

## FEATURED ARTICLE

# APOE and dementia – resequencing and genotyping in 105,597 individuals

Katrine L. Rasmussen<sup>1,2</sup> | Anne Tybjærg-Hansen<sup>1,2,3,4</sup> | Børge G. Nordestgaard<sup>2,3,4,5</sup> | Ruth Frikke-Schmidt<sup>1,2,4</sup><sup>1</sup> Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark<sup>2</sup> Copenhagen General Population Study, Herlev and Gentofte Hospital, Herlev, Denmark<sup>3</sup> Copenhagen City Heart Study, Frederiksberg Hospital, Frederiksberg, Denmark<sup>4</sup> Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark<sup>5</sup> Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Herlev, Denmark**Correspondence**Ruth Frikke-Schmidt, Professor, Chief Physician, Department of Clinical Biochemistry, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.  
Email: [ruth.frikke-schmidt@regionh.dk](mailto:ruth.frikke-schmidt@regionh.dk)**Funding information**

This work was supported by the Research Council at Rigshospitalet and the Lundbeck Foundation, both to [Ruth Frikke-Schmidt]. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the article; and decision to submit the article for publication.

**Abstract****Introduction:** The mechanism behind the strong association between the  $\epsilon 2/\epsilon 3/\epsilon 4$  apolipoprotein E gene (APOE) polymorphism and Alzheimer's disease is not well-characterized. Because low plasma levels of apoE associate with risk of dementia, genetic variants altering apoE levels in general may also associate with dementia.**Methods:** The APOE gene was sequenced in 10,369 individuals, and nine amino acid-changing variants with frequencies  $\geq 2/10,000$  were further genotyped in 95,228 individuals. Plasma apoE levels were measured directly.**Results:** Risk of all dementia and Alzheimer's disease (AD) increased with decreasing genetically determined apoE levels ( $P = 5 \times 10^{-4}$  and  $P = 1 \times 10^{-4}$  after APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  adjustment). Hazard ratios (95% confidence intervals) for all dementia and AD were 2.76 (1.39 to 5.47) and 4.92 (2.36 to 10.29) for the group with the genetically lowest apoE versus  $\epsilon 33$ .**Discussion:** We found that genetically low apoE levels increase and genetically high levels decrease risk, beyond  $\epsilon 2/\epsilon 3/\epsilon 4$ . This underscores that dementia risk more likely relates to variants affecting levels of apoE.**KEYWORDS**

Alzheimer's disease, APOE, apolipoprotein E, dementia, genetics, rare variation

## 1 | BACKGROUND

Dementia is a major cause of disability in later life, with an increasing global prevalence.<sup>1-5</sup> Currently no curative and preventive options are available. Improved understanding of the underlying genetic background is likely to identify relevant targets for future treatment options and more precise risk prediction for targeted preventive interventions.

In genome-wide association studies (GWASs) the apolipoprotein E (APOE) gene stands out as an impressive signal for risk of Alzheimer's

disease (AD),<sup>6,7</sup> thereby confirming the identification of the common APOE  $\epsilon 4$  allele as an important risk marker first reported by Corder et al.,<sup>8</sup> and since validated in major cohorts around the globe.<sup>9,10</sup> ApoE is a central apolipoprotein in both cerebral and peripheral cholesterol metabolism and is among many functions involved in brain amyloid metabolism, blood-brain barrier integrity, and transport of various lipid species to neuronal cells, as well as in hepatic uptake of triglyceride-rich lipoproteins.<sup>11-16</sup> Despite the impressive  $\epsilon 4$  GWAS signal, the exact mechanism behind this observation is not well

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Alzheimer's & Dementia* published by Wiley Periodicals, Inc. on behalf of Alzheimer's Association

characterized. Of interest, low levels of plasma apoE *per se* were recently reported to be associated with increased risk of dementia,<sup>10,17</sup> thus suggesting that APOE variants that affect apoE protein levels in general also associate with risk of dementia.<sup>10,17-19</sup> Because the APOE ε4 allele is both common and has a large risk effect—much higher than generally observed for common variants in common diseases—the contribution from other genetic less-frequent variants in the APOE gene to risk and their contribution to apoE levels has not been studied at the population level.

Therefore, we set out to do a systematic resequencing of the APOE gene with subsequent large-scale genotyping and related these variants to their specific gene product—plasma levels of apoE—and to the risk of dementia. All rare variants were analyzed in the context of the well-known APOE polymorphism, which is a combination of two genetic variants (ε4 rs429358 and ε2 rs7412), defining six common APOE genotypes (ε22, ε32, ε42, ε33, ε43, and ε44), which correspond to the isoform patterns apoE2/2, apoE3/2, apoE4/2 or 3/4+2, apoE3/3, apoE4/3, and apoE4/4. For this purpose, we used two large general population cohorts, the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS), and performed population-based resequencing in 10,369 individuals from the CCHS and further genotyped nine variants with a frequency of ≥2 per 10,000 in 95,228 individuals from the CGPS.

## 2 | METHODS

Studies were approved by institutional review boards and Danish ethical committees, and were conducted according to the Declaration of Helsinki, with written informed consent from participants. All participants were white and of Danish descent. There was no overlap of individuals between studies.

### 2.1 | Participants

We included individuals from two similar studies of the Danish general population: The CCHS and the CGPS. Individuals were selected randomly from the national Danish Civil Registration System to reflect the adult population aged 20 through 100+ years. These studies combined included a total of 105,597 of whom 3444 developed dementia during the follow-up period.

#### 2.1.1 | The Copenhagen City Heart Study

This prospective study of the Danish general population was initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003.<sup>10,20,21</sup> Data collection included a questionnaire, a physical examination, and blood sampling for biochemical and DNA analyses. We included 10,369 individuals from whom blood was obtained for biochemical and DNA analyses at the 1991 to 1994 or 2001 to 2003 examinations; among these, 1093 developed dementia during follow-up.

### RESEARCH IN CONTEXT

1. Systematic review: We searched PubMed with the term “APOE” or “apolipoprotein E” plus “dementia”, “Alzheimer”, “sequencing”, “rare”, or “variation”. We also searched the reference lists of these articles.
2. Interpretation: Structural changes in apoE beyond the common apolipoprotein E gene (APOE) ε2/ε3/ε4 polymorphism contribute to risk of dementia, where genetically low apoE levels increase and genetically high apoE decrease risk. Consequently, APOE-associated dementia risk is not a property solely related to the strong ε4 allele, but more likely relates to variants affecting levels of apoE in general.
3. Future directions: The study underscores apoE as an important target for future therapeutic strategies. Genetically determined low apoE levels, as seen for ε4 and ε4-like variants, may reflect increased binding to heparan sulfate proteoglycans (HSPGs), which is necessary for development of tau pathology. Hence, inhibitors of HSPG binding may be a future path for targeted therapies in the 3% high-risk ε44 group in the general population.

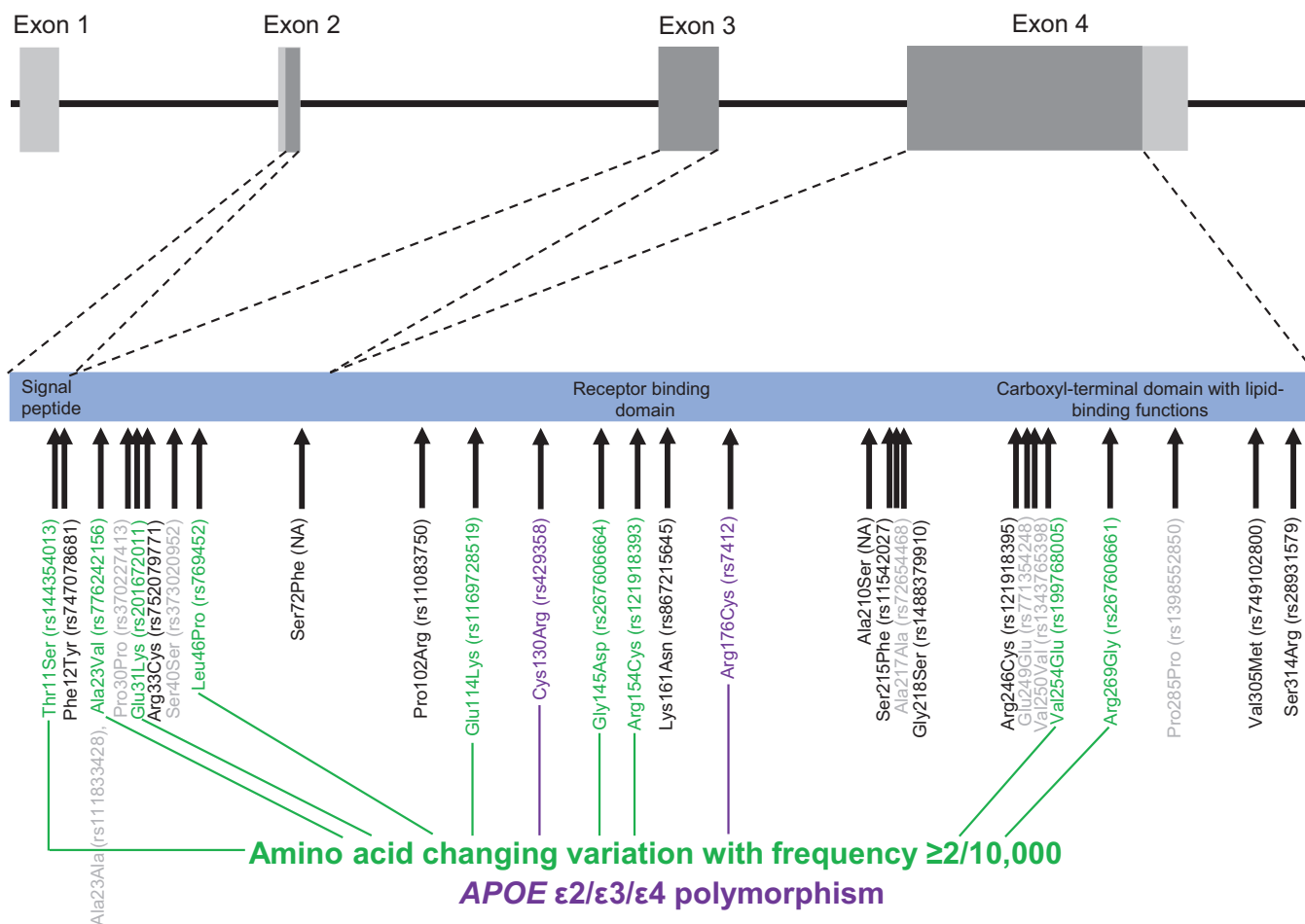
#### 2.1.2 | The Copenhagen General Population Study

This prospective study of the Danish general population was initiated in 2003, with the first enrollment period from 2003 to 2015, and with follow-up examinations ongoing.<sup>10,20,21</sup> Participants were recruited and examined as in the CCHS. We included 95,228 individuals; among these, 2351 developed dementia during follow-up.

### 2.2 | Endpoints

Information on diagnoses was collected from the national Danish Patient Registry, with data on all patient contacts from all clinical hospital departments in Denmark since 1977, and from the national Danish Causes of Death Registry, with data on causes of all deaths in Denmark, as reported by hospitals and general practitioners since 1977. Alzheimer's disease was World Health Organization International Classification of Diseases (ICD) 8th Revision and 10th Revision ICD8 290.10 and ICD10 F00 and G30. All dementia included Alzheimer's disease, vascular dementia (ICD10 F01) and unspecified dementia (ICD8 290.18 and ICD10 F03). For Cox regression models for APOE variants and genetic scores, follow-up began at birth or start of the registries (January 1, 1977), whichever came last. Follow-up ended at occurrence of event, death, emigration, or on April 10, 2018 (last update of the registries), whichever came first. Median follow-up from start of the registries was 40 years (range 0 to 41 years) for all

## Population-based resequencing of 10,369 individuals in the Copenhagen City Heart Study



## Genotyping of 95,228 individuals in the Copenhagen General Population Study

**FIGURE 1** Population-based resequencing of the translated region of the apolipoprotein E gene (*APOE*) in the Copenhagen City Heart Study followed by genotyping in the Copenhagen General Population Study. Gene screening and genotyping are described in the Methods Section. The dark gray regions indicate the translated regions of the *APOE* gene. Of the 27 rare variants found by resequencing, nine amino acid-changing variants with frequencies  $\geq 2/10,000$  are indicated in green. Amino acid-changing variants with frequencies  $< 2/10,000$  are indicated in black, and synonymous variants are indicated in light gray. In addition, the *APOE*  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism is indicated in purple. ApoE consists of two domains connected by a “hinge region” and forms a tetrameric  $\alpha$ -helical bundle, and in the presence of lipids, the bundle “opens” to expose the hydrophobic cores, making the protein available to interact with lipids.<sup>28,42</sup> The amino-terminal domain (amino acids  $\approx 19$  to 209) contains the receptor binding functions (amino acids  $\approx 148$  to 168). This is preceded by a posttranslationally removed 18-residue signal peptide (amino acids 1 to 18).<sup>45</sup> The carboxyl-terminal domain (amino acids  $\approx 243$  to 317) contains the lipid-binding functions. *APOE*, apolipoprotein E gene; NA, rs number not available

dementia, with no individuals lost to follow-up due to the completeness of the Danish nationwide registries.

### 2.3 | Gene screening and genotyping

We screened the translated region of *APOE* by using five polymerase chain reaction (PCR) fragments covering exons 2, 3, and 4 and

exon-intron boundaries (*APOE* consensus sequence NC\_000019.10) (Figure 1). Mutation screening was performed by adding a fluorescent dye (LC Green, Idaho Technology Inc, Salt Lake City, Utah) to the mixture before amplification by PCR which allows for end-point melting (Lightscanner with Lightscanner Instrument & Analysis Software, version 2.0.0.1331, Idaho Technology Inc, Salt Lake City, Utah).<sup>22,23</sup> If two repeated Lightscanner melting analyses indicated variation, the initial PCR was followed by cycle sequencing with a dideoxy-termination

reaction<sup>24</sup> (Sanger sequencing) and further analysis by automated capillary electrophoresis (ABI 3730 DNA Analyzer), and finally verified by K.L.R. (Seqscape version 2.5.0, Applied Biosystems). Identified variants were confirmed by a second round of Sanger sequencing. An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA) and TaqMan-based assays were used to genotype for p.Cys130Arg (rs429358, legacy name Cys112Arg, c.388T>C) defining the  $\epsilon$ 4 allele and p.Arg176Cys (rs7412, legacy name Arg158Cys, c.526C>T) defining the  $\epsilon$ 2 allele. Nine amino acid-changing rare variants with frequency  $\geq 2/10,000$  (allele frequency  $\geq 0.01\%$ ) were further genotyped in the CGPS, using either PCR-based KASP assays (LGC, Teddington, UK) (p.Thr11Ser, p.Ala23Val, p.Glu31Lys, p.Leu46Pro) or ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA) and TaqMan-based assays (p.Glu114Lys, p. Gly145Asp, p.Arg154Cys, p.Val254Glu, p. Arg269Gly). A substantial fraction of heterozygote calls and all homozygote calls for rare variants were validated by Sanger sequencing. Genotypes for three APOE promoter variants (rs449647, rs769446, and rs405509) in 74,940 individuals were determined as described previously.<sup>18,19</sup>

## 2.4 | Biochemical analyses and other covariates

Direct measurement of plasma apoE has previously been described.<sup>10,18,25</sup> Biochemical analyses are described in the Appendix; other covariates are described in the legend to Table S1.

## 2.5 | Statistical analysis

We used Stata/S.E. version 13.1 (Stata Corp., College Station, Texas, USA). *P*-values  $< .0001$  are given as powers of 10. Kruskal–Wallis equality-of-populations rank test and the Pearson  $\chi^2$  test or Fisher exact test were used in comparisons of continuous and categorical variables, respectively. *Z* test evaluated plasma apoE levels for individuals with a rare variant versus individuals without this rare variant: The two-sided *P*-value was derived from the *z*-value, which was calculated as  $z = (\text{mean}_1 - \text{mean}_2) / (\sqrt{(\text{standard error of the mean}_1^2 - \text{standard error of the mean}_2^2)})$ . Missing data ( $< 0.4\%$ ) were imputed from age and sex in each population separately, using multinomial logistic regression for categorical variables and linear regression for continuous variables; however, if only individuals with complete data were included, results were like those reported. No genotypes, endpoints, or plasma apoE levels were imputed.

The apoE-weighted allele score was calculated for each participant using a weighted sum of apoE-changing variants. The weights correspond to the sum of  $\beta$ -coefficients for the 11 apoE-changing variants for each individual obtained from a linear regression for plasma levels of apoE measured directly in 103,898 individuals in CCHS and CGPS, accounting for the effect of the nine rare variants, APOE  $\epsilon 2/\epsilon 3/\epsilon 4$ , sex, age, and cohort (Table 1). By doing so, we ensured that the contribution from both the common APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism as well as

the nine rare variants were captured. Subsequently, we categorized the weighted allele score into five reasonably sized groups (given the large groups of “clean”  $\epsilon 33$  (55%),  $\epsilon 32$  (12%), and  $\epsilon 43$  (25%) without rare variation) with a “clean”  $\epsilon 33$  group as the reference group displaying intermediate levels of genetically determined apoE and being the most prevalent group of individuals in most populations.<sup>15</sup> The “clean”  $\epsilon 33$  group consisted of individuals who were wild-type for the common  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism and with no additional genetic variants in the coding part of APOE, thereby expressing only the apoE3 isoform. The five groups were named “very high,” “high,”  $\epsilon 33$ , “low,” and “very low” weighted allele score group. To illustrate the rare variants in the  $\epsilon 2/\epsilon 3/\epsilon 4$  context we also divided the weighted allele score into 13 groups according to  $\epsilon 2/\epsilon 3/\epsilon 4$  status with and without rare variation. Finally, as a sensitivity analysis an unweighted allele score was created by dividing individuals into 12 groups: the “clean”  $\epsilon 22$ ,  $\epsilon 32$ ,  $\epsilon 42$ ,  $\epsilon 33$ ,  $\epsilon 43$ , and  $\epsilon 44$  genotypes, as well as the same genotypes with additional rare variation ( $\epsilon 22+$ ,  $\epsilon 32+$ ,  $\epsilon 42+$ ,  $\epsilon 33+$ ,  $\epsilon 43+$ , and  $\epsilon 44+$ ).

To test whether rare APOE variants were associated with increased risk of dementia, we used Cox regression models adjusted for known biologically relevant risk factors and markers of lifestyle: age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status, hormonal replacement therapy, and education, and further APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype. For Cox regression models, proportionality of hazards over time was assessed by plotting  $-\ln(-\ln[\text{survival}])$  versus  $\ln(\text{analysis time})$ . There was no suspicion of nonproportionality. Interaction between apoE-weighted allele score in five groups and sex, and between apoE weighted allele score in five groups and cohort were evaluated by the inclusion of two-factor interaction terms in the Cox regression model, using a likelihood ratio test between models excluding and including the interaction term.

For the continuous causal, genetic estimates, we used the weighted genetic score as described above, and further we included the three common APOE promoter variants (rs449647, rs769446, and rs405509) in an alternative weighted score suited to serve as a genetic instrument for plasma apoE levels, as previously applied in Mendelian randomization studies using instrumental variable analyses.<sup>18,19</sup> For comparison with causal, genetic estimates on risk of dementia, we also studied plasma apoE on a continuous scale. These observational and causal, genetic estimates were illustrated both using restricted cubic splines and per 1 mg/dL lower plasma apoE.

## 3 | RESULTS

Twenty-seven rare APOE variants were identified by resequencing APOE in the CCHS, and consequently nine amino acid-changing rare variants with frequencies  $\geq 2/10,000$  were further genotyped in the CGPS (Figure 1). Characteristics of study participants are given in Tables S1, S2, and S3. Forty-five percent of participants were  $\geq 60$  years of age at study entry corresponding to 44% of the person-years in the genetic analyses for all dementia. The three promoter variants and the  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism are associated with expression quantitative

**TABLE 1** Single per allele weights (part A) used for apolipoprotein E-weighted allele score calculation (part B)

Type of variant	Gene region	Nucleotide substitution	Variant	Rs number	Number of individuals (minor allele frequency)			Common allele	Minor allele	Single per allele weights	Contribution (%) <sup>b</sup>
					CCHS N <sub>total</sub> = 10,369	CGPS N <sub>total</sub> = 95,228	CGPS N <sub>total</sub> = 95,228				
Rare	Exon 2	c.31A>T	p.Thr11Ser	rs144354013	2 (0.01)	27 (0.01)	A	T	0.4821579	3	
Rare	Exon 3	c.68C>T	p.Ala23Val	rs776242156	2 (0.01)	7 (0.004)	C	T	-0.6517211	3	
Rare	Exon 3	c.91G>A	p.Glu31Lys	rs201672011	2 (0.01)	6 (0.003)	G	A	-0.5511144	3	
Rare	Exon 3	c.137T>C	p.Leu46Pro	rs769452	74 (0.36)	737 (0.39) <sup>a</sup>	T	C	0.1419617	3	
Rare	Exon 4	c.340G>A	p.Glu114Lys	rs1169728519	11 (0.05)	88 (0.05)	G	A	-1.3534370	3	
Common	Exon 4	c.388T>C	p.Cys130Arg	rs429358	Common (17)	Common (17)	T	C	-0.4460599	8	
Rare	Exon 4	c.434G>A	p.Gly145Asp	rs267606664	4 (0.02)	32 (0.02)	G	A	0.0232725	3	
Rare	Exon 4	c.460C>T	p.Arg154Cys	rs121918393	3 (0.01)	19 (0.01)	C	T	2.8210540	3	
Common	Exon 4	c.526C>T	p.Arg176Cys	rs7412	Common (8)	Common (8)	C	T	1.6478710	26	
Rare	Exon 4	c.761T>A	p.Val254Glu	rs199768005	12 (0.06)	229 (0.12)	T	A	0.1699064	3	
Rare	Exon 4	c.805C>G	p.Arg269Gly	rs267606661	9 (0.04)	102 (0.05)	C	G	-0.4358085	3	

Number of individuals and rare allele frequencies in percent in brackets are shown. The single allele weights correspond to the per-apoE allele  $\beta$ -coefficients adjusted for the impact of the other variants, derived from a linear regression in 103,898 individuals in the Copenhagen City Heart Study and the Copenhagen General Population Study with plasma apoE including all 11 APOE variants, sex, age groups (20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+) and cohort. <sup>a</sup>Includes two homozygotes. <sup>b</sup>The explanatory percentage of the inter-individual variation in plasma apoE from an age, sex and cohort adjusted linear regression for each of the variants separately (R<sup>2</sup> in percent).

TABLE 1 (Continued)

B: Combined single allele weights into a plasma apoE weighted allele score

Thr11Ser	Ala23Val	Glu31Lys	Leu46Pro	Glu114Lys	Cys130Arg	Gly145Asp	Arg154Cys	Arg176Cys	Val254Glu	Arg269Gly	Weights of allele combinations	Weighted allele score in 5 groups	Weighted allele score in 13 groups	N	Percentage of population (%)
AA	CC	GG	TT	GG	TT	GG	CT	CT	TT	CC	4.468925	Very high	3.32-4.47	1	0.0009
AA	CC	GG	TT	GG	TT	GA	CC	TT	TT	CC	3.319015	Very high	3.32-4.47	3	0.003
AA	CC	GG	TT	GG	TT	GG	CC	TT	TT	CC	3.295742	Very high	3.30 (€22)	715	0.7
AA	CC	GG	TT	GG	TT	GG	CT	CC	TT	CC	2.821054	Very high	1.67-2.82	19	0.02
AA	CC	GG	TT	GG	TC	GG	CT	CC	TT	CC	2.374994	Very high	1.67-2.82	2	0.002
AA	CC	GG	TT	GG	TT	GG	CC	CT	TA	CC	1.817777	Very high	1.67-2.82	14	0.01
AA	CC	GG	TC	GG	TT	GG	CC	CT	TT	CC	1.789833	Very high	1.67-2.82	2	0.002
AT	CC	GG	TT	GG	TC	GG	CC	CT	TT	CC	1.683969	Very high	1.67-2.82	5	0.005
AA	CC	GG	TT	GG	TT	GA	CC	CT	TT	CC	1.671144	Very high	1.67-2.82	27	0.03
AA	CC	GG	TT	GG	TT	GG	CC	CT	TT	CC	1.647871	Very high	1.65 (€32)	13,063	12
AA	CC	GG	TC	GG	TC	GG	CC	CT	TT	CC	1.343773	High	1.23-1.34	76	0.07
AA	CC	GG	TT	GG	TC	GA	CC	CT	TT	CC	1.225084	High	1.23-1.34	5	0.005
AA	CC	GG	TT	GG	TC	GG	CC	CT	TT	CC	1.201811	High	1.20 (€42)	2,953	3
AA	CC	GA	TT	GG	TT	GG	CC	CT	TT	CC	1.096757	High	0.02-1.10	1	0.0009
AA	CC	GG	TT	GG	TC	GG	CC	CT	TT	CG	0.766003	High	0.02-1.10	9	0.009
AA	CC	GG	TT	GA	TT	GG	CC	CT	TT	CC	0.294434	High	0.02-1.10	11	0.01
AA	CC	GG	TT	GG	TT	GG	CC	CC	TA	CC	0.169906	High	0.02-1.10	190	0.2
AA	CC	GG	TC	GG	TT	GG	CC	CC	TT	CC	0.141962	High	0.02-1.10	13	0.01
AT	CC	GG	TT	GG	TC	GG	CC	CC	TT	CC	0.036098	High	0.02-1.10	19	0.02
AA	CC	GG	TT	GG	TT	GA	CC	CC	TT	CC	0.023273	High	0.02-1.10	1	0.0009
AA	CC	GG	TT	GG	TT	GG	CC	CC	TT	CC	0	€33	0 (€33)	58,428	55
AA	CC	GG	TC	GG	TC	GG	CC	CC	TA	CC	-0.134192	Low	-0.41 (-0.13)	1	0.0009

(Continues)

TABLE 1 (Continued)

**B: Combined single allele weights into a plasma apoE weighted allele score**

Thr11Ser	Ala23Val	Glu31Lys	Leu46Pro	Glu114Lys	Cys130Arg	Gly145Asp	Arg154Cys	Arg176Cys	Val254Glu	Arg269Gly	Weights of allele combinations	Weighted allele score in 5 groups	Weighted allele score in 13 groups	N	Percentage of population (%)
AA	CC	GG	TT	GG	TC	GG	CC	CC	TA	CC	-0.276154	Low	-0.41(-0.13)	35	0.03
AA	CC	GG	TC	GG	TC	GG	CC	CC	TT	CC	-0.304098	Low	-0.41(-0.13)	594	0.6
AT	CC	GG	TT	GG	CC	GG	CC	CC	TT	CC	-0.409962	Low	-0.41(-0.13)	5	0.005
AA	CC	GG	TT	GG	TC	GG	CC	CC	TT	CC	-0.446060	Low	-0.45 (€43)	26,177	25
AA	CC	GA	TT	GG	TT	GG	CC	CC	TT	CC	-0.551114	Very low	-0.88(-0.55)	5	0.005
AA	CC	GG	CC	GG	CC	GG	CC	CC	TT	CC	-0.608196	Very low	-0.88(-0.55)	2	0.002
AA	CT	GG	TT	GG	TT	GG	CC	CC	TT	CC	-0.651721	Very low	-0.88(-0.55)	8	0.008
AA	CC	GG	TT	GG	CC	GG	CC	CC	TA	CC	-0.722213	Very low	-0.88(-0.55)	1	0.0009
AA	CC	GG	TC	GG	CC	GG	CC	CC	TT	CC	-0.750158	Very low	-0.88(-0.55)	122	0.1
AA	CC	GG	TT	GG	TC	GG	CC	CC	TT	CC	-0.881868	Very low	-0.88(-0.55)	80	0.08
AA	CC	GG	TT	GG	CC	GG	CC	CC	TT	CC	-0.892120	Very low	-0.89 (€44)	2898	3
AA	CC	GA	TT	GG	TC	GG	CC	CC	TT	CC	-0.997174	Very low	-1.80(-1.00)	1	0.0009
AA	CT	GG	TT	GG	TC	GG	CC	CC	TT	CC	-1.097781	Very low	-1.80(-1.00)	1	0.0009
AA	CC	GG	TT	GG	CC	GG	CC	CC	TT	CG	-1.327928	Very low	-1.80(-1.00)	21	0.02
AA	CC	GG	TT	GA	TT	GG	CC	CC	TT	CC	-1.353437	Very low	-1.80(-1.00)	73	0.07
AA	CC	GA	TT	GG	TC	GG	CC	CC	TT	CG	-1.432983	Very low	-1.80(-1.00)	1	0.0009
AA	CC	GG	TC	GA	TC	GG	CC	CC	TT	CC	-1.657535	Very low	-1.80(-1.00)	1	0.0009
AA	CC	GG	TT	GA	TC	GG	CC	CC	TT	CC	-1.799497	Very low	-1.80(-1.00)	14	0.01

For all existing genotype combinations, the single allele weights were summarized into a weighted allele score. These weighted allele scores were subsequently categorized into 5 groups ("very high," "high," "€33," "low," "very low") as well as into 13 groups (3.32-4.47, 3.30 (€22), 1.67-2.82, 1.65 (€32), 1.23-1.34, 1.20 (€42), 0.02-1.10, 0 (€33), -0.41(-0.13), -0.45 (€43), -0.88(-0.55), -0.89 (€44), -1.80(-1.00)). In a sensitivity analysis, a similar score obtained from a linear regression without adjustment for sex, age and cohort resulted in identical grouping in 5 and 13 groups.

trait loci (eQTLs) of APOE or nearby genes in linkage disequilibrium with APOE, whereas the rs769452 (Leu46Pro) is not associated with any eQTLs (Table S4). Pairwise linkage disequilibria of APOE variants are given in Table S5. ApoE-weighted allele score in five groups and sex or cohort did not interact in predicting risk of all dementia and AD (all  $P$ -values  $\geq .10$ ). Consequently, all further analyses were performed for sex and cohorts combined.

### 3.1 | Individual rare APOE variants and plasma levels of apoE

Cohort-specific age- and sex-adjusted percentiles of plasma apoE for nine rare variants are given in Figure S1. Plasma apoE levels were increased for Gly145Asp and Arg154Cys ( $P \leq 8 \times 10^{-9}$ ); were decreased for Ala23Val, Leu46Pro, Glu114Lys, and Arg269Gly ( $P \leq 4 \times 10^{-5}$ ); and were unaltered from the population median for Thr11Ser, Glu31Lys, and Val254Glu ( $P \geq .35$ ) (Figure S1). Allele frequencies for the nine rare variants ranged from 0.01% to 0.36%. All nine variants were included in the weighted and in the unweighted allele scores.

### 3.2 | Individual rare APOE variants and risk of dementia

Multifactorially adjusted Cox regression models evaluated risk of all dementia and AD for individuals with each of the rare APOE variants (Figure S2). No events were observed for heterozygote carriers of Thr11Ser, Gly145Asp, and Arg154Cys, and for homozygote carriers of Leu46Pro. Hazard ratios (HRs) (95% confidence intervals [CIs]) for heterozygotes versus non-carriers for all dementia were 0.87 (0.12 to 6.17) for Ala23Val, 3.34 (0.47 to 23.85) for Glu31Lys, 1.87 (1.39 to 2.51) for Leu46Pro, 2.75 (1.37 to 5.51) for Glu114Lys, 0.30 (0.08 to 1.22) for Val254Glu, and 1.04 (0.34 to 3.23) for Arg269Gly. Corresponding HRs for AD were 1.88 (0.26 to 13.37), 5.79 (0.81 to 41.42), 2.36 (1.66 to 3.34), 3.64 (1.63 to 8.13), 0.57 (0.14 to 2.28), and 1.16 (0.29 to 4.64), respectively. Estimates for Glu31Lys and Glu114Lys improved after APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  adjustment, whereas the significant estimates for Leu46Pro heterozygotes disappeared.

### 3.3 | ApoE-weighted allele score, plasma apoE level, and risk of dementia

The apoE-weighted allele score was calculated for each individual (Table 1, panel A) using a weighted sum of apoE-changing variants and subsequently categorized into five reasonably sized groups (Table 1, panel B). The weights correspond to the sum of the individual  $\beta$ -coefficients for the 11 apoE-changing variants for each individual obtained from a linear regression accounting for the effect of the nine rare variants, APOE  $\epsilon 2/\epsilon 3/\epsilon 4$ , sex, age, and cohort. By doing so, we ensured that the contribution from both the common APOE  $\epsilon 2/\epsilon 3/\epsilon 4$

polymorphism and the nine rare variants were captured, and at the same time ensured that linkage disequilibrium between all variants were accounted for (Table 1).

Risk of all dementia and AD increased with decreasing plasma apoE level on the continuous scale, observationally and genetically determined (Figure 2), as well as for genetically determined plasma apoE levels in five groups ( $P$  for trends  $9 \times 10^{-104}$  and  $6 \times 10^{-107}$  from the very high to high to  $\epsilon 33$  to low to very low groups, and  $5 \times 10^{-4}$  and  $1 \times 10^{-4}$  after APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  adjustment). HRs (95% CIs) for all dementia and AD were 5.25 (4.61 to 5.98) and 7.54 (6.46 to 8.79) for the very low weighted allele score group versus  $\epsilon 33$  (Figure 3). Corresponding estimates after APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  adjustment were 2.76 (1.39 to 5.47) and 4.92 (2.36 to 10.29).

### 3.4 | ApoE-weighted allele score arranged according to APOE $\epsilon 2/\epsilon 3/\epsilon 4$ genotype and risk of dementia

For apoE-weighted allele score in 13 groups arranged according to APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype ( $\epsilon 22$ ,  $\epsilon 32$ ,  $\epsilon 42$ ,  $\epsilon 33$ ,  $\epsilon 43$ , and  $\epsilon 44$  without rare variation), there was an overall increased risk with decreasing apoE-weighted allele score groups for both all dementia and AD (Figure 4;  $P$  for trends  $\leq 1 \times 10^{-100}$ ).

HRs for a 1 mg/dL genetic decrease in plasma apoE were 2.18 (1.35 to 3.50) for all dementia and 2.66 (1.49 to 4.77) for AD for the weighted allele score after  $\epsilon 2/\epsilon 3/\epsilon 4$  adjustment (Figure 5). Corresponding HRs for the weighted allele score in  $\epsilon 33$  individuals only were 2.48 (1.44 to 2.25) and 3.63 (1.98 to 6.66), respectively (Figure 5). For comparison, Figure 5 also illustrates similar risk estimated for plasma apoE observationally and for genetically determined plasma apoE from APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype and from APOE promoter variants, respectively.

### 3.5 | Unweighted allele score arranged according to APOE $\epsilon 2/\epsilon 3/\epsilon 4$ genotype and risk of dementia

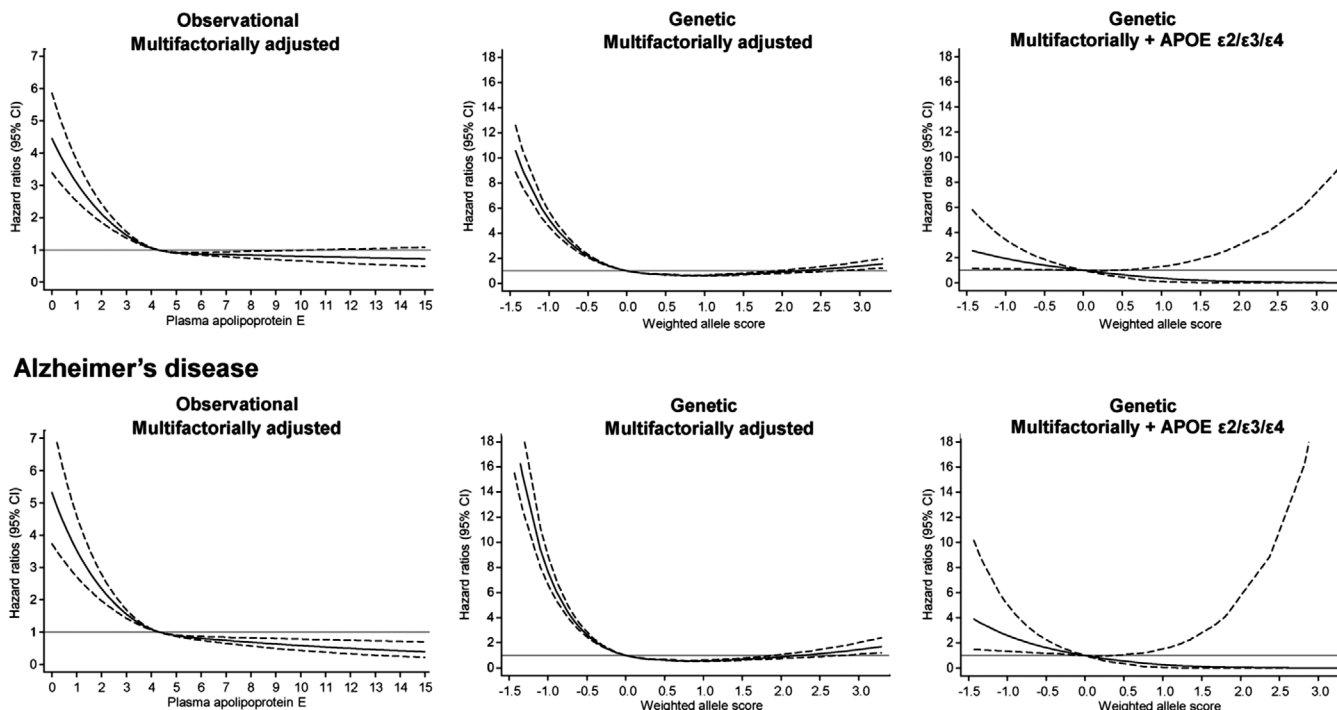
For 12 groups of the clean  $\epsilon 22$ ,  $\epsilon 32$ ,  $\epsilon 42$ ,  $\epsilon 33$ ,  $\epsilon 43$ , and  $\epsilon 44$  genotypes, as well as the same genotypes with additional rare variation ( $\epsilon 22+$ ,  $\epsilon 32+$ ,  $\epsilon 42+$ ,  $\epsilon 33+$ ,  $\epsilon 43+$ , and  $\epsilon 44+$ ) a similar pattern as for the weighted allele score in 13 groups was observed (Figure S3;  $P$  for trends were  $\leq 5 \times 10^{-100}$ ).

### 3.6 | Sensitivity analyses

Excluding three or six variants in the weighted allele score did not change estimates substantially (Figures S4 and S5). The three compound heterozygotes (Glu31Lys/Arg269Gly, Leu46Pro/Glu114Lys, and Leu46Pro/Val254Glu) and the two Leu46Pro homozygotes did not contribute to estimates with any dementia events, and risk estimates for Glu31Lys, Glu114Lys, and Val254Glu did not change after exclusion of the three compound heterozygotes (data not shown).



## All dementia



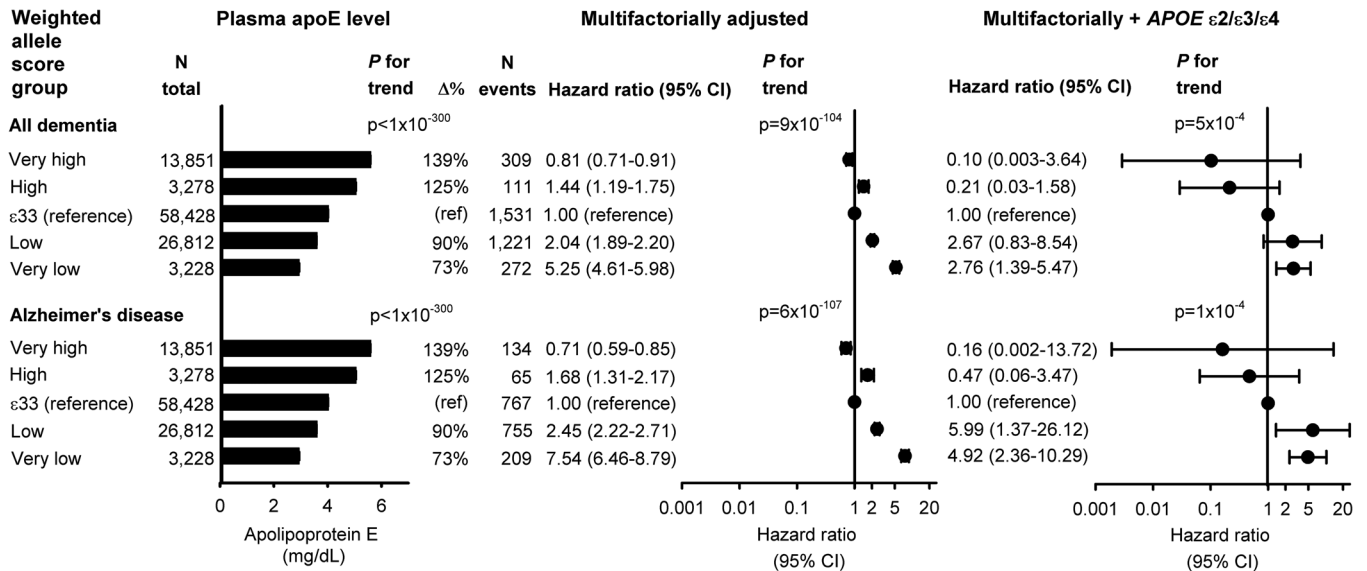
**FIGURE 2** Multifactorially adjusted hazard ratios for all dementia and Alzheimer's disease according to plasma levels of apolipoprotein E (apoE) and weighted allele score in individuals in the general population. Solid lines are multifactorially adjusted hazard ratios, whereas dashed lines indicate 95% confidence intervals derived from restricted cubic splines with three knots, with the reference defined as the mean plasma level of apoE (4.3 mg/dL) or  $\epsilon 33$  (weighted allele score = 0). For the observational estimates, follow-up began at study entry and only if plasma apoE measurements were available. Graphs are truncated at 15 mg/dL for plasma levels of apoE and at  $-1.5$  and  $3.3$  for the weighted allele score due to statistically unstable estimates outside these values, thus including 103,682 and 105,578 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study for these observational and genetic analyses. Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status, hormonal replacement therapy, and education (left and middle columns), and further for APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype (right column). CI, 95% confidence interval

Estimates for single rare variants and for apoE-weighted allele score in five groups remained largely unchanged after adjustment for low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (data not shown and Figure S6, respectively) and after stratification by  $\geq 60$  years at study entry (Figures S7 and S8).

## 4 | DISCUSSION

The principal findings of this study are that rare amino acid-changing variants in the APOE gene contribute to a risk of dementia beyond the strong  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism where genetically low apoE levels increase and genetically high levels decrease risk. A 1 mg/dL decrease in genetically determined plasma apoE levels increased risk of dementia two- to fourfold. These novel findings underscore the importance of structurally well-functioning apoE in dementia risk and that risk increase conferred by the APOE gene is not a property solely related to the common  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism, but more likely relates to variants affecting levels of apoE in general.

Mechanistically, our findings can possibly be understood as follows. First, it is well established that heterozygotes for Arg176Cys ( $\epsilon 32$ ) are associated with a decreased risk of dementia, likely explained by higher apoE levels caused by decreased affinity of the apoE2 isoform to LDL receptors due to the well-established ligand-defect of 176Cys.<sup>15</sup> Of interest, this is highly supported by a novel report on the APOE Arg154Ser mutation (APOE3 Christchurch, old nomenclature Arg136Ser), where the effect of a dominant presenilin 1 mutation was neutralized in a woman from a large Columbian kindred with autosomal dominant AD.<sup>26</sup> Functional studies have suggested that the beneficial effect is related to decreased affinity for LDL receptor family members and heparan sulfate proteoglycans (HSPGs). Consequently, no tau pathology develops because apoE binding may be necessary for neuronal uptake of extracellular tau.<sup>27</sup> Additional support comes from the present data, where Gly145Asp and Arg154Cys are located in the receptor binding region (amino acids  $\approx 148$  to 168)<sup>28</sup> and display a phenotype similar to the  $\epsilon 2$ -defining Arg176Cys residue with high apoE levels and seemingly low risk of dementia (no events). Second, variation near the  $\epsilon 4$ -defining residue Cys130Arg results in an  $\epsilon 4$ -like increased risk of dementia. Glu114Lys is the rare variant located



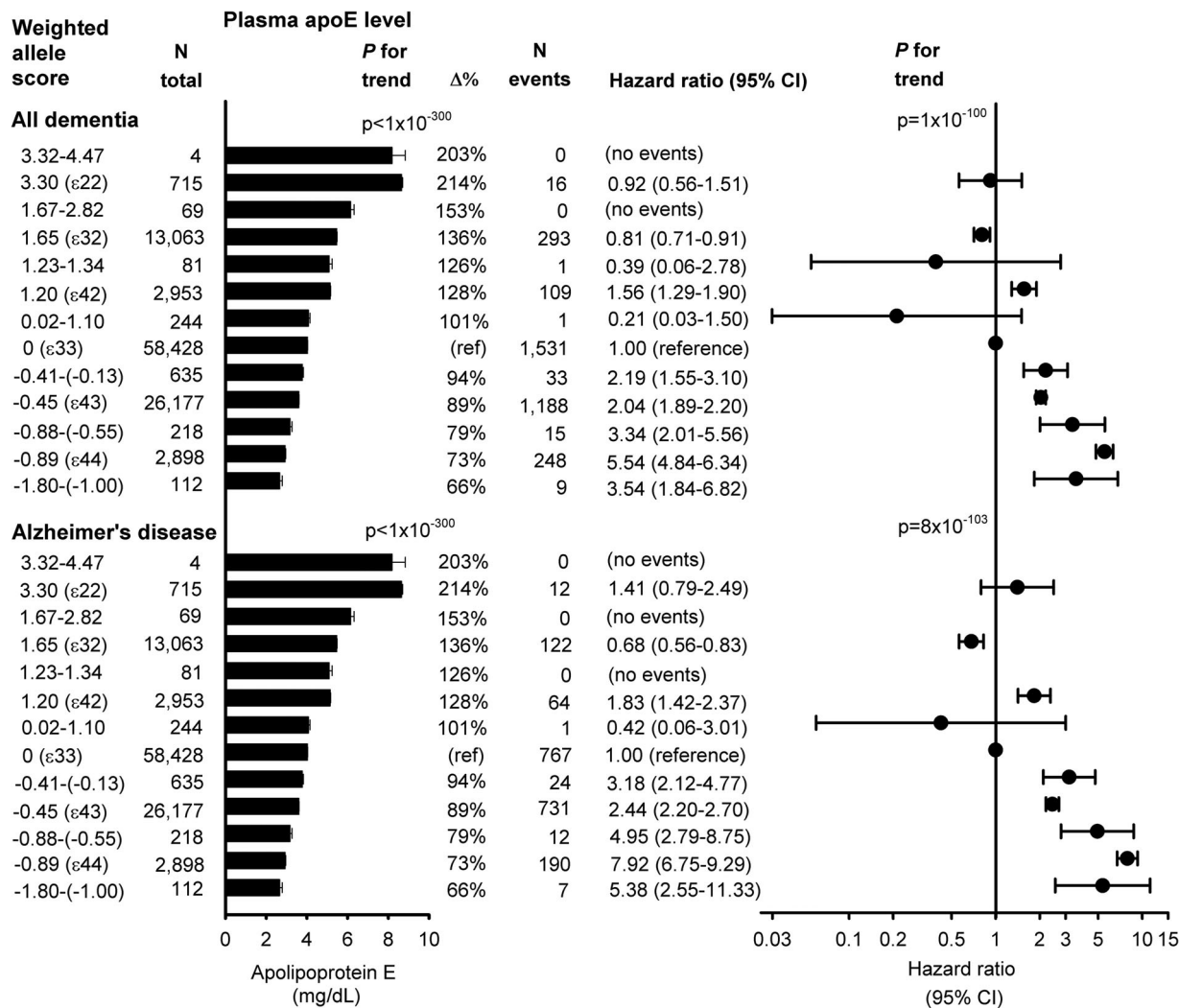
**FIGURE 3** Risk of all dementia and Alzheimer's disease as a function of apolipoprotein E (apoE)-weighted allele score in five groups. Geometric mean  $\pm$  standard errors of the mean are given for plasma apolipoprotein E (left column). Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status, hormonal replacement therapy, and education (middle column), and further for APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype (right column); CI, 95% confidence interval

closest to Cys130Arg but is not in linkage disequilibrium with  $\epsilon 4$ . Of interest, this variant is associated with decreased apoE levels and increased risk of dementia, similar to  $\epsilon 4$ . HSPGs are suggested to play a role in neuronal uptake of tau and in amyloid beta metabolism, and apoE binding may play an important role here.<sup>26,27</sup> It is important to note that HSPGs binding studies have shown that apoE4 had higher affinity than apoE3, apoE2 and apoE3 Christchurch,<sup>29</sup> and antibodies raised against the receptor-binding region mimicked the protective effect of apoE2 and apoE3 Christchurch,<sup>26</sup> thereby raising hope for apoE4-targeted therapies and/or possibly therapies modulating apoE-HSPG interactions.<sup>26</sup> Functional studies of rare variation near the receptor-binding area—near  $\epsilon 4$  as well as  $\epsilon 2$ —would be relevant to explore this further.

Taken together, and in light of the potential amyloid-neutralizing effect of an apoE-increasing mutation,<sup>26</sup> these  $\epsilon 2$ -like and  $\epsilon 4$ -like deviations of the structure of apoE have potential important biologic and therapeutic implications. Whereas brain apoE plays a major role in brain amyloid metabolism, in blood-brain barrier integrity, and in supplying cholesterol and phospholipids to neuronal cells,<sup>12-14,16,30,31</sup> it is also well-known that  $\epsilon 4$  is associated with an adverse lipid profile and that  $\epsilon 22$  carriers have a propensity to develop a recessive form of dysbetalipoproteinemia (formerly known as type III hyperlipoproteinemia).<sup>15,18</sup> Mutations in the LDL receptor-binding domain, as the APOE Arg154Ser Christchurch, as well as the presently identified Gly145Asp and Arg154Cys mutations, are also associated with dysbetalipoproteinemia—a condition that is, however, treatable with standard lipid medications.<sup>32</sup> Therefore, if receptor binding-inhibiting therapies will be developed, a natural side effect will be introduction of dysbetalipoproteinemia, unless the therapy can be limited to the brain.

This is the first time that the full spectrum of genetic variation in the coding parts of the APOE gene, corresponding effects on the direct gene product, levels of apoE, and risk of dementia are presented in a large general population cohort. The current literature is sparse on this issue and consists mostly of case reports and smaller studies. In accordance with previous studies, we found Leu46Pro in linkage disequilibrium with  $\epsilon 4$ , and whereas some studies reported Leu46Pro to add risk beyond  $\epsilon 4$ <sup>33,34</sup> through a destabilized apoE structure,<sup>35</sup> others found that Leu46Pro did not increase risk of AD beyond  $\epsilon 4$ .<sup>36,37</sup> Our study confirmed the latter. A resequencing study of 376 healthy individuals  $\geq 85$  years of age and 376 controls 41 to 54 years of age found no excess of rare variants in the 85+ year olds.<sup>38</sup> However, that study was unable to investigate the effects of the identified rare variants for development of dementia in a large prospective set-up with an unselected population with long follow-up. Furthermore, two case-studies of patients with frameshift mutations and premature stop codons in exon 4 did not observe clear neurocognitive defects, and one case-control study found a higher frequency of a combined Glu262Lys and Glu263Lys variant in patients with memory complaints ( $P = .08$ )<sup>39-41</sup>; however, these variants were not found in our population. Val254Glu and Arg269Gly are located in the lipid-binding carboxyl-terminal domain (amino acids  $\approx 243$  to 317).<sup>28,42</sup> We did not find risk associations, although Val254Glu was previously reported to be protective.<sup>36</sup> Whereas APOE  $\epsilon 4$  is the main contributor to late-onset AD, and has provided the clearest and most consistent signal in GWASs,<sup>6,7</sup> the risk of dementia for carriers of rare APOE variants was also substantial, with effect sizes similar to those for  $\epsilon 4$  as well as other rare variants in dementia risk genes such as TREM2.<sup>43</sup>

Collectively, the present data suggest that rare functional genetic variation in APOE, which causes lifelong changes in apoE levels, affects

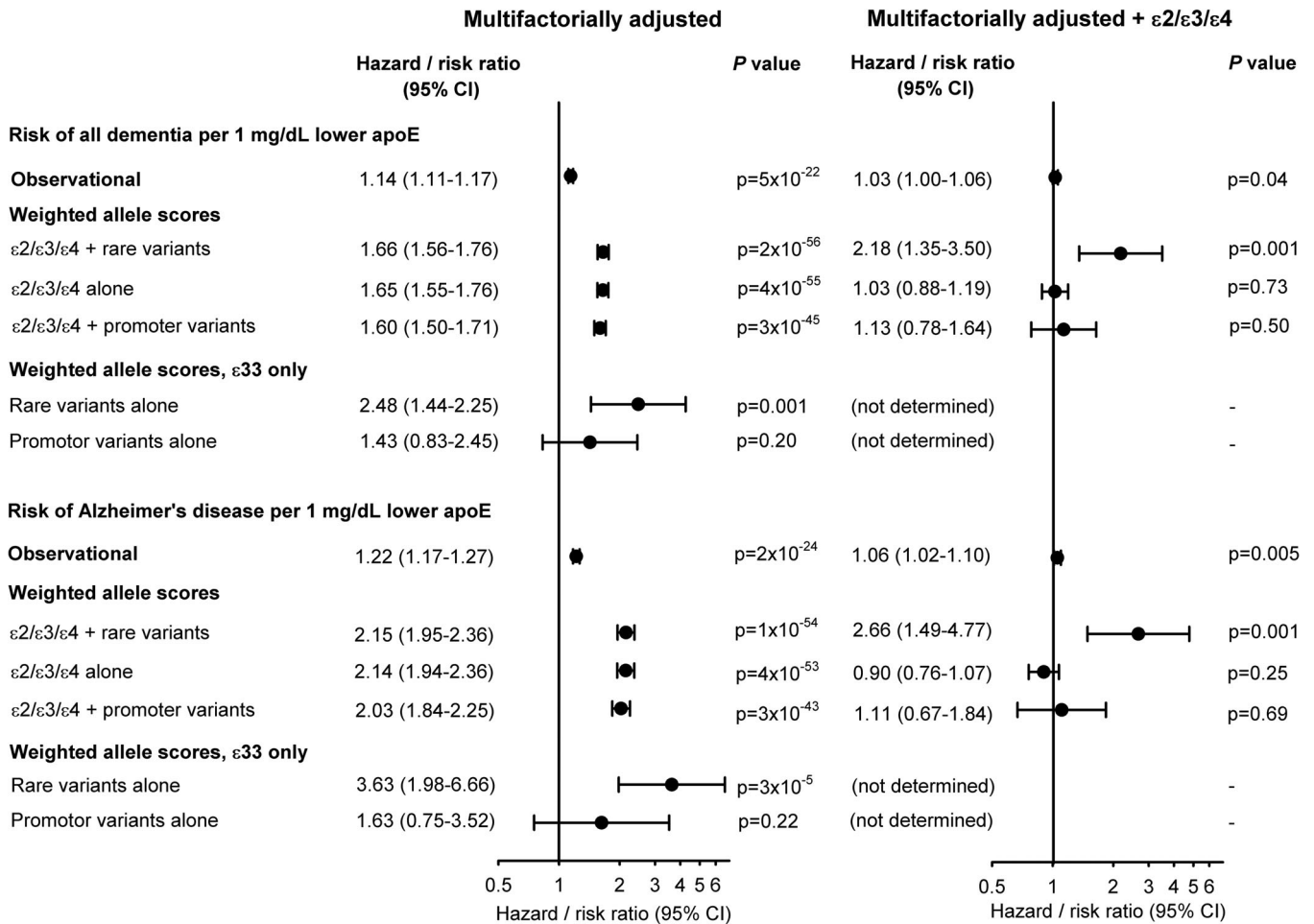


**FIGURE 4** Risk of all dementia and Alzheimer's disease as a function of the weighted allele score stratified according to apolipoprotein E gene (APOE)  $\epsilon 2/\epsilon 3/\epsilon 4$  status. The weighted allele score was divided in 13 groups according to APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype (clean  $\epsilon 22$ ,  $\epsilon 32$ ,  $\epsilon 42$ ,  $\epsilon 33$ ,  $\epsilon 43$  and  $\epsilon 44$  genotypes without rare variation). Geometric mean  $\pm$  standard errors of the mean are given for plasma apoE (left column). Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status, hormonal replacement therapy, and education (middle and right column). *P* for trends were from 3.32-4.47 to 3.30 ( $\epsilon 22$ ) to 1.67-2.82 to 1.65 ( $\epsilon 32$ ) to 1.23-1.34 to 1.20 ( $\epsilon 42$ ) to 0.02-1.10 to 0 ( $\epsilon 33$ ) to -0.41-(-0.13) to -0.45 ( $\epsilon 43$ ) to -0.88-(-0.55) to -0.89 ( $\epsilon 44$ ) to -1.80-(-1.00). ApoE, apolipoprotein E; CI, 95% confidence interval

risk of dementia, both alone and compiled in weighted allele scores. This general statement is highly supported by the fact that the association between the weighted allele score groups and risk of dementia becomes more linear after adjusting for the  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism. Because  $\epsilon 4$  is a strong risk-increasing allele and  $\epsilon 2$  is a strong apoE-increasing allele, the  $\epsilon 42$  genotype displays a higher risk than  $\epsilon 33$  but also higher apoE levels than  $\epsilon 33$ . This results in a less linear trend for the weighted allele score group before adjustment, because the "high" weighted allele score group contains the majority of  $\epsilon 42$  individuals. However, after adjustment, the weighted allele score groups reflect that genetically high apoE levels in general are associated with low risk. These findings are in accordance with our previous work on common APOE variants, where genetically low plasma apoE level was associated with increased risk of dementia and dementia-associated

mortality.<sup>18,19</sup> Hence, we now suggest that both rare and common variants in APOE contribute to plasma levels of apoE, likely mimicking brain apoE levels, and to risk of dementia in the general population.<sup>10,19</sup>

A major strength of the study is the large prospective general population design with no losses to follow-up: that is, every single individual could be followed to occurrence of event, end of follow-up, death, or emigration. In addition, due to the large sample size, we were able to conduct survival analyses with a meaningful power despite the fact that allele frequencies for the nine rare variants were  $\leq 0.4\%$ . Finally, the ability to adjust for and relate estimates for rare variants to the common APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  genotype is a major advantage. One potential limitation concerns the validity of the dementia diagnoses; however, the national Danish Patient Registry includes all hospital visits, and dementia diagnoses in the Danish registries have high diagnostic



**FIGURE 5** Risk for all dementia and Alzheimer's disease for a 1 mg/dL decrease in plasma apolipoprotein E (apoE) levels. Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status, hormonal replacement therapy, and education (left column), and further for APOE ε2/ε3/ε4 genotype (right column). For the observational estimates, follow-up began at study entry and only if plasma apoE measurements were available, thus including 103,744 individuals in these analyses. The first weighted allele score, ε2/ε3/ε4 + rare variants, is the score described in the Methods section (n = 105,597). For the second score, ε2/ε3/ε4 alone, the score was obtained similarly, but in a model only including the ε4 and the ε2 alleles, and not the nine rare variants (n = 105,597). The third score, ε2/ε3/ε4 + promoter variants, was obtained like the main genetic score, but included weights from ε4, ε2, and three common promoter variants (rs449647, rs769446, and rs405509) (n = 74,940). The estimates for rare variants alone and for promotor variants alone were for the same genetic scores for ε33 individuals only (n = 58,737 and 41,789). CI, 95% confidence interval

validity<sup>19,44</sup>; any unregistered events would only lead to decreased power of estimates and bias results toward the null hypothesis. Second, the generalizability of our study results is limited because we studied white individuals only, and consequently our results may not necessarily apply to other populations. In the majority of populations, the ε33 genotype is the most common; however, interestingly allelic frequencies vary in different populations,<sup>15</sup> which could affect the relative contribution. The impact of the APOE polymorphism is well described, however, among many ethnicities, so our results are likely to be applicable to most humans. Third, as the present large prospective cohort study is the only study with measurements of both plasma apoE and genetic variation, the weights for the 11 apoE-changing variants were estimated from the data to which they were applied. Therefore, risk of overfitting is a potential limitation, and as the score is generated in a

very homogeneous sample, it may be less likely to perform as well in other populations.

In conclusion, by resequencing the APOE gene in 10,369 individuals from the general population and genotyping selected variants in up to 105,597 individuals, we found that structural changes in apoE beyond ε2/ε3/ε4 contribute to a risk of dementia where genetically low apoE levels increase and genetically high apoE levels decrease risk. We suggest, that it is useful to genotype more than just the ε2/ε3/ε4 polymorphism, despite its strong impact. Rare genetic variation in APOE may thus add to explain missing heritability for dementia. These findings underscore the importance of structurally well-functioning apoE in dementia risk and that this is not a property related solely to the common ε2/ε3/ε4 polymorphism, but more likely relates to variants affecting levels of apoE.

## ACKNOWLEDGMENTS

We thank the staff and participants of the CGPS and the CCHS for their important contributions.

## AUTHOR CONTRIBUTIONS

Katrine L. Rasmussen: Study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, drafting of the article, and critical revision of the article for important intellectual content. Anne Tybjærg-Hansen: Acquisition of data, critical revision of the article for important intellectual content, obtained funding, and provided administrative, technical, and material support. Børge G. Nordestgaard: Acquisition of data, critical revision of the article for important intellectual content, obtained funding, and provided administrative, technical, and material support. Ruth Frikke-Schmidt: Study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, drafting of the article, critical revision of the article for important intellectual content, obtained funding, and administrative, technical, and material support, study supervision.

## REFERENCES

- Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;385:117-171.
- Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement*. 2013;9:63-75.
- Langa KM, Larson EB, Crimmins EM, et al. A Comparison of the prevalence of dementia in the United States in 2000 and 2012. *JAMA Intern Med*. 2017;177:51-58.
- Satizabal CL, Beiser AS, Chouraki V, Chene G, Dufouil S, Seshadri S. Incidence of dementia over three decades in the Framingham Heart Study. *N Engl J Med*. 2016;374:523-532.
- Ahmedi-Abhari S, Guzman-Castillo M, Shipley MJ, et al. Temporal trend in dementia incidence since 2002 and projections for prevalence in England and Wales to 2040: modelling study. *BMJ*. 2017;358:j2856.
- Lambert JC, Ibrahim-Verbaas CA, Naj AC, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45:1452-1458.
- Kunkle BW, Grenier-Boley B, Bis JC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet*. 2019;51:414-430.
- Corder EH, Saunders AM, Schmechel DE, 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.
- Livingston G, Sommerlad A, Costafreda SG, et al. Dementia prevention, intervention, and care. *Lancet*. 2017;390:2673-2734.
- Rasmussen KL, Tybjærg-Hansen A, Borge G, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol*. 2015;77:301-311.
- Cramer PE, Cirrito JRR, Lee CY, et al. ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. *Science*. 2012;335:1503-1506.
- Bell RD, Winkler EA, Sagare AP, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature*. 2012;485:512-516.
- Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: a review. *Atherosclerosis*. 2016;255:145-144.
- Wellington CL, Frikke-Schmidt R. Relation between plasma and brain lipids. *Curr Opin Lipidol*. 2016;27:225-232.
- Mahley RW, Jr, Rall SC. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York, NY: McGraw-Hill; 2001:2835-2862.
- Mahley RW. Central nervous system lipoproteins: ApoE and regulation of cholesterol metabolism. *Arterioscler Thromb Vasc Biol*. 2016;36:1305-1315.
- Wolters FJ, Koudstaal PJ, Hofman A, Duijn CM, Ikram AF. Serum apolipoprotein E is associated with long-term risk of Alzheimer's disease: The Rotterdam Study. *Neurosci Lett*. 2016;617:139-142.
- Rasmussen KL, Tybjærg-Hansen A, Frikke-Schmidt R. Plasma levels of apolipoprotein E, APOE genotype, and all-cause and cause-specific mortality in 105 949 individuals from a white general population cohort. *Eur Heart J*. 2019;40:2813-2824.
- Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma apolipoprotein E levels and risk of dementia: a Mendelian randomization study of 106,562 individuals. *Alzheimers Dement*. 2018;14:71-80.
- Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32-41.
- Frikke-Schmidt R, Nordestgaard BG, Sethi AA, et al. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA*. 2008;299:2524-2532.
- Liew M, Pryor R, Meadows C, et al. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. *Clin Chem*. 2004;50:1156-1164.
- Wittwer CT, Reed GH, Gundry CM, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chem*. 2003;49:853-860.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;74:5463-5467.
- Rasmussen KL, Tybjærg-Hansen A, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of ischemic heart disease in the general population. *Atherosclerosis*. 2016;246:63-70.
- Arboleda-Velasquez JF, Lopera F, Delgado-Tirado S, et al. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nat Med*. 2019;25:1680-1683.
- Rauch JN, Chen JJ, Miller GM, et al. Tau internalization is regulated by 6-O sulfation on Heparan Sulfate Proteoglycans (HSPGs). *Sci Rep*. 2018;8:6382.
- Weisgraber KH. Apolipoprotein E: structure-function relationships. *Adv Protein Chem*. 1994;45:249-302.
- Yamauchi Y, Deguchi N, Tanaka M, et al. Role of the N- and C-terminal domains in binding of apolipoprotein E isoforms to heparan sulfate and dermatan sulfate: a surface plasmon resonance study. *Biochemistry*. 2008;47:6702-6710.
- Hirsch-Reinshagen V, Burgess BL, Wellington CL. Why lipids are important for Alzheimer disease? *Mol Cell Biochem*. 2009;326:121-129.
- Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron*. 2009;63:287-303.
- Hegele RA, Boren J, Arca M, et al. Rare dyslipidaemias, from phenotype to genotype to management: a European Atherosclerosis Society task force consensus statement. *Lancet Diabetes Endocrinol*. 2019.
- Scacchi R, Gambina G, Ferrari G, Corbo RM. Screening of two mutations at exon 3 of the apolipoprotein E gene (sites 28 and 42) in a

- sample of patients with sporadic late-onset Alzheimer's disease. *Neurobiol Aging*. 2003;24:339-343.
34. Kamboh MI, Aston CE, Kokmen K, et al. A novel mutation in the apolipoprotein E gene (APOE\*4 Pittsburgh) is associated with the risk of late-onset Alzheimer's disease. *Neurosci Lett*. 1999;263:129-132.
  35. Argyri L, Dafnis I, Gantz D, Chroni A. Molecular basis for increased risk for late-onset Alzheimer disease due to the naturally occurring L28P mutation in apolipoprotein E4. *J Biol Chem*. 2014;289:12931-12945.
  36. Medway CW, Abdul-Hay S, Zou F, et al. ApoE variant p.V236E is associated with markedly reduced risk of Alzheimer's disease. *Mol Neurodegener*. 2014;9:11.
  37. Baron M, Jimenez-Escrig A, Simon J, et al. Apolipoprotein E Pittsburgh variant is not associated with the risk of late-onset Alzheimer's disease in a Spanish population. *Am J Med Genet B Neuropsychiatr Genet*. 2003;120B:121-124.
  38. Tindale LC, Leach S, Daley D, et al. Rare and common variants in the Apolipoprotein E gene in healthy oldest old. *Neurobiol Aging*. 2014;35:727-3.
  39. Youn YC, Lim YK, Giau VV, et al. Apolipoprotein epsilon7 allele in memory complaints: insights through protein structure prediction. *Clin Interv Aging*. 2017;12:1095-1102.
  40. Mak AC, Pullinger CR, Schwarz JM, et al. Effects of the absence of apolipoprotein e on lipoproteins, neurocognitive function, and retinal function. *JAMA Neurol*. 2014;71:1228-1236.
  41. Lohse P, Brewer HB, III, Meng MS, Skarlatos SI, LaRosa JC, Brewer HB, Jr. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. *J Lipid Res*. 1992;33:1583-1590.
  42. Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res*. 2009;50(Suppl):S183-S188.
  43. Jonsson T, Stefansson H, Steinberg P, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*. 2013;368:107-116.
  44. Phung TK, Andersen BB, Høgh P, Kessing LV, Mortensen PB, Waldemar G. Validity of dementia diagnoses in the Danish hospital registers. *Dement Geriatr Cogn Disord*. 2007;24:220-228.
  45. Rall SC, Jr, Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. *J Biol Chem*. 1982;257:4171-4178.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. APOE and dementia – resequencing and genotyping in 105,597 individuals. *Alzheimer's Dement*. 2020;16:1624–1637.  
<https://doi.org/10.1002/alz.12165>