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The Neurobiology and Age-Related Prevalence of the $\epsilon 4$ Allele of Apolipoprotein E in Alzheimer's Disease Cohorts

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by amyloid beta ($A\beta$) plaques and tau neurofibrillary tangles in the brain. Human apolipoprotein E (ApoE) is a lipid transport protein coded by the polymorphic *APOE* gene, with three major alleles: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. After age, the $\epsilon 4$ allele is the greatest risk factor for developing sporadic AD, conferring an increased risk of 3–4 and 8–12 times for one or two copies of the allele, respectively. This risk is reported to vary by demographic factors including sex, ethnicity and geography. In order to understand the risk of ApoE $\epsilon 4$ in relation to age, the primary risk factor for developing AD, we need to understand how the prevalence of *APOE* genotypes changes with age. Here, we present the first data on age-related prevalence of *APOE* $\epsilon 4$ in AD in three AD cohorts in Australia and the USA. There is a significant association between age and $\epsilon 4$ prevalence, particularly for $\epsilon 4$ homozygotes, such that as age increases the prevalence of $\epsilon 4$ decreases. Further studies on a random, population-based sample of the population are needed to provide more generalizable data, particularly in the >90-year-old age group.

Keywords

Alzheimer's disease; APOE; Apolipoprotein E; Population demographics

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by amyloid beta ($A\beta$) plaques (Masters et al. 1985) and tau neurofibrillary tangles in the brain

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and is the most common form of dementia. Early-stage AD symptoms include short-term memory loss, disorientation and language and behavioural problems, with an average life expectancy of less than 10 years following diagnosis (Liu et al. 2013; Todd et al. 2013). The number of people living with dementia globally is expected to rise to 65.7 million by 2030, and the burden is borne disproportionately by females and persons living in low-and middle-income countries (Prince et al. 2013).

Human apolipoprotein E (ApoE) is a ~34-kDa, 299-aminoacid polypeptide, coded by the polymorphic APOE gene, mapped to chromosome 19q13.2. There are three major alleles designated $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, (Das et al. 1985) that encode three isoforms arising from a single cysteine-to-arginine amino acid substitution at positions 112 and 158 (positions 130 and 176, including the signalling peptide), producing ApoE2 (Cys112/ Cys158), ApoE3 (Cys112/Arg158) and ApoE4 (Arg112/ Arg158) (OMIM 2016).

ApoE $\epsilon 4$ is recognised as the major single genetic risk factor for developing sporadic forms of AD (Corder et al. 1993). There is much evidence that *APOE* $\epsilon 4$ moves forward the age of onset of AD (Sweet et al. 2012) and also the rate of cognitive decline in preclinical AD (Pietrzak et al. 2015; Lim et al. 2016). Carrying one copy of the $\epsilon 4$ allele increases risk 3–4 times, whereas homozygous $\epsilon 4/\epsilon 4$ carriers have an 8–12 times increased risk of developing AD compared with $\epsilon 2$ which has a protective effect (Corder et al. 1994; Verghese et al. 2011), and this risk is known to vary by demographic factors including sex and ethnicity (Farrer et al. 1997). However, not all $\epsilon 4$ homozygotes develop the disease. For example, in a study of first-degree relatives of AD patients ($n = 3999$), up to 50 % of $\epsilon 4/\epsilon 4$ carriers survived to age 80 years and did not develop AD (Farrer et al. 1995).

Globally, the prevalence of $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles is estimated as 7, 79 and 14 %, respectively (ALZGENE 2010), with ApoE3 regarded as the neutral parent isoform. In order to understand the risk of *APOE* $\epsilon 4$ in AD in relation to the primary risk factor for developing AD, age, we need to understand how the prevalence of *APOE* allotypes changes with age. Farrer et al. (1997) demonstrated an *APOE* $\epsilon 4$ -AD effect at all ages between 40 and 90 years, but the effect diminished after 70 years of age. Similarly, a study of Finnish centenarians ($n = 179$) showed significantly lower $\epsilon 4$ allele frequency (8.4 %) compared to Finnish adults ($n = 655$, 20–55 years, 22.7 %) and children/adolescents ($n = 1577$, 3–18 years, 19.4 %) (Louhija et al. 1994). Here, we examine the prevalence of *APOE* $\epsilon 4$ with age in three AD cohorts ($n = 21,513$ AD cases + healthy controls) in Australia and the USA.

Biological Function of APOE

To interpret the demographics of ApoE, we first need to contextualise its neurobiology. Plasma lipoproteins are spherical moieties composed of a hydrophobic lipid core and an exterior hydrophilic phospholipid and apolipoprotein monolayer. The general function of apolipoproteins, including apoE, is to stabilise, solubilise and transport triglycerides, cholesterol and other lipoproteins in blood and other tissues. Their role in lipid metabolism includes serving as cofactors in enzymatic reactions and acting as ligands for lipoprotein receptors (Eichner et al. 2002). ApoE is synthesised and secreted by many tissues, primarily by hepatic synthesis and astrocytes in the brain, and occurs in all lipoprotein fractions in

human plasma, constituting 10–20 % of very-low-density lipoproteins (VLDLs) and 1–2 % of high-density lipoproteins (HDLs).

Protein Structure and Polymorphisms

ApoE was first described in 1973 by Shore and Shore (1973) during an investigation of VLDLs in human plasma. Despite early insights into the ApoE structure gained through x-ray crystallography (reviewed in Frieden and Garai 2013), the complete protein structure was only recently determined by Chen et al. (2011) using nuclear magnetic resonance. ApoE consists of two independently folded domains separated by a flexible hinge region: the N-terminal domain (amino acids 20-165) arranged in an anti-parallel elongated, four-helix bundle, and the C-terminal domain (amino acids 225-299), which is predicted to contain three helices (Wilson et al. 1991; Nolte and Atkinson 1992), one of which contains the major lipid binding region (amino acids 244-272) (Sparrow et al. 1992) (Fig. 1). The N-terminus contains a region of basic amino acids (amino acids 140-150) clustered at one end of one of the helices and is involved in ApoE receptor binding (Innerarity et al. 1983), such as the low-density lipoprotein (LDL) receptor. In the ApoE4 isoform, interaction between these two domains via the formation of a salt bridge at the Arg112-substituted position results in significant conformational changes compared with ApoE2 and ApoE3 (Huang 2010), and these isoform-specific differences in protein structure directly affect their role in disease (Mahley et al. 2009).

Lipid Transport and Cardiovascular Disease

Cardiovascular disease is the leading cause of death and disability globally (WHO 2011) and is responsible for 30 % of all deaths in both Australia and the USA (ABS 2014; Mozaffarian et al. 2016). There is a well-established risk of heart disease associated with high cholesterol and high LDL, and ApoE is an essential ligand for the clearance of triglyceride, cholesterol-rich lipoproteins and lipoprotein remnants from plasma (Mahley et al. 2009). Large-scale genome-wide association studies have identified *APOE* as one of the main risk factors for cardiovascular disease (Visvikis-Siest and Marteau 2006; Deloukas et al. 2013), and ApoE has been extensively studied in relation to cardiovascular disease—either ApoE polymorphism as an independent risk factor for disease or through ApoE contribution to cholesterol levels (Eichner et al. 2002).

In cardiovascular disease, ApoE2 is thought to be protective, and ApoE4 carries the most risk (Davignon et al. 1988). The Arg112 substitution in ApoE4 alters the lipid-binding region to favour large triglyceride-rich VLDLs over small phospholipid-rich HDLs seen in ApoE2 and ApoE3. This preferential binding leads to accelerated clearance of VLDLs, downregulation of LDLs and thus increased LDL levels (Huang 2010). Elevated cholesterol levels in ApoE4 patients compared with ApoE3 patients results in increased risk of cardiovascular disease, and ApoE4 is significantly overrepresented in hyperlipidemic and cardiovascular disease populations (Mahley and Rall 2000; El-Lebedy et al. 2016).

Conversely, the Cys158 substitution in ApoE2 results in severely impaired function of the receptor-binding region compared to ApoE3 (Weisgraber et al. 1982). This causes delayed clearance of remnant lipoproteins and results in increased triglyceride and cholesterol levels

(Havel and Kane 1973; Mahley and Rall 2000). Hyperlipoproteinemia III (HLP III), a genetic lipid disorder, is characterised by an accumulation of remnant lipoproteins in the plasma, leading to the development of premature atherosclerosis, and virtually all patients with HLP III are $\epsilon 2$ homozygotes (Giau et al. 2015). Despite causing increased cholesterol levels, ApoE2 may be protective in atherosclerosis as it is associated with lower LDL levels (Davignon et al. 1988; Elosua et al. 2004; Huang 2010). If ApoE is a cardiovascular disease risk factor, then that must be taken into account in prevalence studies of AD.

Role of APOE in AD

Although ApoE $\epsilon 4$ is generally accepted as playing a critical role in the age at onset of AD, there is no consensus on the mechanism by which this occurs. This may be related to the role of ApoE in the following:

1. The effect on A β deposition and clearance
2. A regulatory role for tau phosphorylation (Kim et al. 2009; Mohamad et al. 2016)
3. Neuronal maintenance and repair, including cerebral energy metabolism, neurovascular function, neurogenesis and synaptic plasticity (Kim et al. 2014)
4. Neuroinflammation and the association of $\epsilon 4$ with impaired lymphatic drainage of the brain (Weller et al. 2015)

ApoE and A β Clearance in AD Brain

Neuroinflammation is widely accepted as an important mechanism contributing to AD progression and increased levels of A β , including in the form of A β plaques, which is a major component of neuronal dysfunction in AD (Tai et al. 2015). There is evidence that ApoE isoforms differentially modulate neuroinflammation (Keene et al. 2011), but there is little information available on *APOE* modulation of A β -induced neuroinflammation (Tai et al. 2015).

The 244-272 amino acid region of ApoE binds to A β at residues 12-28 to form an ApoE-A β complex (Strittmatter et al. 1993; Golabek et al. 1996). The binding occurs primarily with the oligomeric state of A β , which subsequently affects progress of these oligomers into fibrils (Garai et al. 2014). The influence of ApoE, via formation of the ApoE-A β complex, is thought to be through (1) promotion of the deposition and production of A β or (2) alteration of amyloid clearance rates, and isoform-specific effects have been reported (Mahley et al. 2006; Holtzman et al. 2012). It is generally accepted that ApoE3 forms a more stable complex and in greater quantities than that of E4. With respect to E4, the results are inconsistent: some studies report an association with increased quantity of the ApoE-A β complex (Strittmatter et al. 1993), while others report a corresponding decrease (LaDu et al. 1994). This discrepancy could be the result of experimental conditions; for example, a comparison of ApoE-A β complex formation under native and reducing conditions demonstrated a significant decrease in formation of ApoE4-A β under the latter (Tai et al. 2015).

The majority of evidence supports an ApoE-mediated alteration in amyloid clearance rates (LaDu et al. 1994; Holtzman et al. 2000; Jiang et al. 2008; Castellano et al. 2011). For instance, Jiang et al. (2008) proposed that ApoE has an integral role in promoting proteolytic degradation of A β , a role that is significantly influenced by ApoE lipidation status, which is in turn isoform dependent. Jiang et al. (2008) report more rapid A β degradation through proteolytic cleavage in E2 and E3 compared with E4. Similarly, Castellano et al. (2011) observed an isoform-specific effect, with ApoE4 displaying the slowest clearance rate of A β compared with E2 and E3, which were not significantly different from each other. Additionally, fractional synthesis rates, based on leucine-normalised increase of labelled to unlabelled A β between the various isoforms, were not significantly different, suggesting that the ApoE genotype has minimal effect on A β aggregation. Similarly, following administration of an ApoE-dependent therapeutic in an AD mouse model, Cramer et al. (2012) showed enhanced clearance of brain-soluble A β levels, with a >50 % reduction in the plaque area within 72 h, with increasing ApoE levels. Conversely, using genetically-modified haploinsufficient ϵ 3 and ϵ 4 mice, Kim et al. (2011) demonstrated that decreased levels of ApoE resulted in significantly decreased amyloid deposition, independent of isoform status. Considering the evidence, it seems likely that ApoE influences A β through multiple mechanisms.

APOE Allele Frequency

In Caucasian populations <65 years of age, *APOE* ϵ 3 is the most common allele (75 %), followed by ϵ 4 (15 %) and ϵ 2 (8 %), but this is known to vary widely with demographic factors such as sex, ethnicity and geography (Zannis et al. 1993; Eisenberg et al. 2010). A number of meta-analyses of *APOE* allele frequency have previously been published. For instance, Farrer et al. (1997) analysed information from 40 contributing research teams to examine the association between *APOE* genotype and AD (n = 5930 cases; n = 8607 healthy controls), including data collected prior to July 1995. The *APOE* ϵ 4 allele frequency was 37 %/14 %, 32 %/19 %, 19 %/11 % and 28 %/8.9 % for Caucasian, African American, Hispanic and Japanese in AD cases/controls, respectively. An elevated frequency of ϵ 4 in AD patients versus controls was observed in every ethnic group, but the association was weaker among African Americans than Caucasians (OR 5.7 and 12.5-14.9, respectively, for ϵ 4/ ϵ 4), and the relative increase in frequency of ϵ 4 in AD was substantially less in African Americans (Farrer et al. 1997). The ϵ 4 allele was less prevalent in Japanese participants, but the ratio of cases to controls (3.1) was similar to that of Caucasians (2.7). It is important to note that the relatively weaker association between ϵ 4 and AD in non-Caucasian populations may be due to the lower diagnostic error in Caucasian populations due to socioeconomic variables such as access to healthcare, smoking status and other lifestyle factors that are known to vary by ethnicity and may affect accurate AD diagnosis.

More recently, Bang et al. (2003) conducted a meta-analysis of 78 case-control studies (n = 7812 AD cases; n = 10,654 controls), including data collected prior to January 2000. Similar ethnic differences of *APOE* ϵ 4 allele frequency to those reported by Farrer et al. (1997) were observed in Caucasian, Southern European and East Asian participants (39 %/14 %, 30 %/11 % and 27 %/9.2 % in AD cases/controls, respectively). Interestingly, significant differences were observed in ϵ 4 allele frequency and odds ratios of AD between the different

subtypes of AD ($p < 0.01$), such that $\epsilon 4$ was more prevalent in late-onset AD compared with early-onset AD and, further, among late-onset AD patients, $\epsilon 4$ was more frequent in familial rather than sporadic cases (48 and 31 %, respectively). Bang et al. (2003) note that although many studies report an increased risk of AD with $\epsilon 4$ status, the strength of the association varies widely and may be dependent on selection bias, including the source of the cases (clinic versus population based), the diagnostic criteria used, sample size and a failure to account for the demographic factors discussed above.

Singh et al. (2006) conducted an investigation of global geographical distribution of *APOE* allele frequency, including data from 297 healthy populations only collected prior to March 2004. At the continental level, Indian populations showed the highest proportion of *APOE* $\epsilon 3$, while Oceanic populations showed the lowest. The authors observed a higher frequency of $\epsilon 4$ in dark-skinned populations than in those with less melanin or in populations living in regions with low solar radiation (e.g. Inuit) and proposed that despite its deleterious effects in later life, a relative advantage of the $\epsilon 4$ allele is superior intestinal absorption of lipid-soluble vitamins, such as vitamin D, which is known to be lower in dark-skinned populations (Singh et al. 2006).

The Ancestral Allele and Evolutionary Advantage

Based on evidence from animals, which are ApoE4-like and do not have polymorphisms, it is likely that $\epsilon 4$ is the ancestral allele. The increased risk of cardiovascular and neurodegenerative disease associated with *APOE* $\epsilon 4$ begs the question, "Why is the allele frequency so high?" *APOE* $\epsilon 4$ has also been associated with improved perinatal health and survival outcomes (Eisenberg et al. 2010), but the issue is complicated by the fact that the deleterious effects of $\epsilon 4$ arise after the reproductive age. The answer may lie in the ability to modulate susceptibility to infectious disease, and a selective advantage in immune regulation (see above). For example, $\epsilon 4$ is more common in Africa, where it may have offered protective against specific endemic diseases (Mahley et al. 2009).

Conversely, there is evidence of some survival advantage associated with *APOE* $\epsilon 2$. A recent study by Ryu et al. (2016) of 450 centenarians revealed that *APOE* $\epsilon 2$ is associated with longevity and that the individuals in this cohort were significantly less likely ($p = 0.005$) to carry the $\epsilon 4$ allele, with a frequency of 9.6 and 15.7 % in centenarians versus controls, respectively. This association has been observed in other cohorts (Louhija et al. 1994; Schachter et al. 1994; Mayila et al. 2005) and may be due to a reduced risk of both cardiovascular disease and AD (Eichner et al. 2002). For a review of evolution and allele variation of *APOE*, see Singh et al. (2006).

APOE Allele Frequency by Decade of Life

Because *APOE* $\epsilon 4$ has been implicated in two principle diseases, cardiovascular atherosclerosis and AD, manifesting in the fifth and sixth decades of life, we were interested to see how the prevalence of *APOE* genotypes changed with decade of life. Specifically, we hypothesised that the proportion of $\epsilon 4$ carriers would decrease with increasing age, due to the combined mortality from cardiovascular disease and AD. Here, we present new data on *APOE* genotypic frequencies in the *Australian Imaging, Biomarkers and Lifestyle Flagship*

Study of Ageing (AIBL) and Alzheimer's Disease Neuroimaging Initiative (ADNI) cohorts. Due to the relatively small sample size of these studies, a third study (Uniform Data Set of the Alzheimer's Disease Centers (UDS)) was included for comparison. The recruitment protocols and cohort description are detailed in Table 1.

Figure 2 compares $\epsilon 4$ allele frequency and cognition. Cognitive status was assigned as normal, mild cognitive impairment (MCI) or severe based on the total score of the minimental state examination (MMSE) (24-30, 23-18 and 17, respectively), a standardised screening test for cognitive impairment (Tombaugh and McIntyre 1992). While there are other more suitable tests to identify and measure cognitive impairment (e.g. DSM-III-R and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria), and these scores have not been corrected for age and education level, the MMSE was the only assessment available for each of the three studies and provides a quick and simple comparison of the available data.

There is a greater proportion of $\epsilon 4$ s with MCI versus normal cognition in ADNI, AIBL and UDS datasets. In UDS, there is a marked increase in the proportion of $\epsilon 4^{+/-}$ carriers in MCI and severe categories versus controls (55, 60 and 33 %, respectively) (Fig. 2c). While the proportion of $\epsilon 4^{+/+}$ carriers is increased with MCI and impaired (sum of MCI and severe groups) compared to normal cognition in ADNI (9.2 and 20% for normal and MCI, respectively) and UDS (3.6, 11 and 14% for normal, MCI and severe, respectively) (Fig. 2), it remains relatively consistent for AIBL (6.1, 6.0 and 5.6 %, respectively). Contrary to expectation, within AIBL there is a greater proportion of $\epsilon 4^{+/-}$ carriers classified as MCI rather than severe (46 and 36 %, respectively). This may be due to the relatively small number of participants with severely impaired cognition ($n = 86$) or due to insufficient power of the MMSE in differentiating between different degrees of cognitive impairment. For example, comparing normal and impaired (sum of MCI and severe groups) cognitive groups, there is a greater proportion of $\epsilon 4^{+/-}$ carriers in the impaired group versus controls for each of the three cohorts.

A limitation of this analysis is that the ADNI data is from baseline assessment. At baseline, there are potentially fewer cognitively impaired participants (i.e. *APOE* $\epsilon 4$ carriers) because of barriers to participation or due to insufficient time since inception of the study for symptoms to manifest. For this reason, there are relatively few participants classified as MCI ($n = 163$) and no participants classified as having severe cognitive impairment in this cohort (Fig. 2). Whilst the normal/MCI/severe classification is not a measure of AD, the allele frequencies observed are consistent with existing data on allele frequencies in AD populations and with the increased risk conferred with $\epsilon 4$ status.

Summary statistics of the number of $\epsilon 4$ carriers by decade of life in ADNI, AIBL and UDS AD cohorts are presented in Table 2 and Fig. 3. The influence of the *APOE* genotype and age on the odds of developing AD was assessed using logistic regression. Two independent models of genotype ($\epsilon 4$ negative, [$\epsilon 4^{-/-}$], and $\epsilon 4$ homozygous, [$\epsilon 4^{+/+}$]) versus age were applied to each of the three datasets, and the results are summarised in Table 3. All

regression analyses were conducted using R version 3.2.0 (2015-04-16) (R Core Team 2015). Criteria for significance were set as $p < 0.05$.

In UDS, the largest and most statistically powerful dataset examined here, the proportion of $\epsilon 4^{+/+}$ carriers decreases with increasing age, from 10 % ($n = 401$ of 3955) to 1.6 % ($n = 18$ of 1126) of participants aged 61–70 and 91–100 years, respectively (Fig. 3). There is a significant association between age and $\epsilon 4^{-/-}$ ($p = < 2 \times 10^{-16}$) and $\epsilon 4^{+/+}$ ($p = < 2 \times 10^{-16}$) status, such that the odds of positive $\epsilon 4$ status ($\epsilon 4^{+/-}$ or $\epsilon 4^{+/+}$) decrease with increasing age. The same trend is observed in ADNI, with significantly decreased odds of $\epsilon 4^{+/+}$ status with increasing age ($p = 6 \times 10^{-8}$), but not with AIBL, where the odds of being an $\epsilon 4$ carrier increase with increasing age ($p = 0.0330$) (Table 3). This may be due to small sample size, particularly for participants >80 years ($n = 240$), or due to confounding factors in UDS and ADNI datasets such as evidence of heart disease, diabetes or familial history of AD, which were specific exclusion criteria for AIBL.

The interpretation of these results is confounded by multiple uncertainties inherent in the study design: (1) there is a general bias in recruitment because the studies are all AD cohorts, so there will be an inherent enrichment of $\epsilon 4$ s, and they are therefore not representative of the general Australian and US populations; (2) there was no standardised recruitment protocol for enrolment, with each study, including each of the 34 centres contributing to the UDS dataset, developing their own inclusion and exclusion criteria; and (3) AIBL and UDS included demographic and clinical data from the most recent visit, whereas the corresponding ADNI data was from the baseline assessment, which may reduce the relative prevalence of $\epsilon 4$ (see above).

Here, we present the first data on age-related prevalence of *APOE* $\epsilon 4$ in AD. There is evidence of a significant association between age and $\epsilon 4$ prevalence, particularly for $\epsilon 4$ homozygotes, such that as age increases the prevalence of $\epsilon 4$ decreases, but the effect is small and may be confounded by mortality from cardiovascular disease, in which *APOE* is known to play a role. Future studies should focus on a large, random, community-based sample of the population in order to provide more generalised evidence for the age-related prevalence of *APOE* $\epsilon 4$ in AD and to understand cardiovascular disease as a risk factor for AD.

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Fig. 1. Structure of human ApoE (Protein Data Bank 2L7B, Chen et al. 2011), with 10-kDa C-terminal shown in *blue* (**a**) and removed (**b**), and 22-kDa N-terminal separated by a flexible hinge region. The major lipid-binding region is shown in *green*, and the LDL receptor-binding region is shown in *pink* (**b**). The sites of isoform-specific cysteine-to-arginine amino acid substitution is shown in *red* at positions 112 and 158, such that ApoE2 (Cys112/Cys158), ApoE3 (Cys112/Arg158) and ApoE4 (Arg112/Arg158). Graphics were prepared using The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC

Fig. 2.

APOE $\epsilon 4$ allele frequency by cognitive status (normal/MCI/ severe) for three Alzheimer's disease cohorts. **a** the Alzheimer's Disease Neuroimaging Initiative (ADNI) study. **b** the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing (AIBL). **c** Uniform Data Set of the Alzheimer's Disease Centers (UDS) program. Assignment of cognitive status was based on total MMSE score (normal 24–30, MCI 23–18, severe 17). Allele frequency (%) of $\epsilon 4^{+/+}$ carriers is indicated at the top of each bar. Total number of

participants per group is indicated below the *x-axis*. *MCI*: mild cognitive impairment;
MMSE: mini mental state exam

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Fig. 3. *APOE* $\epsilon 4$ allele frequency by decade of life for three Alzheimer's disease cohorts. **a** The Alzheimer's Disease Neuroimaging Initiative (ADNI) study. **b** the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing (AIBL). **c** Uniform Data Set of the Alzheimer's Disease Centers (UDS) program. Allele frequency (%) of $\epsilon 4^{+/+}$ carriers is indicated at the *top* of each *bar*. Total number of participants per decade of life is indicated below the *x-axis*

Table 1

Description of three independent datasets used to examine the APOE allele frequency in Alzheimer's disease

The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a prospective, longitudinal study of participants aged 55–90 years, launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD) (for more information, see <http://www.adni-info.org>). The dataset includes data up to December 2014 ($n = 1566$ individuals), collected at baseline visit.

The Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing (AIBL) commenced in 2009 and is a longitudinal, prospective study of volunteers aged >60 years who are undergoing assessment for research into ageing and AD. Further details regarding recruitment, assessment and inclusion and exclusion criteria are described in Ellis et al. (2009). The dataset includes data up to March 2016 ($n = 1479$ individuals), collected during baseline visit.

The Uniform Data Set of the Alzheimer's Disease Centers (UDS) program of the US National Institute of Aging consists of prospective, standardised and longitudinal clinical data from approximately ~33,000 individuals collected from 34 past and present contributing centres since 2005 and is collated by the National Alzheimer's Coordinating Center (for more information, see www.alz.washington.edu/WEB/researcher_home). Each centre enrolls subjects according to its own protocol, and these subjects may come from clinical or self-referral, active recruitment or volunteers. The dataset includes data up to March 2016 ($n = 17,983$ individuals), collected during most recent visit.

Table 2

Number of *APOE* $\epsilon 4$ carriers by decade of life in ADNI, AIBL and UDS Alzheimer's disease cohorts

Decade of life	Age range	ADNI		AIBL		UDS	
		$\epsilon 4$	$\epsilon 4^{+/-}$	$\epsilon 4^{+/+}$	$\epsilon 4$	$\epsilon 4^{+/-}$	$\epsilon 4^{+/+}$
6	51–60	34 (47)	28 (38)	11 (15)	21 (55)	13 (34)	4 (11)
7	61–70	203 (47)	160 (37)	71 (16)	404 (68)	150 (25)	38 (6)
8	71–80	405 (52)	303 (39)	67 (9)	365 (62)	186 (32)	36 (6)
9	81–90	176 (62)	93 (33)	13 (5)	133 (55)	96 (40)	11 (4)
10	91–100	1 (N.C)	0 (N.C)	0 (N.C)	15 (75)	4 (20)	1 (5)
Total		819	584	162	938	449	90
					10,347	6323	1313

Percentage allele frequency per decade indicated in parentheses

ADNI Alzheimer's Disease Neuroimaging Initiative, *AIBL* The Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing, *UDS* Uniform Data Set of the Alzheimer's Disease Centers program, *N.C* not calculated

Table 3

Summary of the effect of age (β (standard error)) on *APOE* genotype ($\epsilon 4$ negative [$\epsilon 4^{-/-}$] and $\epsilon 4$ homozygous [$\epsilon 4^{+/+}$]) in ADNI, AIBL and UDS Alzheimer's disease cohorts

	Intercept	Age (years)	Z
ADNI			
$\epsilon 4^{-/-}$	-2.258 (0.5271)	0.03182 *** (0.007102)	4.480
$\epsilon 4^{+/+}$	2.343 (0.8207)	-0.06198 *** (0.01144)	-5.419
AIBL			
$\epsilon 4^{-/-}$	1.642 (0.5138)	-0.01485 * (0.006964)	-2.132
$\epsilon 4^{+/+}$	-1.544 (1.045)	-0.01638 (0.01438)	-1.139
UDS			
$\epsilon 4^{-/-}$	-0.6483 (0.1095)	0.01270 *** (0.001437)	-2.906
$\epsilon 4^{+/+}$	-0.2948 (0.1951)	-0.0304 *** (0.002652)	-11.45

ADNI Alzheimer's Disease Neuroimaging Initiative, *AIBL* The Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing, *UDS* Uniform Data Set of the Alzheimer's Disease Centers program

*
 $p < 0.05$

**
 $p < 0.01$

 $p < 0.001$