APOE-ε4 Genotype and Dementia Before and After Transient Ischemic Attack and Stroke
Population-Based Cohort Study

Sarah T. Pendlebury, FRCP, DPhil; Debbie Poole, HNC; Annette Burgess, DPhil; Julia Duerden, PhD; Peter M. Rothwell, FMedSci; for the Oxford Vascular Study

Background and Purpose—APOE-ε4 genotype is a risk factor for sporadic Alzheimer disease and reduced recovery from brain injury. Since data on APOE genotype and dementia associated with transient ischemic attack/stroke are sparse, we determined the associations in a longitudinal population-based cohort.

Methods—All patients with transient ischemic attack or stroke (2002–2012) in a defined population of 92 728 OxVASC (Oxford Vascular Study) had follow-up to 5-years. Pre-event and incident postevent dementia were ascertained through direct patient assessment and follow-up, supplemented by review of hospital/primary care records. Associations between pre- and post-event dementia and APOE genotype (ε4/ε4-homozygous and ε4/ε3-heterozygous versus ε3/ε3) were examined using logistic regression and Cox regression models, respectively, adjusted for age, sex, education, cerebrovascular burden (stroke severity, prior stroke, white matter disease), diabetes mellitus, and dysphasia.

Results—Among 1767 genotyped patients (mean/SD age, 73.0/13.0 years, 901 [51%] male, 602 [34%] transient ischemic attack), 1058 (59.9%) were APOE-ε3/ε3, 403 (22.8%) were ε4/ε3 and 30 (1.7%) were ε4-homozygous. Homozygosity was associated with both pre-event (adjusted odds ratio, 5.81 [95% CI, 1.93–17.48]; P=0.002) and postevent dementia (adjusted hazard ratio, 3.64 [95% CI, 1.90–7.00]; P<0.0001). Association with postevent dementia was maintained after further adjustment for baseline cognitive impairment (hazard ratio, 2.41 [95% CI, 1.19–4.89]; P=0.01). There were no associations overall between ε4/ε3 and pre-event dementia (adjusted odds ratio, 1.47 [95% CI, 0.88–2.45]; P=0.14) or postevent dementia (hazard ratio, 1.11 [95% CI, 0.84–1.48]; P=0.47).

Conclusions—In patients with transient ischemic attack and stroke, APOE-ε4 homozygosity was associated with both pre- and post-event dementia. Associations were independent of cerebrovascular burden and may be mediated through increased neurodegenerative pathology or vulnerability to injury. (Stroke. 2020;51:751-758. DOI: 10.1161/STROKEAHA.119.026927.)

Key Words: apolipoprotein E4 ■ brain injuries ■ cohort studies ■ dementia ■ genotype ■ stroke

APOE-ε4 genotype is the major genetic risk factor for sporadic Alzheimer disease: ε4/ε4 homozygotes have an odds ratio (OR) of 9 to 13 compared with ε3/ε3 individuals, although risks are substantially lower in ε4 heterozygotes (OR, ≈3). Dementia free carriers of APOE-ε4 have lower baseline cognitive function and faster rates of cognitive decline, although effects attenuate in the oldest old. APOE-ε4 homozygosity has been linked to white matter disease load and progression, but neuropathological studies suggest that effects are mediated by increased Alzheimer pathology. APOE-ε4 genotype might, therefore, be expected to impact the risk of dementia associated with stroke. However, there

See related article, p 699

APOE is a lipoprotein produced in several organs including the brain with 3 major isoforms (APOE 2, 3, and 4) encoded by 3 different alleles located on chromosome 19: APOE-ε4, APOE-ε3, and APOE-ε2. Allergic frequencies in whites are 15% for APOE-ε4 and 8% for APOE-ε2 with the remainder being APOE-ε3. The 3 isoforms differ by single amino acid residues resulting in structural and functional differences. The APOE4 isoform has neuropathological effects on neurons, the blood-brain barrier, and blood vessels resulting in various clinical manifestations including worse outcomes after brain injury.
are relatively few data, and findings from previous studies are conflicting. Positive association,\textsuperscript{16–21} no effect,\textsuperscript{22} and protective effects have been reported,\textsuperscript{23–24} but numbers with stroke were small (<200) often with limited adjustment for confounders. We, therefore, undertook a longitudinal population-based study of all transient ischemic attack (TIA) and stroke with standardized assessment of confounders and follow-up for dementia to 5-years to determine the association between APOE4 ε4 genotype and dementia before and after TIA and stroke. The current study builds on our previous work validating our methodology to reliably estimate dementia associated with TIA and stroke.\textsuperscript{25–28}

**Methods**

**Data Availability**

Requests for access to data should be submitted for consideration to the OxVASC (Oxford Vascular Study) Study Director (peter.rothwell@ndcn.ox.ac.uk).

**Patient Cohort and Eligibility**

Consecutive patients with TIA or stroke were prospectively recruited from April 1, 2002 to March 31, 2012 as part of the OxVASC, a population-based cohort study of all acute vascular events occurring within a defined population of 92,728 covered by around 100 primary care physicians in 9 primary care practices in Oxfordshire, United Kingdom.\textsuperscript{29,30} The OxVASC population is 94% white, 3% Asian, 2% Chinese, and 1% Afro-Caribbean.\textsuperscript{4} Please see the online-only Data Supplement for further details on the study population and case ascertainment methodology.

Ascertainment of all TIA and ischemic or hemorrhagic stroke events, including in those with early deaths and TIA/stroke occurring in nonhospitalized/nonrefrained patients, was achieved by multiple methods and has been shown to be near-complete,\textsuperscript{9} and to minimize selection biases in determining dementia risk.\textsuperscript{23} Informed consent (or assent from relatives) was obtained for study interview and follow-up, including ongoing review of all primary care/hospital records and death certificates. The study was approved by the local research ethics committee. Patients eligible for APOE genotype testing were defined as patients surviving to acute assessment and with consent or assent for blood sampling. Patients who were moribund/unlikely to survive were considered not eligible for genotyping.

**Baseline Data Collection**

Patients were assessed by a study clinician as soon as possible after their TIA/stroke. TIA and stroke were defined using the World Health Organization criteria\textsuperscript{42} (ie, patients with relevant infarction on brain imaging but focal neurological symptoms lasting <24 hours were classed as TIA) with review of all cases by the same senior (vascular) neurologist (P.M. Rothwell) throughout the study. Please see the online-only Data Supplement for further details on the definitions of cerebrovascular events. Patient data, including education and vascular risk factors, were collected by interview using a standardized form, supplemented by primary care records (see the online-only Data Supplement).\textsuperscript{29,30} Premorbid functional status was assessed using modified Rankin and Barthel scores, and stroke severity assessed with the National Institutes of Health Stroke Scale (NIHSS) score.\textsuperscript{29,30} Baseline brain and vascular imaging and other investigations were performed as reported previously.\textsuperscript{29,30}

**Brain Imaging and White Matter Disease Severity Grading**

For further details on the brain imaging methodology see the online-only Data Supplement. Assessments were made blind to clinical data. A qualitative scale was used (Oxford scale) based on the white matter disease severity score (absent, mild, moderate, or severe) of the Blennow scale for computed tomography scans and a modified version of the Fazekas scale for magnetic resonance imaging scans.\textsuperscript{33}

**Follow-Up Methodology and Dementia Diagnosis**

Multiple methods of follow-up were used to reduce attritional biases in identification of dementia.\textsuperscript{25} Follow-up interviews were done by trained nurses or study physicians at 1 and 6 months and 1 and 5 years. If clinic follow-up was not possible, patients were assessed at home or via telephone. Mini-mental-state-examination\textsuperscript{44} was done at face-to-face interview and telephone-montréal cognitive assessment and telephone interview for cognitive status-modified for those unable to have face-to-face follow-up.\textsuperscript{36–37}

Dementia was defined as pre- or post-event according to whether the diagnosis was made before or after the index event, as described previously (see the online-only Data Supplement).\textsuperscript{25–28} Briefly, pre-event dementia diagnosis was made using the following information: (1) baseline clinical assessment by study physician and discussion with relatives or other informant; (2) any dementia diagnosis, and related consultations and investigations, where available, in the primary care record, with hand-searching of the entire record including individual consultations, clinic letters, and hospitalization documentation. The diagnosis of pre-event dementia was made by an experienced consultant physician/geriatrician with subspeciality interest in dementia (S.T. Pendlebury) using the Diagnostic and Statistical Manual-IV criteria after review of the baseline clinical assessment and the medical records.

In patients without pre-event dementia, postevent dementia was diagnosed by S.T. Pendlebury using the same methodology.\textsuperscript{25–28} Mini-mental-state-examination was done at each follow-up interview, and dementia was diagnosed if mini-mental-state-examination was <24 and remained <24 for all subsequent follow-ups.\textsuperscript{25–28} In patients with problems interfering with testing, incomplete testing, telephone follow-up, or untestability at study interview,\textsuperscript{45} or without a study follow-up assessment, dementia was diagnosed on the basis of study records where available and hand-searching of primary care, hospital and death records, based on Diagnostic and Statistical Manual-IV criteria.\textsuperscript{25–28}

**APOE Genotype Testing**

Laboratory staff undertaking APOE genotyping were blinded to clinical data. Genomic DNA was extracted from whole blood (collected into vacutainers, K2E, Becton Dickinson) using the QiAamp DNA Blood Midi Kit (Qiagen). The concentration and purity of the DNA were measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, United Kingdom). The DNA was analyzed using the StepOne Real-Time Polymerase Chain Reaction analyzer (Applied Biosystems) to determine the allelic variants of APOE. The 6 APOE genotypes (ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4, and ε4ε4) were determined using SNPs rs429358 and rs7412 (Life Technologies). The standard StepOne genotyping method was followed using a 96-well MicroAmp Fast reaction plate (Life Technologies). Each well contained 50 ng DNA and one of the 2 SNPs, in a TaqMan Universal Polymerase Chain Reaction master mix (Applied Biosystems) in a total of 25 µL. Results were analyzed using the StepOne software. The validity of genotyping was assessed by repeating analyses in 100 samples in an independent lab with investigators blinded to the initial results. Agreement for APOE genotype was found to be 100%.

**Statistical Analysis**

Baseline characteristics between eligible and tested, eligible but not tested, and not eligible patients were compared using ANOVA or χ² test as appropriate. For tested patients, baseline characteristics between groups with the different APOE genotypes were also compared using ANOVA or χ² test. Cumulative incidence of dementia postevent (ie, after exclusion of pre-event dementia) was calculated by Kaplan-Meier methods censoring at death as described previously.\textsuperscript{28} To account for the competing risk of death, we also used Cumulative Incidence Competing Risk methods.\textsuperscript{28}
Associations between APOE genotype and dementia were determined by comparing heterozygous (APOE ε4/ε3) or homozygous (APOE ε4/ε4) individuals with ε3/ε3 as the reference group. We excluded those with ε2/ε4 since ε2 is reported as protective against dementia, and this group was very small. We also examined APOE-ε2 genotype (ε2/ε3 and ε2/ε2) associations with ε3/ε3 as the reference group.

Associations between APOE-ε4 and APOE-ε2 genotypes and pre-event dementia were determined by binary logistic regression to generate ORs adjusted for (1) age, sex, and education (model 1) and (2) age, sex, education, and other factors previously reported as associated with pre-event dementia, including white matter disease, prior stroke, diabetes mellitus, and also index stroke event severity (NIHSS) and dysphasia occurring after the index stroke (model 2). Stroke severity and dysphasia may be linked to preexisting dementia because of a shared susceptibility to stroke and dementia. Cox regression was used to determine hazard ratios (HRs) for associations between overall 5-year risk of postevent dementia, and separately for early (≤1 year) and late (>1 year) postevent dementia (patients with late dementia were excluded from analyses of early dementia and vice versa). Regression analyses were adjusted (1) for age, sex, and education (model 1), (2) age, sex, education, and other factors reported as associated with dementia after TIA/stroke, including stroke severity (NIHSS), white matter disease, dysphasia, prior stroke, and diabetes mellitus, and (3) with further adjustment for baseline cognitive test score (model 3). Similar analyses were done restricted to TIA and minor stroke (defined as NIHSS <3 as per OxVASC protocol) and major stroke (NIHSS ≥3). We also performed competing risks regressions to account for the competing risk of death using cumulative incidence function covariate analysis and generated subdistribution HRs for comparison.

We did not examine primary intracerebral hemorrhage (PICH) separately owing to small numbers with this stroke subtype, but we undertook sensitivity analyses for pre- and post-event dementia in which PICH was excluded. We performed further sensitivity analyses excluding recurrent stroke on follow-up in analyses of postevent dementia.

### Results

Among 1881 consenting, eligible participants from the source population of 2305 patients recruited from 2002 to 2012 (mean/SD age, 74.4/13.0 years), 1767 (94%) had APOE genotype testing (Figure). Nonelgible patients included those with death before interview (n=60), moribund state/severe illness at ascertainment (n=94), declining study bloods/interview or unable to give informed consent and no consent (n=173), and late ascertainment (n=97; Figure). Noneligible patients and eligible but not tested patients were older with less TIA, greater premorbid dependency/disability, moderate/severe white matter disease, more pre-event dementia, higher rates of early death (<31 days), and shorter overall survival time (Figure I in the online-only Data Supplement). Noneligible patients also had higher mean NIHSS and more dysphasia.

#### APOE Genotype

Among the 1767 tested patients (mean/SD age, 73.0/13.0 years, 901 [51%] male, 192 [11%] prior stroke, 602 [34%] TIA, 1100 [62%] ischemic stroke, and 65 [4%] PICH), 1058 (59.9%) were APOE-ε3/ε3, 403 (22.8%) were ε4/ε3, and 30 (1.7%) were homozygous (ε4/ε4, Figure 1, Table 1). The 30 homozygous patients were better educated but with lower baseline cognition and more pre-event dementia than heterozygous or APOE-ε3/ε2 patients (Table 1). Homozygous patients also tended to be younger (mean/SD age, 70.8/11.9 years) versus 72.3/12.9 and 72.9/13.2 years, respectively; P=0.55) with higher premorbid dependency but less severe disability, less severe stroke, more postevent dementia, and more severe white matter disease than the other groups (Table 1).

#### Pre-Event Dementia

Pre-event dementia was present in 110/1767 (6%) of genotyped patients of whom 93 were ε4/ε4, ε4/ε3, or ε3/ε3 (Table 1). Compared with APOE-ε3/ε3, APOE-ε4 homozygosity was strongly associated with pre-event dementia (OR, 4.81 [95% CI, 1.65–14.06]; P=0.004) overall after adjustment for age, sex, and education (model 1). After additional adjustment for other factors associated with pre-event dementia (model 2), the OR was broadly similar. Associations were stronger for TIA and minor stroke and did not reach significance for
There was no association overall between ε4/ε3 status and pre-event dementia after adjustment for all factors in model 2, although there was a significant association for major stroke (OR, 2.30 [95% CI, 1.12–4.71]; P=0.02, Table 2). Exclusion of PICH did not impact the results (Table 2).

### Postevent Dementia

In the 1767 APOE genotyped patients, <5% did not complete study interview follow-up until death or end of study (69 [4%] at 6 months, 71 [4%] at 1 year, and 73 [4%] at 5 years) in whom all available study data, and information from medical records was used to assign dementia diagnoses. Postevent dementia occurred in 345/1657 (21%) genotyped patients without pre-event dementia. APOE ε4/ε4 was strongly associated with all postevent dementia to 5-years follow-up (HR, 2.94 [95% CI, 1.55–5.57]; P=0.001) after adjustment for age, sex, and education (model 1, Table 3, please see the online-only Data Supplement). When other factors associated with postevent dementia were entered (model 2), the association strengthened (3.64 [95% CI, 1.90–7.00]; P<0.0001). After further adjustment for baseline cognitive score (model 3), the HR remained broadly similar (Table 3). Associations were stronger for major stroke than for minor stroke and TIA after full adjustment. In contrast, no associations were seen in ε4/ε3 individuals after adjustment for age, sex, and education or after adjustment for other factors associated with postevent dementia. Results were similar when PICH was excluded and when the 194 patients with recurrent stroke on follow-up were excluded (Table 3). Competing risk analyses and subdistribution HRs showed similar results (please see https://www.ahajournals.org/journal/str).

Looking separately at early (<1 year) versus late (>1 year) postevent dementia, homozygous-ε4 status was overall more strongly associated with late versus early postevent dementia (OR, 4.42 [95% CI, 2.04–9.59]; P<0.0001 versus 2.71 [95% CI, 0.86–8.61]; P=0.09) after adjustment for age, sex, and education (model 1, Table 4). Associations were maintained after adjustment for other factors associated with postevent dementia (model 2, OR, 4.88 [95% CI, 2.23–10.70]; P<0.0001 for late dementia versus 3.45 [95% CI, 1.06–11.16]; P=0.04 for early dementia) although the association with early dementia was no longer significant after adjustment for baseline cognitive score (model 3, HR, 2.11 [95% CI, 0.62–7.15]; P=0.23, Table 4). However, when patients were further stratified by severity of the event, homozygous-ε4 status appeared to impact more on early dementia risk after major stroke and later dementia risk after TIA and minor stroke (please see the online-only Data Supplement).

### Table 1. Distribution of APOE-ε4 and APOE-ε3/ε3 Genotypes in Tested Patients and Associated Clinical Characteristics

| APOE-ε4 Genotype | ε3/ε3, N=1058 | ε4/ε3, N=403 | ε4/ε4, N=30 | P Value |
|------------------|---------------|---------------|-----------|---------|
| Age, y, mean/SD  | 72.9/13.2     | 72.3/12.9     | 70.8/11.9 | 0.55    |
| Age ≥75 y        | 525 (49.6)    | 199 (49.4)    | 12 (40.0) | 0.58    |
| Male sex         | 521 (49.2)    | 213 (52.9)    | 14 (46.7) | 0.43    |
| Education ≤12 y  | 721 (68.1)    | 255 (63.3)    | 16 (53.3) | 0.06    |
| Rankin ≥3*       | 163 (15.4)    | 55 (13.6)     | 7 (23)    | 0.31    |
| Barthel <20*     | 219 (20.7)    | 68 (16.9)     | 2 (6.7)   | 0.04    |
| Prior stroke     | 109 (10.3)    | 41 (10.2)     | 2 (6.7)   | 0.81    |
| Mod./severe WMD  | 276/1034 (26.7)| 128/399 (32.1)| 11 (36.7) | 0.07    |
| TIA              | 354 (33.5)    | 142 (35.2)    | 9 (30)    | 0.91    |
| Minor stroke     | 394 (37.2)    | 149 (37.0)    | 13 (43)   |         |
| Major stroke     | 310 (29.3)    | 112 (27.8)    | 8 (27)    |         |
| PICH             | 38 (3.6)      | 14 (3.5)      | 2 (6.7)   | 0.86    |
| NIHSS mean/SD    | 2.8/3.8       | 2.4/3.9       | 2.1/3.8   | 0.45    |
| Dysphasia        | 144 (13.6)    | 53 (13.2)     | 5 (16.7)  | 0.86    |
| Low baseline cognitive score | 168/917 (18.3) | 59/366 (16.1) | 10/27 (37.0) | 0.02 |
| Pre-event dementia | 60 (5.7)     | 28 (6.9)      | 5 (16.7)  | 0.04    |
| Postevent dementia | 202/998 (20.2)| 71/375 (18.9)| 9/25 (30) | 0.12    |
| Postevent dementia, early | 97/998 (9.7) | 32/375 (8.5) | 3/25 (12) | 0.11    |
| Postevent dementia, late | 105/998 (10.5)| 39/375 (10.4)| 6/25 (27) |         |
| Time to death, y, mean/SD | 3.8/2.0 | 3.9/1.9 | 3.8/1.9 | 0.39 |
| Death <31 d      | 35 (3.3)      | 10 (2.5)      | 0 (0)     | 0.44    |

Numbers are n (%) unless otherwise specified. Event refers to the index TIA or stroke event. Mod indicates moderate; NIHSS, National Institutes of Health Stroke Scale; PICH, primary intracerebral hemorrhage; TIA, transient ischemic attack; and WMD, white matter disease.

*Pre-TIA/stroke function.
Discussion

Homozgyosity for the APOE-\(\varepsilon 4\) allele was associated with both pre- and post-event dementia in patients with TIA and stroke. Associations were strengthened after adjustment for other factors, including stroke severity and white matter disease, suggesting that effects are mediated through mechanisms other than cerebrovascular disease burden. Associations with postevent dementia were also robust to adjustment for baseline cognitive score suggesting that postevent APOE-\(\varepsilon 4\) effect was not simply mediated through worse cognition at baseline.

The frequencies of the different APOE alleles in our study were broadly similar to those reported in population-based volunteer cohorts of stroke and dementia, although poststroke cohort studies showed no consistent relationships, and event severity and early/late poststroke dementia were not examined.\(^{16,42,43}\)

\(\varepsilon 4/\varepsilon 4\) associations appeared overall stronger for dementia after major stroke than for TIA/minor stroke and for late versus early dementia. Adjustment for baseline cognitive score attenuated the association with dementia more strongly in those with early versus late dementia and with more minor events. Although numbers in these analyses were small, this supports a role for increased susceptibility/reduced brain reserve in \(\varepsilon 4/\varepsilon 4\)-associated early poststroke dementia in keeping with neuropathological evidence that subclinical Alzheimer disease may be revealed by the occurrence of stroke.\(^{44-46}\) In addition, our findings indicate that low baseline cognition in those with \(\varepsilon 4/\varepsilon 4\) is relatively more important in dementia after TIA/minor stroke whereas faster cognitive decline may be the more dominant factor after major stroke. Stroke may accelerate cognitive decline in \(\varepsilon 4/\varepsilon 4\) individuals through facilitating aberrant \(\beta\)-amyloid processing as suggested by traumatic brain injury studies\(^5\) or through amyloid-associated poststroke inflammation and increased infarct size.\(^6\) Such processes may also contribute to worse recovery after stroke as is seen after other neurological injury.\(^5,6\)

The association between APOE-\(\varepsilon 4/\varepsilon 4\) and dementia might not be mediated solely by Alzheimer pathology. Recent studies have demonstrated other independent detrimental effects: APOE-\(\varepsilon 4\) increases TDP-43 (transactive response DNA-binding protein of 43 kDa) and thereby hippocampal sclerosis.\(^37\) APOE-\(\varepsilon 4\) is also linked to risk of macroinfarcts and vascular disease in general.\(^10\) It should be noted that although our findings were robust to adjustment for stroke severity and

| Table 2. ORs for Pre-Event Dementia According to APOE Status, Unadjusted, and Adjusted for Age, Sex, Education (Model 1), and for Age, Sex, Education, Stroke Severity, Prior Stroke, White Matter Disease, Diabetes Mellitus, Dysphasia (Model 2) |
|---|---|---|---|---|---|---|
| OR (95% CI) | Unadjusted | \(P\)-Value | Model 1 | \(P\)-Value | Model 2 | \(P\)-Value |
| All patients, N=1767 | | | | | | |
| \(\geq \varepsilon 2\) | 0.96 (0.52–1.79) | 0.90 | 0.81 (0.52–1.79) | 0.53 | 0.72 (0.37–1.43) | 0.36 |
| \(\varepsilon 4/\varepsilon 3\) | 1.24 (0.78–2.00) | 0.37 | 1.37 (0.85–2.22) | 0.19 | 1.47 (0.88–2.45) | 0.14 |
| \(\varepsilon 4/\varepsilon 4\) | 3.20 (1.19–8.62) | 0.02 | 4.81 (1.65–14.06) | 0.004 | 5.81 (1.93–17.48) | 0.002 |
| Excluding PICH, N=1702 | | | | | | |
| \(\geq \varepsilon 2\) | 0.82 (0.43–1.59) | 0.56 | 0.70 (0.35–1.37) | 0.30 | 0.60 (0.29–1.25) | 0.18 |
| \(\varepsilon 4/\varepsilon 3\) | 1.21 (0.76–1.94) | 0.43 | 1.33 (0.82–2.16) | 0.26 | 1.41 (0.84–2.38) | 0.19 |
| \(\varepsilon 4/\varepsilon 4\) | 3.39 (1.25–9.20) | 0.02 | 4.98 (1.69–14.70) | 0.004 | 6.06 (2.00–18.48) | 0.002 |
| TIA and minor stroke only, N=1251 | | | | | | |
| \(\geq \varepsilon 2\) | 0.59 (0.21–1.69) | 0.33 | 0.49 (0.16–1.45) | 0.20 | 0.34 (0.09–1.22) | 0.10 |
| \(\varepsilon 4/\varepsilon 3\) | 0.99 (0.50–2.00) | 0.98 | 1.01 (0.51–2.04) | 0.97 | 0.83 (0.38–1.80) | 0.63 |
| \(\varepsilon 4/\varepsilon 4\) | 3.47 (0.98–12.28) | 0.05 | 5.00 (1.26–19.73) | 0.02 | 7.24 (1.66–31.60) | 0.008 |
| Major stroke (NIHSS \(\geq 3\)) only, N=516 | | | | | | |
| \(\geq \varepsilon 2\) | 1.26 (0.57–2.79) | 0.56 | 1.22 (0.54–2.76) | 0.64 | 1.23 (0.52–2.91) | 0.64 |
| \(\varepsilon 4/\varepsilon 3\) | 1.62 (0.84–3.10) | 0.15 | 1.98 (1.00–3.92) | 0.05 | 2.30 (1.12–4.71) | 0.02 |
| \(\varepsilon 4/\varepsilon 4\) | 3.23 (0.62–16.74) | 0.16 | 4.38 (0.76–25.17) | 0.10 | 5.13 (0.80–32.67) | 0.08 |

\(\varepsilon 3/\varepsilon 3\) is the reference group for all analyses. NIHSS indicates National Institutes of Health Stroke Scale; OR, odds ratio; PICH, primary intracerebral hemorrhage; and TIA, transient ischemic attack.
leukoaraisois, we were not able to take account of other measures of cerebrovascular burden including silent/microinfarcts, microbleeds, perivascular spaces, and superficial siderosis, and thus a vascular effect cannot be completely excluded.

The impact of other risk factors for dementia may also be moderated by the presence of APOE-ε4. Female sex increases the risk of Alzheimer disease, but differential effects of sex by APOE genotype is controversial, and reports are inconsistent.3 In our study, we did not see different effects of APOE-ε4 in women versus men. Similarly, vascular risk factors may act differently on the brain according to APOE status. Diabetes mellitus is a risk factor for dementia, but the effect of poor glycemic control on white matter disease may be exacerbated by APOE-ε4.48 Multiple vascular risk factors may also be more detrimental to white matter disease integrity in the presence of APOE-ε4.49 However, the high risk of APOE-ε4 alone may mask additional effects of other risk factors on cognitive outcomes.

### Table 3. HRs for 5-Year Incidence of Postevent Dementia According to APOE Status, Unadjusted and Adjusted for Age, Sex, Education (Model 1), and for Age, Sex, Education, Stroke Severity, Prior Stroke, White Matter Disease, Diabetes Mellitus, Dysphasia (Model 2), and Model 2 Adjusted for Baseline Cognitive Score (Model 3)

|                      | HR (95% CI)                  | Unadjusted | P Value | Model 1 | P Value | Model 2 | P Value | Model 3 | P Value |
|----------------------|------------------------------|------------|---------|---------|---------|---------|---------|---------|---------|
| All patients, N=1657 | ≥ε2                          | 1.36 (1.02–1.83) | 0.04    | 1.12 (0.83–1.51) | 0.45    | 1.18 (0.87–1.61) | 0.28    | 1.12 (0.81–1.56) | 0.49    |
|                      | ε4/ε3                        | 0.92 (0.70–1.20) | 0.52    | 0.91 (0.69–1.19) | 0.49    | 1.02 (0.77–1.33) | 0.91    | 1.11 (0.840–1.48) | 0.47    |
|                      | ε4/ε4                        | 2.05 (1.09–3.87) | 0.03    | 2.94 (1.55–5.57) | 0.001   | 3.64 (1.90–7.00) | <0.0001 | 2.41 (1.19–4.89) | 0.01    |
| Excluding PICH, N=1596 | ≥ε2                          | 1.32 (0.98–1.80) | 0.07    | 1.08 (0.80–1.47) | 0.61    | 1.16 (0.85–1.59) | 0.35    | 1.05 (0.74–1.47) | 0.79    |
|                      | ε4/ε3                        | 0.92 (0.70–1.22) | 0.57    | 0.91 (0.69–2.00) | 0.49    | 1.02 (0.77–1.35) | 0.90    | 1.08 (0.80–1.44) | 0.62    |
|                      | ε4/ε4                        | 1.94 (1.00–3.79) | 0.05    | 2.75 (1.40–5.39) | 0.003   | 3.34 (1.69–6.60) | 0.001   | 2.08 (0.99–4.38) | 0.05    |
| Excluding recurrent stroke on follow-up, N=1463 | ≥ε2                          | 1.34 (0.97–1.87) | 0.08    | 1.10 (0.79–1.54) | 0.57    | 1.09 (0.77–1.54) | 0.63    | 0.95 (0.65–1.38) | 0.78    |
|                      | ε4/ε3                        | 0.90 (0.66–1.22) | 0.50    | 0.92 (0.67–1.25) | 0.58    | 0.99 (0.72–1.35) | 0.94    | 1.14 (0.82–1.57) | 0.44    |
|                      | ε4/ε4                        | 1.60 (0.710–3.62) | 0.26    | 3.04 (1.33–6.93) | 0.008   | 4.03 (1.74–9.34) | 0.001   | 3.51 (1.48–8.34) | 0.004   |
| TIA and minor stroke only, N=1199 | ≥ε2                          | 1.52 (1.00–2.32) | 0.05    | 1.24 (0.89–1.90) | 0.33    | 1.30 (0.84–2.00) | 0.24    | 1.38 (0.87–2.18) | 0.17    |
|                      | ε4/ε3                        | 1.12 (0.78–1.62) | 0.52    | 1.07 (0.74–1.55) | 0.71    | 1.05 (0.72–1.52) | 0.80    | 1.10 (0.75–1.61) | 0.62    |
|                      | ε4/ε4                        | 2.34 (1.02–5.33) | 0.04    | 3.49 (1.52–8.01) | 0.003   | 3.46 (1.49–8.00) | 0.004   | 1.82 (0.69–4.82) | 0.23    |

rε3/ε3 is the reference group for all analyses. HR indicates hazard ratio; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; PICH, primary intracerebral hemorrhage; and TIA, transient ischemic attack.

### Table 4. HR for Early (≤1 Year) and Late (>1 Year) Postevent Dementia According to APOE-ε4 Status, Unadjusted and Adjusted for Demographic Factors (Model 1), and for Age, Sex, Education, Stroke Severity, Prior Stroke, White Matter Disease, Diabetes Mellitus, Dysphasia (Model 2), and Model 2 Adjusted for Baseline Cognitive Score (Model 3)

|                      | HR (95% CI)                  | Unadjusted | P Value | Model 1 | P Value | Model 2 | P Value | Model 3 | P Value |
|----------------------|------------------------------|------------|---------|---------|---------|---------|---------|---------|---------|
| All patients, N=1657 | ≥ε2                          | 1.34 (0.97–1.87) | 0.08    | 1.10 (0.79–1.54) | 0.57    | 1.09 (0.77–1.54) | 0.63    | 0.95 (0.65–1.38) | 0.78    |
|                      | ε4/ε3                        | 0.90 (0.66–1.22) | 0.50    | 0.92 (0.67–1.25) | 0.58    | 0.99 (0.72–1.35) | 0.94    | 1.14 (0.82–1.57) | 0.44    |
|                      | ε4/ε4                        | 1.60 (0.710–3.62) | 0.26    | 3.04 (1.33–6.93) | 0.008   | 4.03 (1.74–9.34) | 0.001   | 3.51 (1.48–8.34) | 0.004   |
| Major stroke (NIHSS ≥3) only, N=458 | ≥ε2                          | 1.14 (0.75–1.71) | 0.55    | 1.01 (0.67–1.53) | 0.96    | 1.07 (0.69–1.66) | 0.77    | 1.02 (0.62–1.66) | 0.95    |
|                      | ε4/ε3                        | 0.75 (0.51–1.12) | 0.17    | 0.81 (0.54–1.20) | 0.29    | 0.94 (0.63–1.42) | 0.78    | 1.14 (0.73–1.77) | 0.57    |
|                      | ε4/ε4                        | 3.04 (1.118–8.28) | 0.03    | 4.21 (1.51–11.74) | 0.006   | 6.38 (2.15–18.62) | 0.001   | 5.44 (1.78–16.67) | 0.003   |

rε3/ε3 is the reference group for all analyses. HR indicates hazard ratio.
Our study has some limitations. TIA/stroke and dementia diagnoses were made by P.M. Rothwell and S.T. Pendlebury, respectively rather than by a consensus panel but this ensured consistency of approach over the 15-year time period of this study. We were not able to adjust for other potential associates of stroke-related dementia, for example, social and lifestyle factors, depression, treatments, specific stroke subtypes, and lesion characteristics. We were also not able to examine the relationship between APOE genotype and specific subtypes of dementia, although mixed pathology is common in older subjects.13,14,45,46 Similarly, we could not examine the associations of APOE genotype with dementia in PICH versus ischemic stroke owing to small numbers with hemorrhage. The number of patients with the ε4/ε4 genotype was small and; therefore, the findings, particularly in the relations to the subgroups including minor versus major cerebrovascular events and early versus late dementia, should be interpreted with caution. Our study may also have been underpowered to show the reported protective effect of ε2 genotypes. Finally, APOE genotype testing was not possible in patients who died acutely, or in whom consent/assent was unavailable, and these patients had more severe events and more dementia. This may have resulted in an under-estimation of the association between APOE-ε4 and particularly, pre-event dementia, but this was unavoidable even with our inclusive study design.

In conclusion, APOE-ε4 homozygosity is strongly related to both pre- and post-event dementia probably through neurodegenerative processes including Alzheimer pathology, increased TDP-43, and enhanced stroke injury/reduced recovery. APOE-ε4 status should be included in matching of baseline dementia risk in stroke trials to prevent cognitive decline. Also, the effect of risk factor interventions may differ by APOE status. Further studies are required to determine the impact of APOE genotype on cognitive trajectory after stroke and on dementia sub-type. Pooled analysis of individual patient data from multiple studies will be needed to properly determine associations between stroke-associated dementia and the 6 different APOE genotypes and with stroke subtype and early versus late postevent dementia.

Acknowledgments
We acknowledge the use of the facilities of the Acute Vascular Imaging Centre, Oxford. We thank Dr Ramon Luengo-Fernandez for assistance with the cumulative incidence competing risk analyses.

Sources of Funding
The Oxford Vascular Study is funded by the Wellcome Trust, Wolfson Foundation, British Heart Foundation, National Institute of Health Research (NIHR), and the NIHR Oxford Biomedical Research Centre (NIBRC. S.T. Pendlebury is supported by the NIHR Oxford Biomedical Research Centre.

Disclosures
None.

References
1. Zannis VI, Kardassis D. Zannis EE. Genetic mutations affecting human lipoproteins, their receptors, and their enzymes. Adv Hum Genet. 1993;21:145–319. DOI: 10.1007/978-1-4615-3010-7_3
2. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer’s disease and other neurological disorders. Lancet Neurol. 2011;10:241–252. doi: 10.1016/S1474-4422(10)70325-2
3. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. JAMA. 1997;278:1349–1356.
4. Zlokovic. Cerebrovascular effects of Apolipoprotein E. JAMA Neurol. 2013;70:440–444. doi: 10.1001/jamaneurol.2013.2152
5. Rannikmäe K, Kalaria RN, Greenberg SM, Chui HC, Schmitt FA, Samarasakera N, et al. APOE associations with severe CAA-associated vasopathologic changes: collaborative meta-analysis. J Neurol Neurosurg Psychiatry. 2014;85:300–305, doi: 10.1136/jnnp-2013-306485
6. Alberts MJ, Graffagnino C, McClenney C, DeLong D, Strittmatter W, Saunders AM et al. APOE genotype and survival from intracerebral haemorrhage. Lancet. 1995;346:575.
7. Lawrence DW, Comper P, Hutchison MG, Sharma B. The role of apolipoprotein E epsilon (ε)-4 allele on outcome following traumatic brain injury: a systematic review. Brain Inj. 2015;29:1018–1031. doi: 10.3109/02699052.2015.1005131
8. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science. 1993;261:921–923. DOI: 10.1126/science.8346443
9. Cappelli RJ, Ducél AC, Osborne D, Sabhaagh MN, Connor DJ, Ahern GL, et al. Longitudinal modeling of age-related memory decline and the APOE ε4 onf4 effect. N Engl J Med. 2009;361:255–263. doi: 10.1056/NEJMoa0809437
10. Praetorius M, Thorvaldsen V, Hasson JB, Johansson B. Substantial effects of apolipoprotein E ε4 on memory decline in very old age: longitudinal findings from a population-based sample. Neurobiol Aging. 2013;34:2734–2739. doi: 10.1016/j.neurobiolaging.2013.06.002
11. Ganguli M, Lee CW, Snitz BE, Hughes TF, McDade E, Chang CC, Rates and risk factors for progression to incident dementia vary by age in a population cohort. Neurology. 2015;84:72–80. doi: 10.1212/WNL.0000000000001113
12. Godin O, Tzourio C, Maillard P, Alpérovitch A, Mazoyer B, Dufouil C. Apolipoprotein E genotype is related to progression of white matter lesion load. Stroke. 2009;40:3186–3190. doi: 10.1161/STROKEAHA.109.555839
13. Rojas S, Brugulat-Serrat A, Bargalló N, Minguillón C, Tucholka A, Falcon C, et al. Higher prevalence of cerebral white matter hyperintensities in homozygous APOE-ε4 allele carriers aged 45-75: results from the ALFA study. J Cereb Blood Flow Metab. 2018;38:250–261. doi: 10.1002/ jcbf.187073597
14. Mortimer JA, Snowden DA, Markesbery WR. The effect of APOE-ε4 on dementia is mediated by Alzheimer neuropathology. Alzheimer Dis Assoc Disord. 2009;23:152–157. doi: 10.1097/WAD. 0b013e318190a855
15. Lamar M, Yu L, Rubin LH, James BD, Barnes LL, Farfel JM, et al. APOE genotypes as a risk factor for age-dependent accumulation of cerebrovascular disease in older adults. Alzheimer’s Diment. 2015;15:256–266. doi: 10.1016/j.jalz.2018.08.007
16. Slooter AJ, Tang MX, van Duijn CM, Stern Y, Ott A, Bell K, et al. Apolipoprotein E epsilon4 and the risk of dementia with stroke. A population-based investigation. JAMA. 1997;277:818–821. doi: 10.1001/jama.1997.277.11.818
17. Wagle J, Farner L, Flekkøy K, Wyller TB, Sandvik L, Eikeland KL, et al. Cognitive impairment, and the role of the APOE epsilon4-allele after stroke–a 13 months follow-up study. Int J Geriatr Psychiatry. 2010;25:833–842. doi: 10.1002/gps.2425
18. Wagle J, Farner L, Flekkøy K, Wyller TB, Sandvik L, Eikeland KL, et al. Association between ApoE epsilon4 and cognitive impairment after stroke. Dement Geriatr Cogn Disord. 2009;27:525–533. doi: 10.1159/000223320
19. Sachdev PS, Lipnicki DM, Crawford JD, Wen W, Brodaty H. Progression of cognitive impairment in stroke/TIA patients over 3 years. J Neurol Neurosurg Psychiatry. 2014;85:1324–1330. doi: 10.1136/jnnp-2013-306776
20. Zhu L, Fratiglioni L, Guo Z, Basun H, Corder EH, Winblad B, et al. Incidence of dementia in relation to stroke and the apolipoprotein E epsilon4 allele in the very old. Findings from a population-based longitudinal study. Stroke. 2000;31:53–60. doi: 10.1161/01. str.31.1.53
21. Ballard CG, Morris CM, Rao H, O’Brien JT, Barber R, Stephens S, et al. APOE epsilon4 and cognitive decline in older stroke patients with early cognitive impairment. *Neurology*. 2004;63:1399–1402. doi: 10.1212/01.wnl.0000148151.93193.17

22. Arpa A, del Ser T, Goda G, Barba R, Bornstein B. Apolipoprotein E, angiotensin-converting enzyme and alpha-1-antichymotrypsin genotypes are not associated with post-stroke dementia. *J Neurol Sci*. 2003;210:77–82. doi: 10.1016/s0022-510x(03)00026-1

23. Dik MG, Deeg DJ, Bouter LM, Corder EH, Kok A, Jonker C. Stroke and apolipoprotein E epsilon4 are independent risk factors for cognitive decline: a population-based study. *Stroke*. 2000;31:2431–2436. doi: 10.1161/01.str.31.10.2431

24. Reitz C, Luchsinger JA, Tang MX, Mayeux R. Stroke and memory performance in elderly persons without dementia. *Arch Neurol*. 2006;63:571–576. doi: 10.1001/archneur.63.4.571

25. Pendlebury ST, Chen PJ, Bull L, Silver L, Mehta Z, Rothwell PM; Oxford Vascular Study. Methodological factors in determining rates of dementia in transient ischemic attack and stroke: (I) impact of baseline selection bias. *Stroke*. 2015;46:641–646. doi: 10.1161/STROKEAHA.115.010290

26. Pendlebury ST, Chen PJ, Welch SJ, Cuthbertson FC, Wharton RM, Mehta Z, et al; Oxford Vascular Study. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet*. 2005;365:1773–1783. doi: 10.1016/s0140-6736(05)67102-1

27. Pendlebury ST, Kraus SP, Thomson RJ, Mehta Z, Wharton RM, Rothwell PM; Oxford Vascular Study. Methodological factors in determining risk of dementia after transient ischemic attack and stroke: (II) effect of attrition on follow-up. *Stroke*. 2015;46:1494–1500. doi: 10.1161/STROKEAHA.115.009065

28. Pendlebury ST, Coul A, Giles MF, Howard SC, Silver LE, Bull LM, et al; Oxford Vascular Study. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). *Lancet*. 2004;363:1925–1933. doi: 10.1016/S0140-6736(04)16405-2

29. Rothwell PM, Coul A, Silver LE, Fairhead JF; Giles MF, Lovelock CE, et al; Oxford Vascular Study. Population-based study of event-rate, incidence, case-fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet*. 2005;365:1773–1783. doi: 10.1016/S0140-6736(05)67102-1

30. Rothwell PM, Coul A, Silver LE, Fairhead JF; Giles MF, Lovelock CE, et al; Oxford Vascular Study. Direct assessment of completeness of ascertainment in a stroke incidence study. *Stroke*. 2005;34:2041–2045. doi: 10.1161/01.str.0000137605.48864.2f

31. Advisory Council for the National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health, Bethesda, Maryland. A classification and outline of cerebrovascular diseases II. *Stroke*. 1975;6:564–566. DOI: 10.1161/01.str.6.5.564

32. Simoni M, Li L, Paul NL, Gruter BE, Schulz UK, Küker W, et al. Age- and sex-specific rates of leukoaraiosis in TIA and stroke patients: population-based study. *Neurology*. 2012;79:1215–1222. doi: 10.1212/WNL.0b013e3182693e51

33. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”: A practical method for grading the state of patients for the clinician. *J Psychiatr Res*. 1975;12:189–198. doi: 10.1016/0022-3956(75)90026-6

34. Nasreddine ZS, Phillips NA, Bédard V, Charbonneau S, Whitehead V, Collin I, et al. The montreal cognitive assessment, MoCa: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53:695–699. doi: 10.1111/j.1532-5415.2005.33221.x

35. Pendlebury ST, Mariz J, Bull L, Mehta Z, Rothwell PM, MoCA, ACE-R, and MMSE versus the national institute of neurological disorders and Stroke-Canadian stroke network vascular cognitive impairment harmonization standards neuropsychological battery after TIA and stroke. *Stroke*. 2012;43:464–469. doi: 10.1161/STROKEAHA.111.633586

36. Pendlebury ST, Welch SJ, Cuthbertson FC, Mariz J, Mehta Z, Rothwell PM. Telephone assessment of cognition after transient ischemic attack and stroke: modified telephone interview of cognitive status and telephone montreal cognitive assessment versus face-to-face montreal cognitive assessment and neuropsychological battery. *Stroke*. 2013;44:227–229. doi: 10.1161/STROKEAHA.112.673384

37. Khan TA, Shah T, Prieto D, Zhang W, Price J, Fowkes GR, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. *Int J Epidemiol*. 2013;42:475–492. doi: 10.1093/ije/dyt034

38. Niu W, Yi Q, Yian Q, Gao P, Zhu D. The relationship between apolipoprotein epsilon2/epsilon3/epsilon4 polymorphisms and hypertension: a meta-analysis of six studies comprising 1812 cases and 1762 controls. *Hypertens Res*. 2009;32:1060–1066. doi: 10.1088/hr/09.2009.164

39. van Bockxmeer FM, Mamotte CD. Apolipoprotein epsilon4 homozygosity in young men with coronary heart disease. *Lancet*. 1992;340:879–880. doi: 10.1016/0140-6736(92)93288-x

40. Whitehead SN, Cheng G, Hachinski VC, Cechetto DF. Progressive increase in infant size, neuroinflammation, and cognitive deficits in the presence of high levels of amyloid. *Stroke*. 2007;38:3245–3250. doi: 10.1161/STROKEAHA.107.492660

41. Whitehead SN, Cheng G, Hachinski VC, Cechetto DF. Progressive increase in infant size, neuroinflammation, and cognitive deficits in the presence of high levels of amyloid. *Stroke*. 2007;38:3245–3250. doi: 10.1161/STROKEAHA.107.492660

42. Collins I, et al. Evaluation of TDP-43 proteinopathy and hippocampal sclerosis in relation to APOE epsilon4 haplotype status: a community-based cohort study. *Lancet Neurol*. 2018;17:773–781. doi: 10.1016/S1474-4422(18)30251-5

43. Cox SR, Ritchie SJ, Dickie DA, Pattie A, Royle NA, Corley J, et al. Interaction of APOE ε4 and poor glycemic control predicts white matter hyperintensity growth from 73 to 76. *Neurolivia Aging*. 2017;5:54–58. doi: 10.1097/WAD.0000000000000173

44. Wang R, Fratiglioni L, Laukka EJ, Lövdén M, Kalpouzos G, Keller L, et al. APOE ε4 allele and incident stroke on cognitive decline and mortality. *Alzheimer Dis Assoc Disord*. 2016;30:318–323. doi: 10.1097/WAD.0000000000000173

45. Stanwood DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. *The Num Stud*. 1997;277:813–817.

46. Taylor J, et al. The reduction in neuropsychological performance in mild cognitive impairment by APOE ε4 is independent of vascular risk factors. *Neurology*. 2013;81:1594–1600. doi: 10.1212/01.wnl.0000453537.90983.a8