene in the Caucasian-American (CEU) sample. It shows that rs6347 forms a haplotype block with two other variants: rs3776511 ( $D^0$  5 .70) and rs3776512 ( $D^0$  5 .71), both of which are intronic SNPs with no known effects on gene function.

Regulatory elements can be located by mapping DNase I Hypersensitive Sites (DHSs), which indicate open or accessible chromatin where DNA is not tightly wrapped within a nucleosome [23]. The first extensive map of human DHSs was recently identified through genome-wide profiling in more than 100 human cell types [24]. The DNase I data are publicly accessible through the UCSC Genome Browser. Sheffield and colleagues created a database and web interface to help visualize results from genome browsers [25]. The Regulatory Elements Database can be used to make predictions about the linkage between regulatory regions and

Table 5

Total baseline ventricular volumes ( $V_B$ ) and total volume loss ( $V_L$ ) over a period of 2 years (in cubic mm) by diagnostic and genotype groups: mean  $\pm$  SEM

	CON	MCI	AD	Total
TT				
$V_B$	49,041 (6 1562)	49,253 (6 1197)	47,052 (6 1454)	48,735 (6 811)
$V_L$	3552 (6 292)	6482 (6 499)	11,363 (6 1060)	6364 (6 361)
TC				
$V_B$	47,605 (6 2133)	48,629 (6 1341)	45,798 (6 1972)	47,647 (6 989)
$V_L$	3627 (6 454)	5951 (6 512)	10,572 (6 1270)	6252 (6 434)
CC				
$V_B$	42,169 (6 4503)	47,680 (6 3505)	46,775 (6 3511)	46,202 (6 2167)
$V_L$	2352 (6 1085)	6923 (6 1769)	12,660 (6 1729)	8039 (6 1197)
Total				
$V_B$	48,167 (6 1215)	48,915 (6 868)	46,527 (6 1124)	48,145 (6 602)
$V_L$	3525 (6 242)	6304 (6 355)	11,205 (6 744)	6437 (6 271)

Abbreviations: SEM, standard error of the mean; CON, healthy elderly controls; MCI, mild cognitive impairment; AD, Alzheimer's disease.

genes, based on the statistical association of DHSs and gene expression across more than 100 samples consisting of over 70 diverse cell types [25]. We searched a region located 6 10 kb of rs6347 and a list of four DHSs found in this region was outputted. Two of these sites were predicted to regulate the expression of the adjacent LPCAT1 gene (P 5 .012 and P 5 .019), which encodes lysophosphatidylcholine acyltransferase. The other two were expected to regulate the neighboring CLPTM1L gene (P 5 .006 and P 5 .011), which codes for the cisplatin resistance-related protein 9. Fig. 4 illustrates the relative positions of the DAT1 gene (SLC6A3, red arrow) and flanking LPCAT1 and CLPTM1L genes on chromosome 5.

## 4. Discussion

This study is the first to report that a genetic variant in the DAT gene is associated with several brain-related health factors, including diagnostic, cognitive, and anatomical indices of brain aging and neurodegeneration. Genotype at the rs6347 locus was related to the trajectory of ventricular volume expansion within each hemisphere, assuming both recessive and additive models of minor allele effects, and all results remained significant after controlling for APOE status. Genotype and diagnosis were not significant predictors of participants' ages.

We reported a statistically significant association of the minor allele at rs6347 with greater cognitive impairment, but the effect size was small, and we did not establish the clinical significance of this association [26]. These differences in MMSE scores between genotype groups remained significant after controlling for diagnosis, suggesting that

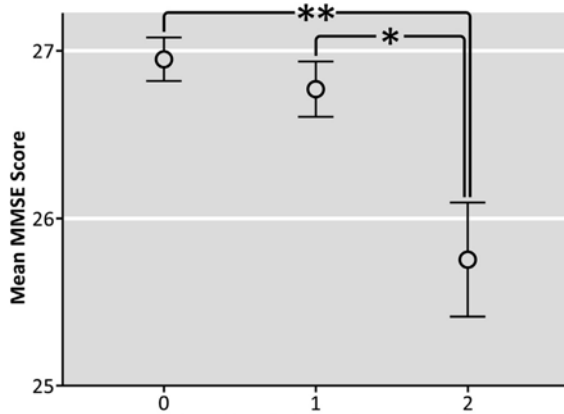


Table 6  
Results of multiple regression analyses: associations between rs6347 genotype and ventricular expansion (recessive and additive models)

	Genotype effects (3 covariates)*	Genotype effects (four covariates) <sup>y</sup>	Genotype by diagnosis interaction <sup>z</sup>
<b>Recessive model</b>			
Total expansion (cubic mm)	<b>F-ratio 5 5.710<sup>x,ij</sup></b> P 5 .017	F-ratio 5 6.198 P 5 .013	F-ratio 5 0.134 P 5 .715
Left expansion (cubic mm)	F-ratio 5 6.230 P 5 .013	F-ratio 5 5.817 P 5 .016	F-ratio 5 0.075 P 5 .784
Right expansion (cubic mm)	F-ratio 5 4.460 P 5 .035	F-ratio 5 4.684 P 5 .031	F-ratio 5 0.123 P 5 .726
<b>Additive model</b>			
Total expansion (cubic mm)	F-ratio 5 3.330 P 5 .037	F-ratio 5 3.873 P 5 .021	F-ratio 5 1.142 P 5 .320
Left expansion (cubic mm)	F-ratio 5 3.384 P 5 .035	F-ratio 5 3.630 P 5 .027	F-ratio 5 0.390 P 5 .677
Right expansion (cubic mm)	F-ratio 5 3.407 P 5 .034	F-ratio 5 3.744 P 5 .024	F-ratio 5 2.216 P 5 .110

\*rs6347 genotype (top: recessive model; bottom: additive model) was used to predict variations in ventricular expansion, with age, sex, and diagnosis regressed out.

<sup>y</sup>rs6347 genotype (top: recessive model; bottom: additive model) was used to predict variations in ventricular expansion, with age, sex, diagnosis, and APOE status regressed out.

<sup>z</sup>Significance of the genotype by diagnosis interaction term using the following equation: expansion measure 5 constant 1 genotype 1 age 1 sex 1 diagnosis 1 genotype ! diagnosis.

<sup>x</sup>In multiple regressions, the F-ratio is used to test the hypothesis that the slopes of the regression lines are 0. The F is large when the independent variable helps to explain the variation in the dependent variable, independently of the other explanatory variables that are regressed out. For instance, here we reject the hypothesis that the slope of the regression line is 0 (F-ratio 5 5.710, P 5 .017), meaning that there is a significant linear relation between rs6347 genotype and total ventricular expansion, independent of age, sex, and diagnosis.

<sup>ij</sup>Bold font indicates significant results (P < .05), and regular font indicates results that did not reach statistical significance.

on the rate of brain aging, which may in turn mediate its weaker association with cognitive decline and diagnostic categories, as these are influenced by many other factors. Cognitive dysfunction in MCI does not always reflect neurodegenerative processes, and may be related to reversible conditions such as depression, delirium, vitamin B<sub>12</sub> deficiency, hypothyroidism, and anticholinergic drug use [27]. This may be why allele frequency significantly differed across the three diagnostic groups, but pairwise comparisons (in a less well powered sample) revealed no significant differences between the smaller MCI and CON groups.

By showing that the minor C allele at rs6347 was more common in AD than both in MCI and CON in the ADNI cohort, we replicated the association between this allele and severe dementia (initially reported in a Taiwanese sample [3]) in individuals of European descent. The minor C allele is more frequent in Caucasians than in Asians, and AD prevalence tends to be higher in individuals of European descent than in people of Asian ancestry. This is true both

when comparing ethno-racial groups within the United States [28] and in studies of AD prevalence across countries [29]. In this respect, our findings are consistent with the epidemiology of AD in different populations. In individuals of African descent, the minor allele is not the C but the T allele. In light of our results, people of African ancestry would be expected to have higher rates of AD than Caucasians and Asians, which is the case within the United States [30,31]. Intriguingly, however, worldwide studies of AD prevalence show that Sub-Saharan African countries have

Table 7  
Baseline age by diagnostic and genotype groups: mean 6 SEM

	CON	MCI	AD	Total
TT	76.45 (6 0.421)	75.59 (6 0.505)	75.61 (6 0.857)	75.86 (6 0.329)

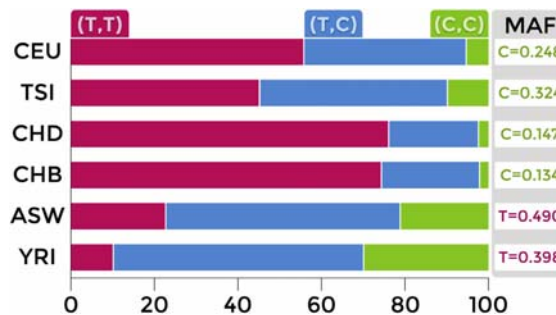


Fig. 2. Ethno-racial differences in genotype and minor allele frequencies at

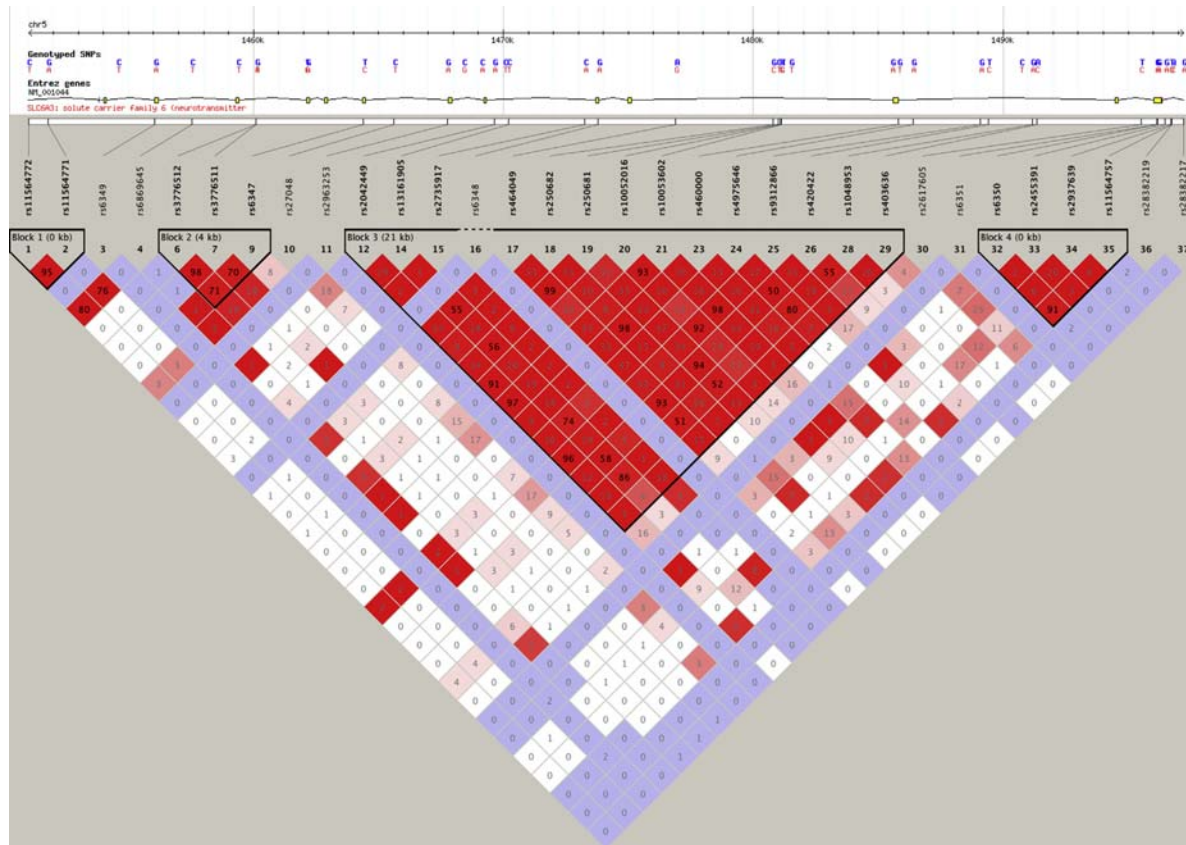


Fig. 3. Patterns of linkage disequilibrium (LD) in the DAT1 (SLC6A3) gene genotyped in the HapMap Caucasian-American (CEU) sample. The single nucleotide polymorphisms (SNPs) are shown on a scale representing their position on chromosome 5. The rs6347 variant is located in haplotype block 2. Figure modified from the HapMap website (<http://hapmap.ncbi.nlm.nih.gov/>).

lower AD rates than North American, European, and Asian countries [29]. Results from studies of AD prevalence across countries are complicated by a number of factors, most importantly life expectancy; therefore, a possible explanation for this discrepancy may be the much lower life expectancy in Sub-Saharan Africa [32]. This is only a conjecture, however, as cultural and educational differences, gene-environment interactions, admixture, and other factors make it difficult to compare reports of AD prevalence in various ethno-racial groups with findings of allele frequency differences between diagnostic categories.

The minor C allele at rs6347 is associated with increased DAT1 expression in vitro [33]. Synonymous mutations can have direct functional effects, but rs6347 may also act as a

surrogate marker for another regulatory polymorphism in high LD with this variant. Rs6347 is in high LD with a variable number tandem repeat (VNTR) in intron 8 of DAT1 ( $D^0 \cdot 0.7$ ) [33,34]. The six-repeat variant is often co-inherited with the major rs6347 T allele, and the five-repeat variant with the minor rs6347 C allele [8]. Consistent with the in vitro findings of Pinsonneault and colleagues [33], the five-repeat is associated with a 32%–34% increase in DAT expression in cell cultures [34]. It is still unclear which polymorphism (the exon 9 rs6347 SNP or the intron 8 VNTR) is the actual regulatory variant. In addition, even if the pattern of LD in the DAT1 gene suggests that rs6347 forms a haplotype block with two intronic SNPs that have no known effects on gene function, we cannot rule out the

possibility that rs6347 may be flagging a region that contains another functional variation.

Regardless of which SNP is the actual regulatory variant, individuals carrying these mutations in DAT1 are likely to express higher levels of DAT proteins, which pump DA into the cytoplasm, where DA is highly prone to spontaneous and enzymatic degradation. Free cytosolic DA has protons that dissociate from their corresponding hydroxyl groups, promoting the oxidation of DA to dopamine quinones (DAQ) [35] and other free radicals. DAQ are known to be involved in methamphetamine-induced neurotoxicity [36] and can lead to the stabilization of alpha-synuclein protofibrils [37], which may be involved in Lewy body formation and the etiology of Parkinson's disease [38]. Furthermore, DAQ can cause microglial activation [39].

In AD and other dementias, it has been shown that reduced striatal uptake *in vivo* using single photon emission computed tomography (SPECT) results from nigral dopaminergic cell loss [40]. It is thus possible that increased DAT levels associated with these variants may result in elevated DAQ concentrations and degeneration of DA neurons. Interestingly, striatal DAT loss is much more characteristic of dementia with Lewy bodies (DLB) than of AD [41], and autopsy studies show that DLB is very commonly misdiagnosed as AD [42]. An important tool for differential diagnosis during a patient's lifetime is the depiction of the dopaminergic system using SPECT [43]. In the ADNI cohort, SPECT was not used during the diagnostic process. It is unlikely that a large number of AD subjects in this cohort actually had "pure" DLB as patients with DLB show ventricular expansion rates comparable with those of healthy controls [44]—but we cannot rule out the possibility that some participants had mixed AD/DLB pathology. The rs6347 C allele may confer an increased risk for AD/DLB or other types of cognitive decline rather than AD. This would offer an alternative explanation for our finding that allele frequency did not significantly differ between the MCI and the control group, although all these comparisons are limited in statistical power.

While free cytosolic DA is associated with neurotoxic processes, several classes of compounds that increase dopaminergic transmission appear to have various neuroprotective effects. Monoamine oxidase B inhibitors can prevent neuronal loss by inducing neuroprotective genes, antioxidant enzymes, and redox proteins [45]. In addition, some dopamine D2/D3 receptor agonists block neuronal cell death under oxidative stress [46], and protect against glutamate toxicity via the inactivation of pro-apoptotic factors [47] and the upregulation of glutamate transporters [48]. Moreover, DA plays an important role in neuroplasticity [49–51], and the activation of D1/D5 receptors can prevent

in reduced DA transmission, which may offer a possible explanation for the findings reported here. In carriers of the minor allele, the reduction in neuroprotective effects resulting from decreased DA transmission may drive the faster ventricular expansion, as ventricular expansion reflects increasing neuronal, myelin, and other cellular loss in neighboring tissues [12].

Another possible mechanism underlying the observed association of rs6347 with brain aging and neurodegeneration may be related to the predicted target genes. CLPTM1L seems mainly involved in the etiology of lung cancer [56], but LPCAT1 is highly expressed in the brain [57]. Its gene product LPCAT is involved in the synthesis of phosphatidylcholine (PC) [58], a major component of biological membranes, and a precursor of choline used for the synthesis of acetylcholine (ACh) [59], implicated in many cellular processes [60], including neurogenesis [61]. PC also plays a role in Ab peptide aggregation [62], acts in conjunction with vitamin E to provide neuroprotection against oxidative damage, and can counteract the diminished oxidative buffering capacity of brains of APOE-deficient mice [63]. The involvement of PC in ACh synthesis and neuroprotection against AD pathology suggests that rs6347 may affect brain atrophy rates through its predicted regulation of LPCAT1 expression. Moreover, as DLB, but not AD, is associated with early and profound cholinergic depletion [64], this hypothesis is also consistent with the minor C allele conferring an increased risk for AD/DLB rather than for AD. These two major mechanisms may well occur in parallel, with rs6347 affecting both DAT and LPCAT expression. In fact, several lines of evidence suggest numerous types of interactions between cholinergic and dopaminergic transmission. PC is involved in the activation and regulation of protein kinase C [65], and protein kinases play important roles in DA receptor signaling [66]. Moreover, the ACh and DA systems are related at the cortical level [67], and DA and DA receptor agonists increase cortical excitability and restore central cholinergic transmission in AD [67,68]. Our theoretical framework remains speculative, but our findings highlight possible links between DA, ACh, and AD neuropathology, suggesting potential novel strategies to prevent dementia by targeting more than one neurotransmitter system.

We found that a DAT1 SNP, which was a strong predictor of brain atrophy rates, also showed a significant association with cognitive decline, and may confer an increased risk for dementia. Future studies should determine whether this polymorphism is indeed a regulatory variant (and if so, how it may affect protein expression) or whether it is simply flagging a region containing another functional variant. In the ADNI cohort, the minor C allele at rs6347 was more prevalent in AD than in both healthy control and MCI sub-

this variant may lead to disrupted transmission in specific brain circuits, which, over the years, results in a pattern of neuropathology associated with a mixed AD/DLB phenotype. Alternatively, this SNP may affect a range of neurodegenerative processes, which in turn mediates its association with AD, assuming that in some MCI subjects, the observed cognitive decline did not have a neurodegenerative etiology. In this case, this polymorphism may confer an increased risk for various types of dementias, even possibly for a range of degenerative brain disorders, and each individual's unique combination of genetic and environmental risk and protective factors ultimately results in the emergence of a specific disease phenotype.

This last possibility underscores the need to devise novel and personalized approaches for the prevention of degenerative brain—as opposed to the symptomatic treatment of a specific syndrome. A therapeutic approach that interferes directly with neurodegenerative processes could override the detrimental effects of certain genetic variants, before the onset of neuropathology [69]. Our findings, combined with earlier reports describing the involvement of DA transmission in neuroprotective processes, suggest that dopaminergic agents may be a useful addition to cholinergic agents for the prevention of neurodegeneration and associated cognitive decline, particularly in carriers of this and other mutations thought to affect DAT expression.

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The authors declare no conflict of interest.

#### RESEARCH IN CONTEXT

- 1 Systematic review: Genetic variants in DAT1, the gene encoding the dopamine transporter protein (DAT), have been implicated in many brain disorders. Genetic factors play a role in most Alzheimer's disease (AD) cases. We searched PubMed for published association studies of DAT1 variants and AD, and also reviewed studies about the role of dopaminergic transmission in neurodegenerative processes.
- 2 Interpretation: This study reveals that a common genetic variant in DAT1, previously linked with the clinical stages of dementia in humans, and with increased DAT expression in vitro, is associated with several brain-related health factors, including diagnostic, cognitive, and anatomical indices of neurodegeneration, providing new insight into the pathogenesis of AD.
- 3 Future directions: Findings from this study provide a strong basis to generate novel hypotheses and devise new experiments. In particular, it is important that future investigations: (a) clarify whether this polymorphism is indeed a regulatory variant (and if so, how it may affect protein expression) or is simply flagging a region that contains another functional variant; (b) elucidate the nature of this variant's association with dementia phenotypes; (c) further characterize the role of dopamine, and the significance of its interactions with cholinergic transmission, in the etiology and pathophysiology of AD and other dementias; and (d) investigate the safety and efficacy of dopaminergic agents—alone and in combination in cholinergic agents—in the prevention of cognitive decline and neurodegeneration, particularly in carriers of this and other mutations affecting dopaminergic transmission.



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