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APOE Genotype and Entorhinal Cortex Volume in Non-Demented Community-Dwelling Adults in Midlife and Early Old Age

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Abstract. The apolipoprotein E (*APOE*) ε4 allele is a risk factor for the neuropathological decline accompanying Alzheimer's disease (AD) while, conversely, the ε2 allele offers protection. One of the brain structures exhibiting the earliest changes associated with the disease is the entorhinal cortex. We therefore investigated the volumes of the entorhinal cortex and other structures in the medial temporal lobe including the parahippocampal gyrus, temporal pole, and inferior, middle, and superior temporal cortices, in relation to *APOE* genotype. Our main objectives were to determine if (a) volumes systematically varied according to allele in a stepwise fashion, ε2 > ε3 > ε4, and (b) associations varied according to age. We investigate this association in 627 non-demented community-dwelling adults in middle age (44 to 48 years; $n = 314$) and older age (64 to 68 years; $n = 313$) who underwent structural MRI scans. We found no evidence of *APOE*-related variation in brain volumes in the age groups examined. We conclude that if a ε2 > ε3 > ε4 pattern in brain volumes does emerge in non-demented adults living in the community in old age, it is not until after the age of 68 years.

Keywords: Age, Alzheimer's disease, *APOE*, entorhinal cortex

Recent work [1] in children, adolescents, and young adults up to age 21 years has shown that the entorhinal cortex thickness varies according to apolipoprotein E (*APOE*) genotype in a stepwise pattern; ε2 carriers' entorhinal cortex thickness exceeds ε3 carriers who, in turn, exceed ε4 carriers. It is well established that in later life, possession of the ε4 allele is associated with cognitive deficits [2, 3], cerebral atrophy, and functional changes [4], and is a risk factor for Alzheimer's disease (AD) (e.g., [5]). By contrast, the ε2 allele may confer protection against age-related neuropathology in old age (e.g., [6]).

If the *APOE*-related variation in entorhinal cortex thickness found in young persons extends into middle and late adulthood, it may provide a

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neurobiological mechanism that mediates vulnerability to cognitive impairment and AD. That is, the greater the entorhinal cortex thickness, the greater the protection offered. This possibility is of some importance as there is evidence that the entorhinal cortex is one of the first areas of the brain to exhibit and be severely affected by the neuropathology associated with AD [7–9]. Indeed, such neuropathological changes have been detected in young adulthood and middle age [10, 11]. It is possible that in ε2 carriers, the critical threshold through which an individual must pass before neurological impairment is manifest may be higher due to the greater cortical thickness. Conversely, in ε4 carriers, that threshold may be lower due to the reduced entorhinal cortex thickness [1].

Studies that have looked specifically at the entorhinal cortex as a function of *APOE* genotype in cognitively intact adults are relatively consistent in their findings. For example, a study [12] of 25 persons with a family history of AD and 25 persons without this risk factor (overall mean age = 62.3 years) found that a family history of AD and possession of the ε4 allele was associated additively with thinner entorhinal cortex thickness. Although not taking family history into account, similar results were obtained in another investigation of 14 ε4 carriers and 16 non-carriers (overall mean age = 57 years); ε4 carriers exhibited thinner entorhinal cortex thickness than non-carriers [13]. Against this work showing ε4 carriers to have reduced entorhinal cortex sizes, longitudinal research produces mixed results. For example, work investigating the entorhinal cortex and hippocampal atrophy over an approximate 3.5-year period in 42 persons aged 58 to 87 years found no evidence that the rate of atrophy varied according to *APOE* genotype [14]. By contrast, another study over a two year period [15] found significantly greater entorhinal cortex thinning in 16 cognitively intact ε4 carriers relative to 16 non-carriers, mean age 61 years.

On balance, much of the work in cognitively intact adults suggests that the entorhinal cortex volume or thickness is reduced in ε4 carriers relative to non-carriers. However, several features of this work are of note. First, all of the studies have relatively small sample sizes. Second, they all compare ε4 carriers to non-carriers and none have contrasted entorhinal cortex volumes or thickness for ε2 versus ε3 versus ε4 genotypes. Finally, the studies have either investigated restricted age ranges in older adults, or where a wider age range has been employed, the sample size is not sufficiently large to allow for a robust test of age effects on *APOE*-entorhinal cortex associations.

While research has shown the entorhinal cortex thickness to vary according to *APOE* genotype (i.e., ε2 > ε3 > ε4) in children, adolescents, and young adults [1], no work has systematically explored whether this association extends into middle age, and becomes more marked in older age in a large-scale population-based sample. As this may provide important information on later life vulnerability to cognitive impairment and AD, we addressed this research shortfall in a large sample of non-demented community-dwelling adults aged 44 to 48, and 64 to 68 years. Specifically, we investigated if entorhinal cortex volumes in these age-groups varied according to *APOE* genotype. Given the neuroanatomical proximity to the entorhinal cortex and potential vulnerability to the early pathological changes accompanying AD, we also examined volumes for parahippocampal gyrus, temporal pole, and inferior, middle, and superior temporal cortices. Our key question was do *APOE* genotype volume differences in the entorhinal cortex and nearby structures occur in middle age in a stepwise fashion (i.e., ε2 > ε3 > ε4), and importantly, do any differences become more marked or change in early old age?

METHODS

Participants

Participants were recruited from the Personality and Total Health (PATH) Through Life study [16]. This is a population-based study where the inclusion criteria were (a) being listed on the electoral roll for Canberra and nearby Queanbeyan, Australia, and (b) being within three narrow age range cohorts of 20–24, 40–44, and 60–64 years at baseline in 1999, 2000, and 2001, respectively. Participants were followed up every four years. In contrast to clinical studies where there are strict inclusion and exclusion criteria, the PATH study adopts an epidemiological approach where participants were recruited to be as representative of the catchment area and age cohort as possible. Study procedures complied with guidelines on human experimentation and approval for the study was obtained from the Human Research Ethics Committee of the Australian National University.

This investigation concerns the second wave of data for the middle aged and early old aged participants, aged 44–48 and 64–68 years, respectively. The initial sample at Wave 2 consisted of 852 (431 + 421) individuals who participated in the MRI substudy. From the initial pool, participants who were suffering from neurological disorders were removed as follows:

epilepsy ($n = 7$), stroke ($n = 26$), head injury ($n = 88$). People with data missing on *APOE* genotype ($n = 45$) along with those with *APOE* genotype ε2/4 (see below) were also removed ($n = 26$). All participants in the older group were screened with the Mini-Mental State Examination [17]. In order to eliminate possible dementia cases, four persons were excluded as their scores were below 24.

MRI acquisition

MRI acquisition parameters have been previously described in detail elsewhere [18, 19]. Briefly, participants were scanned on a 1.5T Philips Gyroscan scanner (ACS-NT, Philips Medical Systems, Best, the Netherlands) for T1-weighted 3D structural MRI in coronal orientation and Fast Field Echo sequence, TR = 8.93 ms, TE = 3.57 ms, flip angle of 8°, matrix size = 256 × 256, slices 160, and field of view (FOV) 256×256 mm, yielding contiguous slices with thickness of 1.5 mm. In addition and due to factors outside the investigators' control, 268 middle aged subjects were scanned on another scanner of the same type. For those, the TR = 8.93 ms and TE = 3.57 ms values were slightly adjusted to improve image quality, but all other parameters were kept constant. In the middle aged group this variation was not associated with significant differences in age, years of education, total intracranial volume, gray matter volume, white matter volume, or cerebrospinal fluid volume.

Image analysis

Volumetric segmentation was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). This processing includes motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure [20], automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures (including for the present investigation, left and right volumes for the entorhinal cortex, parahippocampal gyrus, temporal pole, and inferior, middle, and superior temporal cortices), tessellation of the gray white matter boundary, and topology correction [21–24]. The validity and reliability of the Freesurfer package has been assessed in a number of recent studies and was found to be very good [18]. One scan was lost during acquisition from the middle age-group and the scans of an additional 28 participants were excluded from the sample due to poor scan quality, low signal-to-noise ratio, or movement artifacts which did not allow for normal processing with the standard Freesurfer pipeline. Each segmented volume was inspected slice by slice and reprocessed with additional parameters if errors were detected. Following these exclusions the final sample numbered 627 persons (314 and 313 in the middle aged and older groups, respectively). The sample composition according to age-group and *APOE* genotype is presented in Table 1.

APOE genotyping

Genomic DNA was extracted from buccal swabs using QIAGEN DNA Blood kits (#51162; QIAGEN, Hilden, Germany). Two single-nucleotide polymorphisms (SNPs; *rs429358* and *rs7412*) were genotyped to identify *APOE* genotypes comprised of the *APOE* ε2, ε3, and ε4 alleles using TaqMan assays (Applied Biosystems [ABI], Foster City, CA) as described elsewhere [25]. As ε2 is regarded as a protective factor and ε4 a risk factor (ε3 is regarded as neutral), persons with the ε2/4 genotype were removed from the sample as they may weaken the contrast of primary interest between ε2 and ε4. For this investigation, *APOE* group composition was: ε2 = ε2/2 + ε2/3; ε3 = ε3/3; ε4 = ε3/4 + ε4/4.

Episodic memory

Immediate and *delayed recall* was assessed as part of a wider neuropsychological battery using the first trial of the California Verbal Learning Test [26]. Participants were required to remember 16 shopping list items and to recall them immediately and again after a delay of twenty minutes. As the two scores were highly intercorrelated (0.87, $p < 0.001$), their mean is reported here.

Health variables

Further to the exclusions detailed earlier, several health variables were taken into account in the statistical analyses: heart trouble, thyroid disorders, diabetes, and hypertension. These variables were coded 1 = complaint present, 2 = not present. We adopted a conservative approach and coded missing data 2. Hypertension was defined as either diastolic blood pressure >90 or systolic blood pressure >140.

Table 1
Descriptive data for demographic, health, and neuroanatomical variables

		ε2/2 + 2/3		ε3/3		ε3/4 + 4/4		
		M	SD	M	SD	M	SD	Recall[b]
Age-group	40s	43	1	43	1	43	1	–
	60s	63	1	63	1	62	1	–
Gender (men/women)[a]	40s	18/27	–	76/107	–	35/51	–	–
	60s	23/18	–	99/94	–	43/36	–	–
Years of education	40s	15	2	15	2	15	2	–
	60s	13	3	14	3	14	2	–
APOE[a]	40s	45	–	183	–	86	–	–
	60s	41	–	193	–	79	–	–
Heart trouble[a]	40s	0	–	3	–	2	–	–
	60s	3	–	24	–	9	–	–
Thyroid disorders[a]	40s	1	–	3	–	8	–	–
	60s	3	–	18	–	4	–	–
Diabetes[a]	40s	0	–	5	–	3	–	–
	60s	4	–	20	–	9	–	–
Hypertension[a]	40s	9	–	42	–	17	–	–
	60s	18	–	97	–	34	–	–
L entorhinal	40s	1736	299	1772	374	1726	285	0.14*
	60s	1788	281	1698	315	1661	314	0.08
R entorhinal	40s	1567	292	1618	332	1574	319	0.06
	60s	1591	306	1604	302	1561	351	0.06
L parahippocampal	40s	1736	283	1763	279	1781	233	0.09
	60s	1742	254	1692	242	1691	268	−0.06
R parahippocampal	40s	1565	211	1622	262	1602	241	−0.04
	60s	1571	274	1525	232	1529	235	−0.07
L temporal pole	40s	2259	436	2233	406	2223	469	0.11
	60s	2212	436	2234	401	2214	405	−0.02
R temporal pole	40s	2181	411	2236	401	2176	394	0.12*
	60s	2422	432	2339	442	2340	384	−0.03
L inferior temporal	40s	8991	1498	9210	1532	9319	1700	0.07
	60s	8940	1870	8855	1502	8723	1737	−0.07
R inferior temporal	40s	8383	1490	8933	1499	8569	1738	0.04
	60s	8460	1801	8051	1362	8027	1498	−0.03
L mid temporal	40s	10046	1911	10072	1481	10274	1601	0.13
	60s	9595	1246	9517	1514	9415	1483	−0.07
R mid temporal	40s	11042	1478	10785	1516	11054	1612	0.10
	60s	10723	1626	10548	1597	10499	1574	−0.12
L superior temporal	40s	11547	1858	11497	1533	11713	1647	0.07
	60s	10855	1419	10742	1545	10927	1576	−0.10
R superior temporal	40s	10941	1612	10976	1404	11115	1323	−0.01
	60s	10280	1235	10459	1518	10487	1354	−0.03

Notes. Brain volumes in mm^3; L = Left; R = Right; a = refers to number of cases; b = regression beta weights having controlled for gender and intracranial volume; $^*p < 0.05$.

Statistics

A series of $2 \times 2 \times 3$ Analysis of Covariance (ANCOVA) were run where hemisphere (left, right) served as a within-subjects factor, and age group (middle age, older) and *APOE* group (2/2 + 2/3, 3/3, 3/4 + 4/4) as between-subject factors. We covaried intracranial volume (to control for individual brain size differences) and gender (to control for gender-related differences in cortical volumes). Our main question was whether cortical volumes varied according to *APOE* group, and importantly, whether any significant main effects were modified by age group.

RESULTS

Descriptive data for demographic, health, and brain volume variables according to *APOE* genotype and age are presented in Table 1. Given the association between the temporal lobes and episodic memory, for descriptive purposes, coefficients between the recall

measure and brain volumes are also included in that table. Summary statistics and effect sizes for the ANCOVAs assessing cortical volumes according to hemisphere, age group, and *APOE* genotype are presented in Table 2.

There were several notable findings. First, with one exception, all of the main effects and higher-order interactions involving *APOE* were nonsignificant. Second, for entorhinal cortex volume, none of the other main effects or interactions were significant. Third, all of remaining main effects for age were significant, with the majority indicating middle aged persons recorded higher volumes than older participants. The exception was temporal pole, where opposite was the case. The main effect for hemisphere was also significant for temporal pole with right volumes greater than left volumes. However, this largely stemmed from older persons, as a significant Age × Hemisphere interaction indicated right hemisphere volumes to be larger in this group. That interaction was also significant for the middle temporal volume. Here though, the trend was toward greater right hemisphere volumes in both middle aged and older participants. The effect size for this interaction, however, was small. Finally, there was a significant Age × *APOE* × Hemisphere interaction for inferior temporal lobe volume. ANCOVAs to identify the source of this interaction found the Age group × *APOE* interaction for the left hemisphere was nonsignificant. For the right hemisphere however, that interaction was significant ($p < 0.05$). Although there was a trend suggesting a decline in volumes in the older group that followed a ε2 > ε3 > ε4 pattern, the source of the interaction stemmed from younger ε3 carriers who had greater volumes. This was confirmed by a series of Bonferroni adjusted *T*-tests where only the age group comparison for ε3 carriers was significant ($p < 0.05$). Again though, it should be noted that the effects size relating to this interaction was small.

We repeated the ANCOVAs with handedness and years of education as additional covariates, but this did not change the pattern of the original findings. We also reran the ANCOVAs using the more conservative MMSE cutoff of ≤26 in the older group (13 persons were excluded). With two exceptions, the overall pattern of findings did not change for these analyses using the more homogeneous group. The exceptions were for inferior temporal volume where the Age × *APOE* × Hemisphere interaction became marginally nonsignificant ($p = 0.052$). Similarly, the Age × Hemisphere interaction for middle temporal volume became marginally nonsignificant ($p = 0.051$). As an example of the overlap between *APOE* groups,

Table 2
Summary statistics for Analysis of Covariance: Cortical volumes and Hemisphere × Age × APOE with Intracranial volume and gender as covariates

	df		Entorhinal		Parahippocampal		Temporal pole		Inferior temporal		Middle temporal		Superior temporal	
			F	μ^2	F	μ^2	F	μ^2	F	μ^2	F	μ^2	F	μ^2
Hemisphere[a]	1,619	1.11			0.00		**5.25***	**0.008**	1.85		0.02		1.22	
Hemisphere × Age	1,619	1.15			0.06		**19.39*****	**0.030**	1.23		**4.81***	**0.008**	3.02	0.01
Hemisphere × *APOE*	2,619	0.98			0.28		0.15		0.79		0.77		1.23	
Hemisphere × Age × *APOE*	2,619	0.50			0.40		1.33		**3.15***	**0.010**	0.22		0.24	
Age	1,619	0.70			**6.99****	**0.011**	**4.05***	**0.007**	**16.89*****	**0.027**	**23.52*****	**0.037**	**50.82*****	**0.076**
APOE	2,619	2.58		0.01	0.15		0.39		2.07	0.01	0.41		0.14	
Age × *APOE*	2,619	0.50			1.09		0.14		1.47	0.01	1.19		0.75	

Note. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (significant statistics highlighted in bold); where μ^2 column is blank, effect size is close to zero; a = refers to left versus right hemisphere.

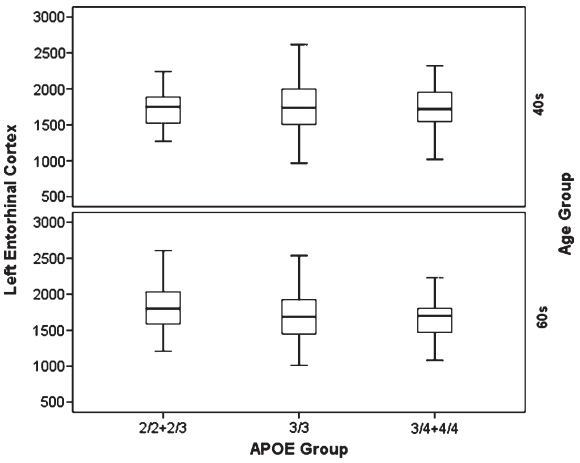

Fig. 1. Left entorhinal cortex volume as a function of age and *APOE*.

Fig. 1 provides a boxplot of left entorhinal cortex volumes in this more homogeneous group according to age and *APOE*.

Finally, we repeated the analyses controlling for the health variables heart trouble, thyroid disorders, diabetes, and hypertension. With one exception, this made no difference to our original findings. The exception was heart trouble where the significant main effect for hemisphere in respect to temporal pole became nonsignificant. Also, as some of the participants were scanned on a different scanner, we repeated the analyses covarying type of scanner. Here, the findings were as in the original analyses except for the Age × Hemisphere interaction for middle temporal pole, which became nonsignificant.

DISCUSSION

This is the largest population-based study of the entorhinal cortex and *APOE* genotype in cognitively intact adults in midlife (44 to 48 years) and early old age (64 to 68 years). Due to their vulnerability to the neuropathological changes associated with AD, other structures in the medial temporal lobe were also examined including the parahippocampal gyrus, temporal pole, and inferior, middle, and superior temporal cortices. Additionally, the sample was sufficiently large to systematically contrast the relative effects of the ε2, ε3, and ε4 alleles. Several notable findings emerged from the study. First, there was no evidence that *APOE* genotype influenced brain volumes in a stepwise fashion (i.e., $\varepsilon 2 > \varepsilon 3 > \varepsilon 4$) in middle age or early old age. Second, there were several significant age effects, the majority indicating that older participants recorded smaller volumes. Additionally, using a more conservative MMSE cutoff of ≤ 26 made little difference to the original pattern of findings. Finally, taking several health variables, handedness and years of education into account in the statistical analyses did not substantially alter our main findings.

The entorhinal cortex is of particular interest in the present context as both neuroimaging [8, 9], and postmortem studies [10] have shown it is one of the main brain structures to exhibit the early neuropathological changes associated with AD. Although there have been previous studies showing that *APOE* genotype is associated with entorhinal cortex size in cognitively intact older adults [12–15], the findings are mixed and none have systematically assessed entorhinal cortex volume in midlife and young old age while contrasting the ε2, ε3, and ε4 alleles. Given evidence that possession of the ε4 allele is a risk factor for AD [5] while the ε2 allele offers protection [6], the present finds are of some importance. Specifically, although there is work suggesting a $\varepsilon 2 > \varepsilon 3 > \varepsilon 4$ pattern in relation to the entorhinal cortex thickness in early life [1], the present findings suggest that in middle age and into early old age, this trend is absent.

There are several possible explanations for this finding. The first is that the absence of this trend reflects a Type II error and therefore, should be treated with caution. This seems unlikely however, as the analyses involved 627 persons and the design offered sufficient statistical power to detect small to medium effect sizes at the five percent alpha level. The second possibility is that the *APOE* associations with brain morphology are related to neurodevelopment in early life and neurodegeneration in later life and the present sample aged 44 to 48, and 64 to 68, years fell between those two extremes. It is of note that studies finding an association between the ε4 allele and entorhinal cortex metrics have either involved participants older than those in the present study [15] or involve persons with a family history of AD who are therefore at greater risk of the disease [12]. Although associations between the ε4 allele and neuroanatomical volumes in other parts of the brain have been detected in middle age persons [27, 28], it is possible that *APOE*-related effects on the entorhinal cortex and nearby structures occur either with older age or in persons with a greater vulnerability to AD.

A related explanation stems from work suggesting the subclinical phase of the disease extends years and perhaps decades in advance of eventual diagnosis. For example, cognitive deficits have been detected up to ten years in advance of diagnosis [29], and

histopathological work suggests that the neuropathology associated with AD is present decades before emergence of the disease [30]. Given the long preclinical phase of AD, and that possession of the ε4 allele increases the risk of the disease, it is possible that where associations with the entorhinal cortex are found, it is in persons who eventually develop AD. As the PATH Through Life project is ongoing, we do not currently have data on AD status as it will be collected over the coming years. However, the lack of *APOE*-entorhinal cortex effects may be due to the largely healthy, cognitively normal population-based sample and the possibility that relatively few individuals will eventually suffer AD. This possibility will be addressed in future research.

Although not the main focus of the present study, there were also some significant hemisphere effects indicating left temporal pole and middle temporal volumes to be smaller. Work suggesting greater left than right atrophy in mild cognitive impairment and AD [19, 31] may explain this finding, although there is also conflicting evidence [32]. However, the lack of information for the present sample on future dementia status already noted makes this explanation uncertain. Additionally, several effects for hemisphere became nonsignificant in repeat analyses with a more stringent MMSE cutoff and when heart trouble was taken into account. Together, given the large sample and that most of the significant statistics involved small effect sizes, the findings in relation to hemisphere should be treated with caution.

Although the study possesses several strengths, there are also some limitations that we should acknowledge. First, in order to robustly test the *APOE* genotype effect, we would have preferred to contrast ε2 and ε4 homozygotes. Although to date, the present study is the largest in non-demented adults to directly contrast the three alleles, there were insufficient numbers possessing the ε2/2 and ε4/4 genotypes to achieve this. However, as with work elsewhere [1], we believe our strategy of removing ε2/4 s, and combining ε2/2 with ε2/3 s, and ε3/4 s with ε4/4 s, and evaluating relative to ε3/3 s provided a sufficiently robust test of the main hypothesis. Additionally, although not a limitation, the present study focused on non-demented persons with a maximum age of 68 years. As noted earlier, it is possible that participants were too young to detect the hypothesized *APOE*-related effects. Finally, for reasons beyond our control, some middle aged participants were scanned on a different scanner to the rest of that group. However, both scanners were 1.5T Philips scanners with the same acquisition parameters, and five controls were scanned on both scanners and no significant volumetric differences were observed. Moreover, as repeating the analyses with scanner type entered as a covariate had little influence on the original findings, it does not appear that scanner differences had a major bearing on the main study outcomes.

To conclude, our findings suggests that *APOE* genotype is not associated with entorhinal cortex volume, or those of other structures in the medial temporal lobe, in cognitively normal community-dwelling adults in midlife and early old age up to 68 years. It is possible that the association between *APOE* genotype and entorhinal cortex metrics is a feature of neurodevelopment in early life and neurodegeneration in late life, and that the ages of the present sample fell between those two extremes. Moreover, associations where they are found in older persons may be related to the subclinical phase of AD that extends years in advance of eventual diagnosis. As the entorhinal cortex is one of the first brain structures to exhibit the pathology associated with the disease and may give an early indication of vulnerability to eventual AD, it is important that further work explores the influence of risk factors such as *APOE* genotype on this brain structure in midlife and early old age.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1226).

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