

Genetic Variation in the Ontario Neurodegenerative Disease Research Initiative

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ABSTRACT: Background/Objective: Apolipoprotein E (*APOE*) E4 is the main genetic risk factor for Alzheimer's disease (AD). Due to the consistent association, there is interest as to whether E4 influences the risk of other neurodegenerative diseases. Further, there is a constant search for other genetic biomarkers contributing to these phenotypes, such as microtubule-associated protein tau (*MAPT*) haplotypes. Here, participants from the Ontario Neurodegenerative Disease Research Initiative were genotyped to investigate whether the *APOE* E4 allele or *MAPT* H1 haplotype are associated with five neurodegenerative diseases: (1) AD and mild cognitive impairment (MCI), (2) amyotrophic lateral sclerosis, (3) frontotemporal dementia (FTD), (4) Parkinson's disease, and (5) vascular cognitive impairment. **Methods:** Genotypes were defined for their respective *APOE* allele and *MAPT* haplotype calls for each participant, and logistic regression analyses were performed to identify the associations with the presentations of neurodegenerative diseases. **Results:** Our work confirmed the association of the E4 allele with a dose-dependent increased presentation of AD, and an association between the E4 allele alone and MCI; however, the other four diseases were not associated with E4. Further, the *APOE* E2 allele was associated with decreased presentation of both AD and MCI. No associations were identified between *MAPT* haplotype and the neurodegenerative disease cohorts; but following subtyping of the FTD cohort, the H1 haplotype was significantly associated with progressive supranuclear palsy. **Conclusion:** This is the first study to concurrently analyze the association of *APOE* isoforms and *MAPT* haplotypes with five neurodegenerative diseases using consistent enrollment criteria and broad phenotypic analysis.

RÉSUMÉ: Étude de variance génétique dans le cadre de l'initiative de recherche sur les maladies neurodégénératives en Ontario. Contexte/ Objectif : L'apolipoprotéine E4 (ApoE4) constitue le principal facteur de risque génétique de la maladie d'Alzheimer. En raison de cette association systématique, il existe un intérêt certain à savoir dans quelle mesure cette classe d'apolipoprotéines peut influencer le risque d'autres maladies neurodégénératives. En outre, le milieu de la recherche n'a cessé d'identifier d'autres biomarqueurs génétiques, par exemple les haplotypes H1 de la protéine tau associée aux microtubules, qui contribuent à certains phénotypes. Dans le cadre de cette étude, des participants à l'initiative de recherche sur les maladies neurodégénératives en Ontario ont été « génotypés » afin de déterminer si l'ApoE4 ou l'haplotype H1 mentionné ci-dessus peuvent être associés à cinq maladies neurodégénératives : 1) la maladie d'Alzheimer et d'autres troubles cognitifs légers ; 2) la sclérose latérale amyotrophique ; 3) la démence fronto-temporale ; 4) la maladie de Parkinson ; 5) et finalement les déficits cognitifs d'origine vasculaire. **Méthodes :** Pour chaque participant, la

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cartographie des génotypes a été établie en fonction de leur ApoE4 respectif et de la présence d'haplotypes H1 de la protéine tau associée aux microtubules. Des analyses de régression logistique ont été ensuite effectuées dans le but d'identifier de possibles liens avec ces maladies neurodégénératives. **Résultats** : Nos travaux ont confirmé l'association entre l'ApoE4 et une plus grande occurrence de cas d'Alzheimer, et ce, en tenant compte de l'effet d'une dose de médicament. Ils ont aussi montré une association entre la seule ApoE4 et des troubles cognitifs légers. Cela dit, il convient de préciser que les quatre autres maladies n'ont pas été associées à cet allèle. Plus encore, nous avons trouvé que l'allèle E2 de l'apolipoprotéine était associé à une occurrence plus faible de cas d'Alzheimer et de troubles cognitifs légers. Fait à souligner, aucune association n'a été détectée entre l'haplotype H1 de la protéine tau associée aux microtubules et nos cohortes atteintes de maladies neurodégénératives. Toutefois, à la suite du sous-typage de la cohorte de participants atteints de démence fronto-temporale, il s'est avéré que l'haplotype H1 était associé de façon notable à la paralysie supra-nucléaire progressive. **Conclusion** : Il s'agit de la première étude à analyser simultanément, au moyen de critères de participation cohérents et d'une analyse phénotypique élargie, les associations entre les isoformes de l'ApoE, l'haplotype H1 de la protéine tau associée aux microtubules et cinq maladies neurodégénératives.

Keywords: Alzheimer's disease, Neurodegenerative disease, *APOE*, *MAPT*

Abbreviations: AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, *APOE* = apolipoprotein E, FTD = frontotemporal dementia, *MAPT* = microtubule-associated protein tau, MCI = mild cognitive impairment, ONDRI = Ontario Neurodegenerative Disease Research Initiative, PD = Parkinson's disease, PSP = progressive supranuclear palsy, SNP = single nucleotide polymorphism, VCI = vascular cognitive impairment

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INTRODUCTION

With the aging of populations, the burden of neurodegenerative diseases is increasing, and substantial effort is directed toward identification of genetic biomarkers with the objective of improved disease prediction and the long-term goal of discovering therapeutic targets. In particular, molecular genetics efforts have focused on identifying common single nucleotide polymorphisms (SNPs) that contribute to disease risk.^{1–3} Although these types of markers usually account for only a small proportion of disease risk, two closely linked common SNPs on chromosome 19 have been identified to jointly impart a relatively large effect on the risk of a particular neurodegenerative phenotype, namely, the apolipoprotein E (*APOE*) E4 allele and Alzheimer's disease (AD).⁴

APOE is found in chylomicrons, very-low-density lipoproteins, intermediate-density lipoproteins, and high-density lipoproteins; it provides structural support to these particles and also governs the catabolism of triglyceride-rich lipoproteins through its role as a receptor ligand. Importantly, *APOE* is the principal cholesterol carrier in the brain.⁵ There are three common protein isoforms of *APOE* – E2, E3, and E4 – historically designated based on protein mobility in isoelectrophoretic focusing gels.⁶ At the DNA level, these three isoforms are encoded by two nonsynonymous SNPs within the *APOE* gene, occurring at amino acid positions 130 and 176 (also numbered as 112 and 158, respectively, if the pro-peptide sequence is excluded), and each involving cysteine or arginine as alternate residues.⁷ The E4 allele, which has arginine at both positions 130 and 176, is the most common genetic risk factor for the development of late onset AD and contributes to disease risk in a dose-dependent manner.⁸ Meta-analyses show that one and two copies of the E4 allele raise AD risk by ~3- to 4- and ~12-fold, respectively.^{9,10} Due to the replicated high-risk association from several meta-analyses of AD and *APOE*,^{8,11,12} researchers have attempted to determine whether the E4 allele is also associated with other neurodegenerative diseases, including amyotrophic lateral

sclerosis (ALS),^{13,14} frontotemporal dementia (FTD),^{15,16} Parkinson's disease (PD),^{17–19} and vascular cognitive impairment (VCI),^{20–22} so far with mixed and inconsistent results.

Another gene less consistently associated with AD risk is the microtubule-associated protein tau gene (*MAPT*) that encodes the protein tau.²³ It remains to be established whether *MAPT* is associated with other neurodegenerative diseases. Within the *MAPT* gene, an ancestral inversion of ~900 kb has resulted in two distinct haplotypes, H1 and H2, and creates a large region of linkage disequilibrium. Apart from the few associations found between H1 *MAPT* and AD, there is debate as to whether the haplotype is associated with PD²⁴ and with the FTD subtype progressive supranuclear palsy (PSP).^{25,26}

The Ontario Neurodegenerative Disease Research Initiative (ONDRI) is a multi-platform, provincial-wide, observational cohort study aiming to characterize multiple attributes of five neurodegenerative diseases, namely, (1) AD and mild cognitive impairment (AD/MCI), (2) ALS, (3) FTD, (4) PD, and (5) VCI.²⁷ In addition to genomic analysis, ONDRI incorporates a comprehensive phenotypic assessment on each participant. The large data set, combined with the consistent enrollment criteria, allows for the unique opportunity to assess the association of *APOE* genotype and *MAPT* haplotype across the respective neurodegeneration phenotypes. Here we aim to replicate the known associations of the *APOE* E4 allele, *APOE* E4/4 genotype, and *MAPT* H1 haplotype with AD, in addition to assessing whether *APOE* E4 and *MAPT* H1 confer risk to ALS, FTD, PD, and VCI within the ONDRI cohort.

METHODS

Blood samples were collected from 519 ONDRI participants after informed consent was obtained, in accordance with the Research Ethics Boards at Hamilton General Hospital (Hamilton, Ontario, Canada); McMaster (Hamilton, Ontario, Canada); Parkwood Hospital (London, Ontario, Canada); London Health Sciences Centre (London, Ontario, Canada); The Ottawa Hospital

Table 1: Demographics of the 519 ONDRI participants and 189 controls genotyped for *APOE* and haplotyped for *MAPT*

	ONDRI	AD/MCI	ALS	FTD	PD	VCI	Controls
Cases	519	126	40	53	139	161	189
Mean Age (years \pm sd)	68.6 \pm 7.7	71.0 \pm 8.2	62.0 \pm 8.7	67.8 \pm 7.0	67.9 \pm 6.3	69.2 \pm 7.4	74.0 \pm 8.2
Min. Age (years)	40.1	53.4	40.1	49.7	55.1	54.9	48.0
Max. Age (years)	87.8	87.8	77.2	80.9	85.9	85.4	92.0
Male: Female	345:174	69:57	24:16	34:19	108:31	110:51	77:112

AD/MCI = Alzheimer's disease/mild cognitive impairment; ALS = amyotrophic lateral sclerosis; *APOE* = apolipoprotein E gene; FTD = frontotemporal dementia; *MAPT* = microtubule-associated protein tau gene; Max = maximum; Min = minimum; ONDRI = Ontario Neurodegenerative Disease Research Initiative; PD = Parkinson's disease; sd = standard deviation; VCI = vascular cognitive impairment.

Table 2: *APOE* allele and genotype frequencies in 519 ONDRI participants and 189 controls. All study participants were genotyped using both the ONDRISeq panel and TaqMan allelic discrimination assay

	<i>APOE</i> Genotype [n (%)]						<i>APOE</i> Alleles [n (%)]		
	E2/2	E3/2	E4/2	E3/3	E4/3	E4/4	E2	E3	E4
AD/MCI	0	5 (4.0)	1 (0.8)	59 (46.8)	43 (34.1)	18 (14.3)	6 (2.4)	166 (65.9)	80 (31.7)
ALS	0	6 (15.0)	0	21 (52.5)	12 (30.0)	1 (2.5)	6 (7.5)	60 (75.0)	14 (17.5)
FTD	1 (1.9)	6 (11.3)	0	26 (49.0)	18 (34.0)	2 (3.8)	8 (7.5)	76 (71.7)	22 (20.8)
PD	1 (0.7)	19 (13.7)	3 (2.2)	86 (67.9)	28 (20.1)	2 (1.4)	24 (8.6)	219 (78.8)	35 (12.6)
VCI	0	21 (13.0)	2 (1.2)	100 (62.1)	34 (21.1)	4 (2.5)	23 (7.1)	255 (79.2)	44 (13.7)
Controls	2 (1.1)	29 (15.3)	6 (3.2)	110 (58.2)	35 (18.6)	7 (3.7)	39 (10.3)	284 (75.1)	55 (14.6)

AD/MCI = Alzheimer's disease/mild cognitive impairment; ALS = amyotrophic lateral sclerosis; *APOE* = apolipoprotein E gene; FTD = frontotemporal dementia; ONDRI = Ontario Neurodegenerative Disease Research Initiative; PD = Parkinson's disease; VCI = vascular cognitive impairment.

(Ottawa, Ontario, Canada); University Health Network-Elizabeth Bruyère Hospital (Ottawa, Ontario, Canada); Baycrest Centre for Geriatric Care (Toronto, Ontario, Canada); Centre for Addiction and Mental Health (Toronto, Ontario, Canada); St Michael's Hospital (Toronto, Ontario, Canada); Sunnybrook Health Sciences Centre (Toronto, Ontario, Canada); and Toronto Western Hospital (Toronto, Ontario, Canada). Formal diagnoses and demographic data were obtained by participants' clinicians upon enrollment in the study, in accordance with ONDRI standard operating protocols.²⁷

Genomic DNA was isolated from blood samples collected from each participant, as described previously.²⁸ DNA samples were also obtained from 189 cognitively normal controls from the GenADA study.²⁹ All samples underwent targeted next-generation sequencing using the ONDRISeq neurodegenerative disease gene panel. Full methodology of DNA isolation, sequencing with the ONDRISeq panel,²⁸ and raw sequencing data processing were previously described.³⁰

Allele calls for the *APOE* risk alleles rs429358(CT):p.Cys130Arg and rs7412(CT):p.Arg176Cys were extracted from the ONDRISeq data files and mapped to their respective *APOE* genotype for each participant using a customized Annotate Variation³¹ script. Allele calls and mapped genotypes were validated with TaqMan allelic discrimination assay,³² as previously described.²⁸

TaqMan was also used to determine the tau haplotype of the ONDRI participants and control samples. DNA samples were genotyped for the intronic SNP rs1800547, which is not covered

by the ONDRISeq panel. Based on a region of linkage disequilibrium, allele calls were mapped to their respective *MAPT* haplotype.³³

Statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, USA). The Wilcoxon Mann-Whitney *U* test was utilized to determine the difference between the ages of the control cohort compared to the five disease cohorts of interest. Chi-square analyses were used to determine the difference between the control cohort and disease cohorts' male-female ratios. Odds ratios (ORs) and confidence intervals (CIs) were obtained using logistic regression, adjusting for participants' age and sex.

RESULTS

Table 1 displays the demographics of the 519 ONDRI participants included in this study as well as the cognitively normal controls. Of the ONDRI participants, 83.0% self-reported their ethnicity as Caucasian. The ALS cohort had the lowest mean age (62.0 \pm 8.7 years), and the control cohort had the highest mean age (74.0 \pm 8.2 years), which was significantly different from the mean age of the five ONDRI disease cohorts ($P < 1.0 \times 10^{-4}$). Additionally, the male-female ratio of the control cohort was significantly different from that of the overall ONDRI cohort ($P < 1.0 \times 10^{-4}$).

Calls of the *APOE* alleles were obtained using the ONDRISeq panel and validated using the TaqMan allelic discrimination assay with 100% concordance. Allele and genotype frequencies were calculated for the five neurodegenerative disease cohorts and the controls (Table 2). As expected, the AD/MCI cohort

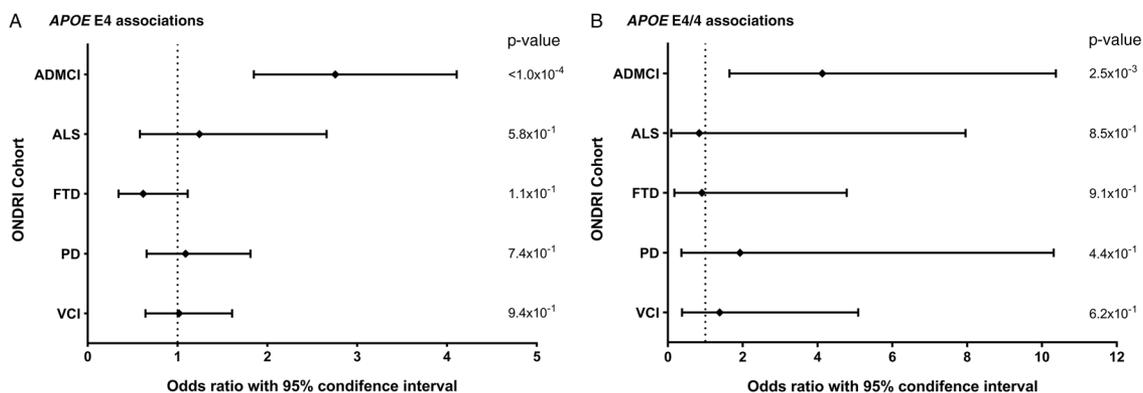


Figure 1: Forest plots of the relationship between *APOE* and risk of each of the diseases encompassed by ONDRI. Logistic regressions adjusting for participant age and sex analyzed the *APOE* E4 allele and E4/4 genotype status of the ONDRI cohorts when compared to controls. (A) Forest plot of the *APOE* E4 allele and associated risk of each ONDRI disease cohort. (B) Forest plot of the *APOE* E4/4 genotype and associated risk of each ONDRI disease cohort.

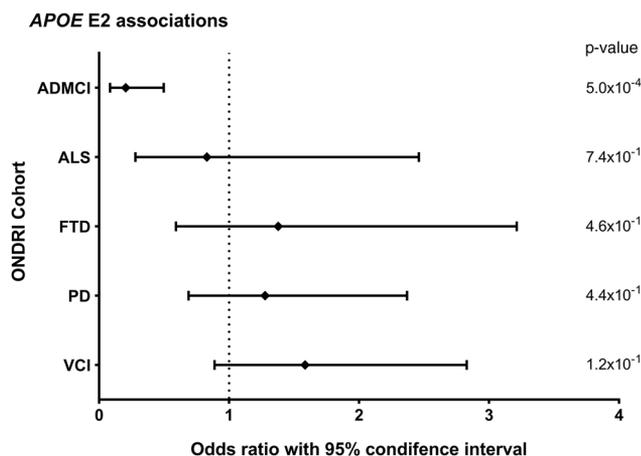


Figure 2: Forest plot of the relationship between the *APOE* E2 allele and risk of each of the diseases encompassed by ONDRI. Logistic regressions adjusting for participant age and sex analyzed the *APOE* E2 allele status of the ONDRI cohorts when compared to controls.

displayed the highest *APOE* E4 allele frequency (31.7%) and E4/4 genotype frequency (14.3%), compared to 14.6% and 3.7%, respectively, in controls. The AD/MCI cohort also displayed the lowest *APOE* E2 allele frequency (2.4%), compared to 10.3% in controls. The lowest *APOE* E4 allele and E4/4 genotype frequencies were observed in the PD cohort (12.6% and 1.4%, respectively), differing marginally from the respective frequencies in controls.

Allele and genotype calls were compared between each ONDRI disease cohort and the control cohort, while adjusting for both age and sex of participants (Figure 1A and 1B). The E4 allele was significantly associated with increased presentation of AD/MCI compared to controls (OR=2.76, 95% CI=1.85–4.11, $P<1.0\times 10^{-4}$). Similarly, the E4/4 genotype significantly increased the presentation of AD/MCI when compared to controls (OR=4.13, 95% CI=1.64–10.37, $P=2.5\times 10^{-3}$). As expected, the E2 allele was associated with a significantly decreased presentation of AD/MCI when compared to controls after adjusting for age and sex (OR=0.21, 95% CI=0.08–0.50, $P=5.0\times 10^{-4}$; Figure 2).

No association with *APOE* was found with the other four phenotypes in the ONDRI data set.

The AD/MCI cohort was split into participants presenting with AD (n=41) and those presenting with MCI (n=85) and *APOE* analyses were repeated. The AD and MCI subcohorts displayed E4 allele frequencies of 46.3% and 24.7% and E4/4 genotype frequencies of 26.8% and 8.2%, respectively. Indeed, the E4 allele was significantly associated with presentation of both AD and MCI compared to the controls (OR=5.24, 95% CI=3.07–8.92, $P<1.0\times 10^{-4}$ and OR=1.94, 95% CI=1.22–3.07, $P=4.9\times 10^{-3}$, respectively), and the E2 allele was significantly associated with decreased presentation of both AD and MCI compared to the controls (OR=0.10, 95% CI=0.01–0.77, $P=0.0268$ and OR=0.26, 95% CI=0.10–0.68, $P=5.8\times 10^{-3}$, respectively). The E4/4 genotype was also significantly associated with increased presentation of AD (OR=10.36, 95% CI=3.55–30.19, $P<1.0\times 10^{-4}$); however, the genotype was not significantly associated with the presentation of MCI.

Allele calls of the intronic *MAPT* variant, rs1800547, were mapped to their respective *MAPT* haplotype for each DNA sample. The ALS cohort had the highest frequencies of H1 haplotype and H1/H1 diplotype (87.5% and 75.0%, respectively), whereas the FTD cohort displayed the lowest frequencies (75.5% and 60.4%, respectively; Table 3). There were no significant associations found between *MAPT* and any of the disease phenotypes in ONDRI when compared to controls following adjustment for both age and sex.

Due to its previous associations with the PSP subtype of FTD, the FTD cohort was split into its respective subtypes, including behavioral variant FTD (bvFTD; n=22), corticobasal syndrome (CBS; n=3), progressive non-fluent aphasia (PNFA; n=8), PSP (n=15), and semantic dementia (SD; n=5), and *MAPT* analyses were repeated. Interestingly, the SD subcohort displayed the greatest *MAPT* H1 haplotype frequency and the CBS subcohort displayed the lowest, at 90.0% and 50.0%, respectively. Similarly, the SD subcohort, along with the PSP subcohort, displayed the greatest H1H1 diplotype frequency of 80.0%, while the CBS subcohort displayed the lowest of 33.3%. We also observed that the H1 haplotype was significantly associated with increased PSP prevalence (OR=7.46, 95% CI=2.39–23.29, $P=5.0\times 10^{-4}$) following adjustment for age and sex; however, the H1H1 diplotype

Table 3: *MAPT* haplotype and diplotype frequencies in 519 ONDRI participants and 189 controls. All study participants were genotyped for the intronic SNP rs1800547 using TaqMan allelic discrimination assay and results were mapped to their respective haplotype

	<i>MAPT</i> Haplotype [n (%)]		<i>MAPT</i> Diplotype [n (%)]		
	H1	H2	H1/H1	H1/H2	H2/H2
AD/MCI	200 (79.4)	52 (20.6)	75 (59.5)	50 (39.7)	1 (0.8)
ALS	70 (87.5)	10 (12.5)	30 (75.0)	10 (25.0)	0
FTD	80 (75.5)	26 (24.5)	32 (60.4)	16 (30.2)	5 (9.4)
PD	227 (81.7)	51 (18.3)	93 (66.9)	41 (29.5)	5 (3.6)
VCI	264 (82.0)	58 (18.0)	108 (67.1)	48 (29.8)	5 (3.1)
Controls	293 (77.5)	85 (22.5)	111 (58.7)	71 (37.6)	7 (3.7)

AD/MCI = Alzheimer's disease/mild cognitive impairment; ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia; *MAPT* = microtubule-associated protein tau gene; ONDRI = Ontario Neurodegenerative Disease Research Initiative; PD = Parkinson's disease; VCI = vascular cognitive impairment.

did not display significant associations with PSP presentation. In addition, there were no significant associations between *MAPT* and any of the other FTD subtypes.

DISCUSSION

This is the first genetic characterization of the ONDRI cohort, which is important for upcoming multimodal, multi-year, prospective observational studies of the five phenotypes (e.g., *APOE/MAPT*-based stratification). The principal findings from the current study are as follows: (1) a dose-dependent association of the *APOE* E4 allele with AD and an association between E4 and MCI, (2) an inverse association of the *APOE* E2 allele with AD and MCI presentation, (3) a lack of associations between the *APOE* alleles and other neurodegenerative diseases included in the ONDRI mandate (i.e. ALS, FTD, PD, or VCI), and (4) no associations between any of the five neurodegenerative disease cohorts and the *MAPT* H1 haplotype, but a significant association between H1 and the PSP subtype of FTD.

Our study design offers a unique opportunity to analyze individuals with one of five neurodegenerative diseases enrolled with strict inclusion criteria and evaluated across a wide range of platforms.²⁷ Because of this robust workflow, we can investigate the effect of the *APOE* alleles and genotypes and *MAPT* haplotypes across multiple diseases with common assessment. The control cohort had a significantly older mean age than the ONDRI disease cohorts, as well as a significantly different male: female ratio. For this reason, logistic regression was applied to obtain ORs adjusted for both the age and sex of participants.

The E4 allele frequency in previously reported AD patients is 28–37%, while in controls it is 8–14%.^{12,34} The results presented here are comparable to these literature values, with E4 allele frequencies of 31.7% and 14.5% in the AD/MCI and control cohorts, respectively. More specifically, we observed an *APOE* E4 allele frequency of 46.3% in individuals with AD and 24.7% in individuals with MCI. Although the E4 allele frequency was significantly increased in cohorts of MCI compared to controls, the increase is not as great as that seen in those with AD only. Interestingly, the E4 allele has been shown to be a predictive risk factor for the clinical conversion from MCI to AD,^{35–37} which,

coupled with the increased E4 allele frequency in the MCI subcohort, may indicate that a portion of the individuals enrolled in ONDRI with MCI will experience disease progression to AD. The longitudinal nature of the ONDRI study will permit follow-up of the individuals with MCI to determine whether their *APOE* status predicts possible progression to AD and to evaluate the phenotypic measures most severely affected by their status.

In accordance with previous literature,⁹ we identified that *APOE* E4 was associated with AD in a dose-dependent manner following adjustment for age and sex. The presence of the E4 allele produced an approximately five-fold increased risk of having AD/MCI, which was marginally greater than the estimates previously found. However, we identified the E4/4 genotype to increase risk just over 10-fold, marginally lower than the commonly reported 12-fold.^{9,10} The slight discrepancies are likely due to the modest number of individuals enrolled in ONDRI with AD.

Previous studies have also suggested that the E2 allele decreases the risk of AD, which we also observed with our AD/MCI cohort.^{12,38} It is hypothesized that the stability provided by the cysteine-to-arginine variant at amino acid 176 may be contributing to this protective effect³⁹ and allows the isoform to more effectively clear amyloid- β ,⁴⁰ protect against synaptic degeneration,⁴¹ and facilitate antioxidant activity.⁴² However, because of the small sample sizes within ONDRI, no individuals in the AD/MCI cohort harbored the E2/2 genotype, and precise genotypic risk associations could not be evaluated. Despite the absence of individuals with the E2/2 genotype in the AD/MCI cohort, it is expected that two copies of the E2 allele would incrementally decrease the risk of the disease in a dose-dependent manner, particularly in those with AD, but larger cohorts would be needed to validate this hypothesis.

Although an association was not observed between *MAPT* H1 and the total FTD cohort, we did observe an association between the haplotype and increased presentation of PSP, as has been previously identified.²⁶ Yet, we were not able to replicate the previously observed increased prevalence of PSP associated with the H1H1 diplotype, again possibly as a result of the modest number of individuals with PSP. Interestingly, the SD subcohort of FTD displayed the highest *MAPT* H1 haplotype frequency and

the same H1H1 diplotype frequency as the PSP subcohort but was not significantly associated with *MAPT*. We expect that the very low number of individuals enrolled in ONDRI with SD may be the driving factor in the lack of association observed here and believe that further analysis into the association between *MAPT* H1 and SD is warranted with larger sample sizes.

Due to the strong association between both the E4 allele and E4/4 genotype and AD status, many studies have attempted to identify associations with other neurodegenerative disorders.⁴³ However, these studies reported inconsistent results regarding the risk associated with *APOE* E4 and onset and/or progression of the other four diseases studied in ONDRI, namely, ALS,^{13,14} FTD,^{15,16} PD,^{17–19} and VCI.^{20–22} Within the ONDRI cohort, no associations were identified for the other disease phenotypes. Similarly, no associations were identified between the *MAPT* H1 haplotype or H1/H1 diplotype and any of the five complete neurodegenerative disease cohorts. Absence of associations could have been due to small sample sizes, and thus false negative inferences, or to the true lack of a biological effect of E4 and H1 in these conditions. Associations previously reported may have been due to the diagnostic challenges associated with neurodegenerative diseases. Admixture of AD pathology in individuals with other neurodegenerative diseases may produce false positive associations with E4, and copathologies within neurodegenerative diseases are far more common than previously appreciated.⁴⁴ Due to the spectrum of overlapping features that can be observed across neurodegenerative phenotypes, it will be important to identify those that are associated with the *APOE* E4 allele and *MAPT* H1 haplotype to better understand patient prognosis. Future analyses will utilize ONDRI's robust assessment of structural and cognitive measures to identify whether common phenotypes across the various diseases are influenced by *APOE* and *MAPT*.

While ONDRI is unique in terms of the number of different clinical conditions evaluated simultaneously within the process, there is still a limitation due to modest sample sizes. Larger cohorts may produce results that align more closely with those previously reported. Additionally, an important limitation to this study is the lack of correlation with cognitive status within the disease cohorts. Assessments of cognitive impairment are ongoing, and future studies will incorporate these measures from the participants in order to assess the effects of the *APOE* E4 allele and *MAPT* haplotype on cognitive status within all five disease cohorts.

In conclusion, E4 allele carriers in the ONDRI study displayed a dose-dependent increased risk of AD/MCI, specifically in those diagnosed with AD, which is consistent with the current *APOE* literature. Similarly, this study was concordant with recent evidence that the *APOE* E2 allele decreases the risk of AD/MCI. Further, the *MAPT* H1 haplotype was significantly associated with the PSP subtype of FTD. The work also confirmed that risks of the other four diseases evaluated within ONDRI, namely, ALS, FTD, PD, and VCI are not associated with the E4 allele or E4/4 genotype and that none of the complete neurodegenerative disease cohorts are associated with the *MAPT* H1 haplotype or H1/H1 diplotype. To our knowledge, this is the first study to analyze *APOE* genotypes and *MAPT* haplotypes across these five neurodegenerative diseases using common enrollment criteria and comprehensive phenotypic analysis. Future studies will investigate the structural and cognitive symptoms of

neurodegeneration influenced by the E4 allele and H1 haplotype and the contributions of other genetic factors to these phenotypes.

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STATEMENT OF AUTHORSHIP

All authors have approved this manuscript.

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