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RESEARCH ARTICLE

The number of cysteine residues per mole in apolipoprotein E affects systematically synchronous neural interactions in women's healthy brains

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Abstract Apolipoprotein E (apoE) is involved in lipid metabolism in the brain, but its effects on brain function are not understood. Three apoE isoforms (E4, E3, and E2) are the result of cysteine–arginine interchanges at two sites: there are zero interchanges in E4, one interchange in E3, and two interchanges in E2. The resulting six apoE genotypes (E4/4, E4/3, E4/2, E3/3, E3/2, E2/2) yield five groups with respect to the number of cysteine residues per mole (CysR/mole), as follows. ApoE4/4 has zero cysteine residues per mole (0-CysR/mole), E4/3 has one (1-CysR/mole), E4/2 and E3/3 each has two (2-CysR/mole), E3/2 has three (3-CysR/mole), and E2/2 has four (4-CysR/mole). The use of the number of CysR/mole to characterize the apoE molecule converts the categorical apoE genotype scale, consisting of 6 distinct genotypes above, to a 5-point continuous scale (0–4 CysR/mole). This allows the use of statistical analyses suitable for continuous variables (e.g. regression) to quantify the relations between various variables and apoE. Using such analyses, here, we show for the first time that apoE affects in a graded and orderly manner neural communication, as assessed by analyzing the relation between the number of CysR/mole and synchronous neural interactions (SNI) measured by magnetoencephalography (MEG) in 130 cognitively healthy women. At the one end of the CysR/mole range, the 4-CysR/mole (E2/2) SNI distribution had the highest mean, lowest variance, lowest range, and lowest coefficient of variation, whereas at the other end, 0-CysR/mole (E4/4) SNI distribution had the lowest mean, highest variance, highest range, and highest coefficient of variation. The special status of the 4-CysR/mole distribution was reinforced by the results of a hierarchical tree analysis where the 4-CysR/mole (E2/2) SNI distribution occupied a separate branch by itself and the remaining CysR/mole SNI distributions were placed at increasing distances from the 4-CysR/mole distribution, according to their number of CysR/mole, with the 0-CysR/mole (E4/4) being farthest away. These findings suggest that the 4-CysR/mole (E2/2) SNI distribution could serve as a reference distribution. When the SNI distributions of individual women were expressed as distances from this reference distribution, there was a substantial overlap among women of various CysR/mole. This refocuses the placement of individual brains along a continuous distance from the 4-CysR/mole SNI distribution, in contrast to the common categorical assignment to a specific apoE genotype. Finally, the orderly variation of SNI with the number of CysR/mole found here is in keeping with recent advances and ideas regarding the molecular mechanisms underlying the differential effects of apoE in the brain which emphasize the healthier stability conferred on the apoE molecule by the increasing number of cysteine–arginine interchanges, with 4-CysR/mole (E2/2) being the best case, as opposed to the instability and increased chance of toxic fragmentation of the apoE molecule with lower number of CysR/mole, with 0-CysR/mole (E4/4) as the worst case

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(Mahley and Huang in Neuron 76:871–885, 2012a). However, our results also document the appreciable variation of SNI properties within the various CysR/mole groups and individuals which points to the existence and important role of other factors involved in shaping brain function at the network level.

Keywords Apolipoprotein E · Magnetoencephalography · Synchronous neural interactions

Introduction

ApoE is a plasma lipoprotein discovered in 1973 (Shore and Shore 1973). It binds low-density lipoprotein (LDL) receptors, thereby facilitating cellular lipoprotein exchange and metabolism (Mahley 1988; Mahley et al. 2006). The human apoE polypeptide consists of 299 amino acids (Rall et al. 1982) and comprises three polymorphisms resulting from single amino acid substitutions. Three isoforms (E4, E3, and E2) are the result of cysteine–arginine interchanges at two sites, namely residues 112 and 158 (Weisgraber et al. 1981); however, other genetic variants have been described (Rall and Mahley 1992). These three isoforms, each differentially affecting protein function, result in six phenotypes: three homozygotes (E4/4, E3/3, E2/2) and three heterozygotes (E4/3, E4/2, E3/2) (Zannis et al. 1982). With respect to the number of cysteine residues per mole, E2/2 contains 4, E3/2 contains 3, E4/2 and E3/3 each contain 2, E4/3 contains 1, and E4/4 contains 0 (Zannis et al. 1982). The number of cysteine residues per mole (CysR/mole) provides a numerical, biochemical scale in lieu of the genotype-based categories.

The 3D structure of the apoE-binding domain (Wilson et al. 1991) helped resolve the apoE isoform-specific binding affinities (Lalazar et al. 1988). Resulting from modulation of receptor-binding activity via conformational changes and plasma lipoprotein concentrations, the role of apoE in cholesterol metabolism has been well characterized (Innerarity et al. 1983; Weisgraber et al. 1983; Mahley 1983; Innerarity et al. 1984a, b). Synthesis and secretion of apoE occurs primarily in the liver and, to a lesser extent, in other organs. In the brain, astrocytes serve as the main source of apoE synthesis and secretion (Boyles et al. 1985; Pitas et al. 1987), although neurons also contribute (Han et al. 1994; Metzger et al. 1996; Xu et al. 1999). Finally, apoE is also involved in neural repair (Ignatius et al. 1986; LeBlanc and Poduslo 1990).

The neural effects of apoE have been studied extensively in neural cell cultures and in apoE-deficient mice. In neural cell cultures, apoE has been shown to be internalized by neurites and growth cones in pheochromocytoma cells (Ignatius et al. 1987) and to increase neurite outgrowth in dorsal root ganglion cells (Handelmann et al. 1992). Differential effects of apoE isoforms have been documented, namely that apoE3 increases neurite outgrowth in the presence of β-very low-density lipoproteins (β-VLDL), whereas apoE4 has an opposite effect (Nathan et al. 1994). Additional studies found similar effects in mice neuroblastoma cells (Nathan et al. 1995; Bellosta et al. 1995) and in a CNS-derived neuronal cell line (Holtzman et al. 1995). ApoE-deficient mice (apoE−/−) exhibited significantly lower neurogenesis in the hippocampus, as compared to wild-type mice (Li et al. 2009). However, neurogenesis was rescued by induced human E3 (but not E4) expression (Li et al. 2009). In addition, age-dependent neurodegeneration and excitotoxin-induced neuronal damage in apoE−/− mice were protected against by E3 expression, whereas E4 was not protective (Buttini et al. 1999); an intermediate effect was observed for E4/3 expression in apoE−/− mice (Buttini et al. 2000). In another study, long-term potentiation measured from hippocampal slices was increased in E3 targeted replacement (TR) mice as compared to apoE−/−, E2-TR, and E4-TR (Trommer et al. 2004).

Several studies have shown increased frequency of apoE4 allele in both sporadic and familial Alzheimer's disease (AD) (Nalbantoglu et al. 1994; Czech et al. 1994; St Clair et al. 1995; Benjamin et al. 1995; Gustafson et al. 1997), a neurodegenerative disease characterized by amyloid plaques and neurofibrillary tangles (Selkoe 1991). In addition, in vivo studies have linked E4 with Aβ deposition (Nicoll et al. 1995). E4 allele dosage effects have been observed in sporadic late-onset AD (Corder et al. 1993; Lucotte et al. 1995). In another study of both AD and older control populations (when compared to no AD and younger control populations, respectively), E4-positive subjects were found to have smaller CA3 and dentate gyrus than E4-negative subjects (Mueller et al. 2008). Hesse et al. (1999) found reduced apoE levels in the hippocampus and frontal cortex of AD subjects, although another study found that the level of apoE in the brain, regardless of genotype, does not differ with the presence of AD (Harr et al. 1996). Overall, there appears to be an association of E4 with AD (Kim et al. 2009); however, E4 homozygotes have been found to be more susceptible to multiple sclerosis (MS) and may also have increased MS disease progression (Høgh et al. 2000) which indicates a more complex role of apoE, particularly the E4 allele, in neurological structure, function, and disease. In addition to neurodegenerative diseases, apoE has been linked to cognition. In general, the presence of E4 is associated with earlier cognitive decline in non-demented individuals (Payton 2009; Norberg et al. 2011), whereas the presence of the E2 allele is associated with increased cognitive performance (Payton 2009). Finally, in subjects with cognitive decline, faster decreases in gray matter volume are found in E4-positive subjects when compared with E4-negative subjects

(Spampinato et al. 2011). Recently, neuroimaging studies have looked into the effect of apoE with the focus mostly on E4 with inconclusive results (Filbey et al. 2006; Trachtenberg et al. 2012a, b; Deeny et al. 2008).

A different issue concerns the assessment of dynamic brain function and the possible effects of apoE on this function. The brain is essentially a communication organ composed of at least 100 billion neurons which are organized in specialized neural ensembles. The function of the brain is processing of information, and this is accomplished by the incessant communication among those neuronal ensembles. First, information is processed by local networks through integrated synaptic cell-to-cell communication, and then transmitted to other ensembles by action potentials. Thus, the brain is a massively interconnected, continuously communicating network. The local, integrated synaptic processing involves continuous, coherent motion of ions (mostly sodium, potassium, and calcium) across the cell membrane, and this gives rise to electromagnetic fields that can be recorded from the cortical surface, given the regular arrangement of the dendrites of the pyramidal cells. MEG records the magnetic signal, which, in contrast to its electrical counterpart, passes undistorted through the soft tissues and arrives at the scalp extremely fast. The MEG signal reflects with the highest fidelity the integrated synaptic activity and, therefore, is the most accurate measure of brain activity. Previously, by sampling the MEG signal every 1 ms from 248 MEG sensors simultaneously, we estimated neural communication by computing 30,628 crosscorrelations from 248 simultaneously recorded MEG signals (Leuthold et al. 2005; Langheim et al. 2006). Two major findings of that study were (a) that communication patterns were very similar among healthy subjects and (b) that the highest strength of communication was at zero-lag, that is, there was a strong synchronicity in neural communications, which we called "Synchronous Neural Interactions" (SNIs). SNIs proved valuable in differentiating brain communication patterns among various diseases (Georgopoulos et al. 2007), including posttraumatic stress disorder (Georgopoulos et al. 2010; Engdahl et al. 2010). In this study, we analyzed the effect of apoE genotype on SNI distributions in cognitively healthy women of various ages in an attempt to determine the relations between apoE genotype and neural communication.

Materials and methods

Subjects

Study subjects were 130 cognitively healthy women (31–97 years old, 64.2 ± 1.38 years, mean ± SEM; see Fig. 1) who participated as paid volunteers. Their cognitive status was assessed using the Montreal Cognitive Assessment (MoCA; http://www.mocatest.org/). All women had MoCA scores >26 (28.05 ± 0.118, mean ± SEM, $N = 130$; range, 26–31). Study participants belonged to the following apoE genotypes: E4/4 ($N = 3$), E4/3 ($N = 26$), E4/2 ($N = 1$), E3/3 ($N = 85$), E3/2 ($N = 14$), E2/2 ($N = 1$). Study protocol was approved by the appropriate institutional review boards, and written informed consent was obtained prior to the study.

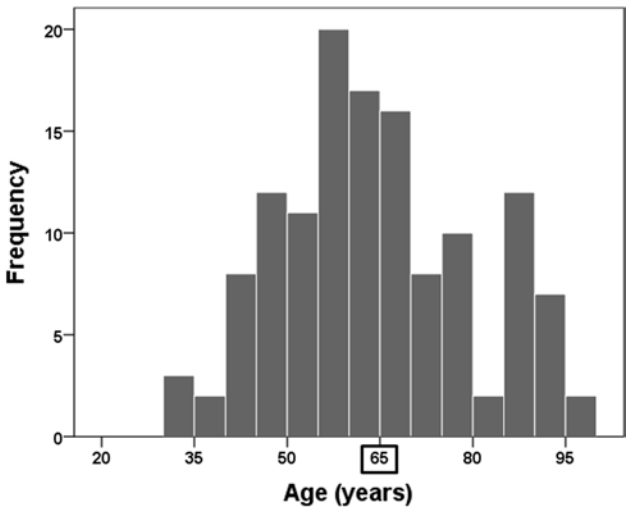

Fig. 1 Age distribution of the women studied ($N = 130$). The *square* around age 65 years indicates the age that divided the distribution in two groups (31–65 and >65 years) for testing apoE effects (see text)

ApoE genotyping

DNA samples were genotyped using PCR amplification followed by restriction enzyme digestion (Reymer et al. 1995). Each amplification reaction contained PCR buffer with 15 mmol/L $MgCl_2$ ng amounts of genomic DNA, 20 pmol apoE forward (5N TAA GCT TGG CAC GGC TGT CCA AGG A 3N) and reverse (5N ATA AAT ATA AAA TAT AAA TAA CAG AAT TCG CCC CGG CCT GGT ACA C 3N) primers, 1.25 mmol/L of each deoxynucleotide triphosphate, 10 % dimethylsulfoxide, and 0.25 μL Amplitaq DNA polymerase. Reaction conditions in a thermocycler included an initial denaturing period of 3 min at 95 °C, 1 min at 60 °C, and 2 min at 72 °C; followed by 32 cycles of 1 min at 95 °C, 1 min at 60 °C, and 2 min at 72 °C; and a final extension of 1 min at 95 °C, 1 min at 60 °C, and 3 min at 72 °C. PCR products were digested with *HhaI* and separated on a 4 % Agarose gel which was stained with ethidium bromide. Known apoE isoform standards were included in the analysis.

MEG recordings

Women fixated a spot of light in the center of a black screen for 60 s, as described previously (Georgopoulos et al. 2007).

MEG data were collected using a 248-channel axial gradiometer system (Magnes 3600WH, 4D-Neuroimaging, San Diego, CA). The cryogenic helmet-shaped dewar of the MEG was located within an electromagnetically shielded room to reduce noise. Data (0.1–400 Hz) were collected at 1,017.25 Hz. The acquired MEG data were time series consisting of 60,000 values per subject and sensor. Individual series were prewhitened using a (50,1,1) AutoRegressive Moving Average (ARIMA) model (Box and Jenkins 1970). Finally, the partial, full-rank zero-lag crosscorrelations PCC^0_{ij} between i and j sensors were computed for all sensor pairs (synchronous neural interactions, SNI); thus, for any given pair of sensors (from a total of 248), the effects of the remaining 246 sensors were partialed out. The PCC^0_{ij} was transformed to z^0_{ij} using Fisher's (1958) z-transformation to normalize its distribution:

$$SNI = z^0_{ij} = \frac{1}{2}\ln\frac{1 + PCC^0_{ij}}{1 - PCC^0_{ij}} \quad (1)$$

Distributions of z^0_{ij} were compared between groups using the Kolmogorov–Smirnov test. In addition, basic statistics of these distributions were computed.

Statistical analyses

The Kolmogorov–Smirnov test (Fig. 2) was used to test differences between SNI distributions. Standard statistical methods were used where needed. A non-metric (ordinal) multidimensional scaling (MDS) analysis was applied on the apoE/SNI proximity data of Table 1 using the PROSCAL procedure of the IBM-SPSS statistical package (version 20).

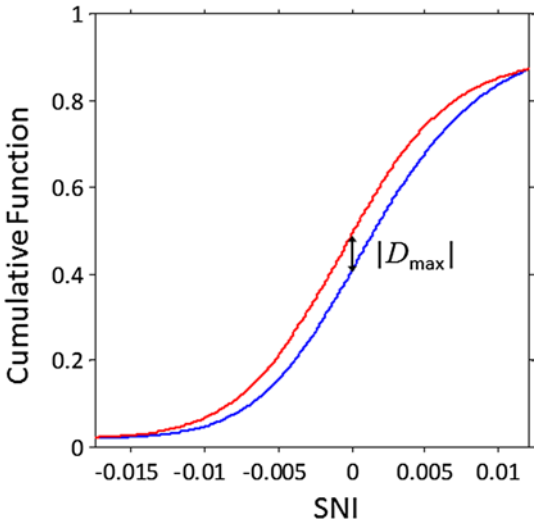

Fig. 2 Illustration of the KS test between the SNI distributions of two subjects. $|D_{max}|$ is the maximum absolute distance between the two cumulative SNI distributions along the vertical axis

Table 1 Comparison of ApoE genotype effect on SNIs

	E4/4	E4/3	E4/2	E3/3	E3/2	E2/2
E4/4	0					
E4/3	2.125	0				
E4/2	2.461	2.556	0			
E3/3	2.674	1.680	2.591	0		
E3/2	3.511	4.680	2.318	4.564	0	
E2/2	8.066	8.400	7.842	8.514	8.851	0

Values are Z-statistic (KS test; Smirnov 1948). All comparisons are significant at $P < 0.001$

Table 2 Effect of ApoE cysteine–arginine interchanges on SNIs

Number of cysteine residues per mole	0	1	2	3	4
0	0				
1	1.968	0			
2	2.738	1.986	0		
3	2.852	2.077	2.501	0	
4	8.066	8.679	8.527	8.463	0

Values are Z-statistic (KS test). All comparisons are significant at $P < 0.001$

A hierarchical tree analysis of the cysteine/SNI proximity data of Table 2 was performed using the Hierarchical Cluster procedure of the IBM-SPSS statistical package (version 20) with the squared Euclidean distance as measure and the average within-groups linkage as the clustering method.

Results

Effect of E4 allele on SNI

There were 100 women without the E4 allele (E4−) and 30 with it (E4+). The brain function measurements (SNI) consisted of pairwise, zero-lag, partial crosscorrelations between resting-state MEG signals (see "Materials and methods" section). The SNI distributions differed highly significantly between the two groups ($P < 0.001$, Kolmogorov–Smirnov (KS) test, $Z = 3.84$). This effect was independent of age and cognitive performance, since neither recombinant E4, the age distributions nor the MoCA scores differed significantly between the two groups (age comparison: $P = 0.998$, $Z = 0.38$, KS test; MoCA comparison: $P = 1.0$, $Z = 0.256$, KS test). Conversely, with respect to age, the SNIs of the two groups (E4+ and E4−) still differed significantly when the analysis was performed separately for a younger and an older age subgroup (younger group, 31–65 years, $N = 73$, $P < 0.001$,

$Z = 3.11$; older group, >65 years, $N = 57$, $P < 0.001$, $Z = 3.19$, KS test).

Effect of apoE genotype on SNI

All apoE genotypes were present in our sample (see "Materials and methods"), and the age did not differ significantly among apoE genotypes (ANOVA, $P = 0.988$, F test). The results of the comparison of the SNI distributions between apoE genotypes are shown in Table 1; all comparisons were statistically significant.

Multivariate analyses

We used the KS Z-statistic as a distance (proximity, dissimilarity) measure to perform two multivariate analyses in order to assess and visualize the effects of the apoE genotypes on the SNI distributions. We first applied multidimensional scaling (MDS), a well-established method (Shepard 1980, 1988; Borg and Groenen 2010; Young et al. 1995; Whang et al. 1999; Tagaris et al. 1998; Tzagarakis et al. 2009), on the apoE proximity matrix of Table 1. The result is shown in Fig. 3, where the following can be seen. First, E2/2 is farthest to the right from all the rest; second, apoE genotypes with presence or absence of E4 are segregated to the lower and upper quadrants on the left hemispace; and third, there seems to be a regularity in the position of the non-E2/2 isoforms along the vertical dimension, starting with E4/4 (lowest) and ending with E3/2 (highest) via E4/3, E4/2, and E3/3, in that sequence. But that sequence corresponds, in

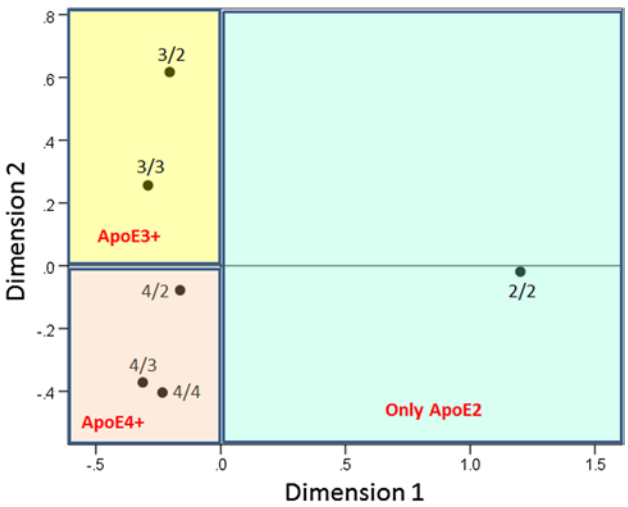

Fig. 3 Two-dimensional plot of E4 isoforms derived from non-metric multidimensional scaling (MDS) of the KS Z-statistic proximity matrix (Table 1). (Normalized raw stress = 0.00009; $R^2 = 0.999$. Very similar plots were obtained using either interval or ratio metric MDS.) (See text for details.)

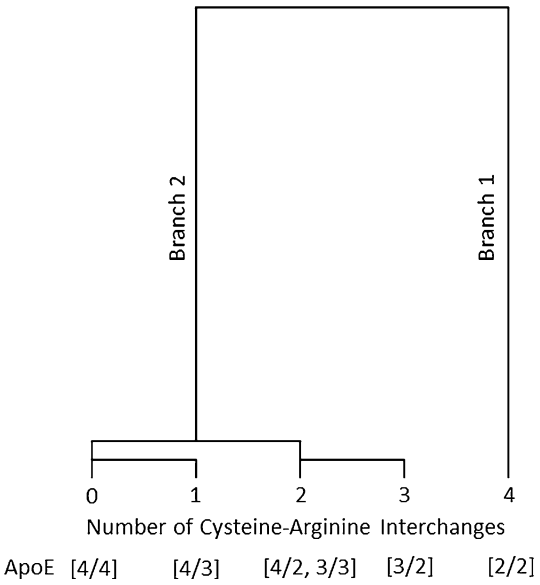

Fig. 4 Dendrogram derived from hierarchical tree clustering of the number of cysteine residues per mole proximity matrix (Table 2). Notice **a** the distinct occupation of Branch 1 by E2/2 (containing 4 cysteine residues per mole), **b** the clustering of all other ApoE isoforms under Branch 2, and **c** the orderly placement in the tree of ApoE genotypes according to the number of cysteine residues per mole, from zero to four

increasing order, to the number of CysR/mole (Zannis et al. 1982; see above) in the various apoE isoforms, namely from zero CysR/mole (in E4/4) to three CysR/mole (in E3/2). This observation led us to compute a new proximity matrix by performing the KS test between SNI distributions for isoforms having the same number of CysR/mole, specifically lumping together the E4/2 and E3/3 SNI distributions, each of which has 2 cysteine residues per mole. The resulting proximity matrix (Table 2) was subjected to a hierarchical tree clustering analysis which yielded the dendrogram shown in Fig. 4. It can be seen that E2/2 with 4-CysR/mole occupies its own branch (#1), whereas all the others populate the second branch. Remarkably, there is an orderly layout in the tree based on the number of CysR/mole, from 0-CysR/mole (left most) to 4-CysR/mole (right most). These results support the idea that the number of CysR/mole is an important quantitative factor underlying the effect of apoE on SNI. A systematic effect of the number of CysR/mole on SNI variance is shown in Fig. 5.

Differences of SNIs of individual women with respect to the E2/2 SNI

The multivariate plots of Figs. 3 and 4 were derived from the analysis of SNI distribution distances (e.g. proximities) between apoE genotypes and associated numbers of CysR/mole. A major finding from this analysis is the unique

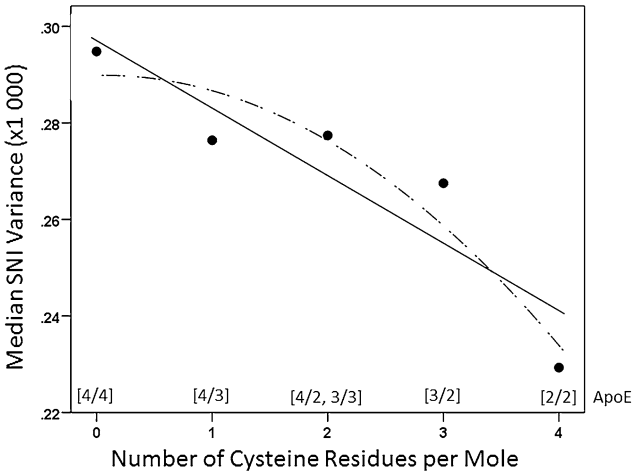

Fig. 5 The average median SNI variance is plotted against the number of cysteine residues. For linear fit (*solid line*): $r^2 = 0.826$, $P = 0.033$; for quadratic fit: $r^2 = 0.903$, $P = 0.097$

position of the E2/2 isoform at the high end of the CysR/mole range, with its 4 cysteine residues. This helped address another issue, namely the placement of individual women within a suitable continuum of variation in the SNI distributions, based on the 4-CysR/mole SNI distribution as a suitable reference. For that purpose, we used the KS Z-statistic as a quantitative measure of distance of a subject's SNI distribution from the reference 4-CysR/mole SNI distribution. The resulting KS Z-values varied widely, ranging from 1.38 to 15.85, a finding that attests to the substantial spread among the SNI distributions of individual, healthy women. These *Z*-values are plotted against their ranks in Fig. 6 for all women, color-coded for the apoE genotype. It can be seen that there is a wide overlap among the various isoforms. For example, even the values of the homozygotic "pure" E3/3 women (light green circles) extend throughout the distance range.

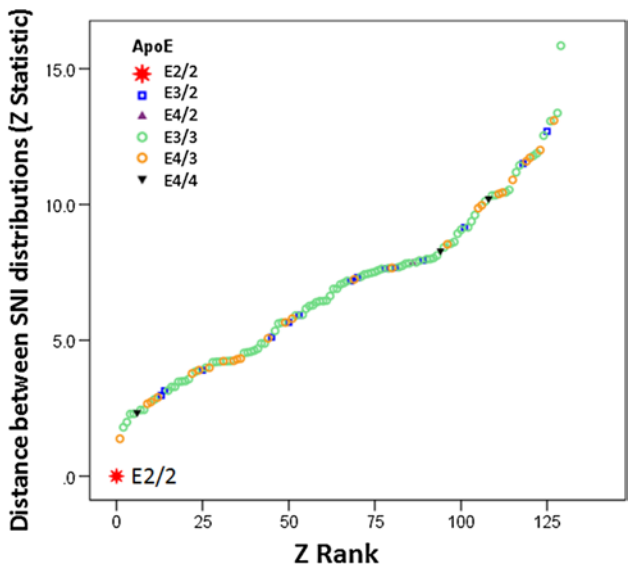

Fig. 6 The Z-statistic derived from a KS test of SNI distributions of all women with the SNI distribution of E2/2 is plotted against its rank, color-coded for apoE genotypes. Notice the broad distribution in the plot of individuals with other apoE genotypes. $N = 130$ women

Characterization of individual SNI distributions

The analysis above was based on a relational measure, namely the distance between the 4-CysR/mole (E2/2) SNI distribution and SNI distributions of other women. It would be important to find out in which aspect(s) the distributions differ. For that purpose, we examined in detail the SNI frequency distribution of every subject; each distribution consisted of >30,000 (up to 30,628, depending on the number of valid sensors) Fisher z-transformed, full-rank partial correlations (see "Materials and methods" section). All distributions were unimodal, positively skewed (i.e. to the right), and leptokyrtotic (i.e. more slender than a normal distribution). We then calculated the average (over women in a CysR/mole group) of the following measures: mean, variance, standard deviation, range, and coefficient of variation (standard deviation/mean). We found that the 4-CysR/mole (E2/2) SNI distribution had the highest mean, lowest variance (and standard deviation), lowest range, and lowest coefficient of variation (Fig. 7). At the other end, the 0-CysR/mole (E4/4) SNI distribution had the lowest mean, highest variance (and standard deviation), highest range, and highest coefficient of variation. This further supported the idea that the 4-CysR/mole (E2/2) SNI distribution could serve as a reference distribution.

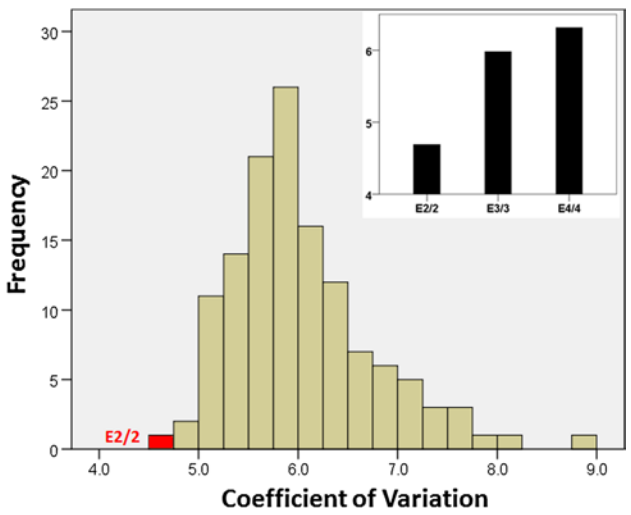

Fig. 7 Frequency distribution of the SNI coefficient of variation. $N = 130$ women. The *inset* shows the average coefficients for the three homozygotic apoE genotypes

Finally, we performed a stepwise multiple linear regression across individual women where the KS Z-distance from the 4-CysR/mole SNI distribution was the dependent variable and several, more inclusive parametric and nonparametric measures of the SNI distribution were the independent variables, namely median, interquartile range, mean, standard deviation, coefficient of variation, range, coefficient of skewness, and coefficient of kurtosis. This analysis yielded a model in which the median ($t_{[126]} = -224$, $P < 10^{-45}$) (Fig. 8) and the coefficient of variation ($t_{[126]} = 6.05$, $P < 10^{-7}$) (Fig. 9) were the only variables with a statistically significant effect. Overall, this two-variable multiple regression model had an excellent fit ($R^2 = 0.977$, adjusted $R^2 = 0.955$) and high statistical significance ($F_{[2,126]} = 1{,}346$, $P < 10^{-84}$).

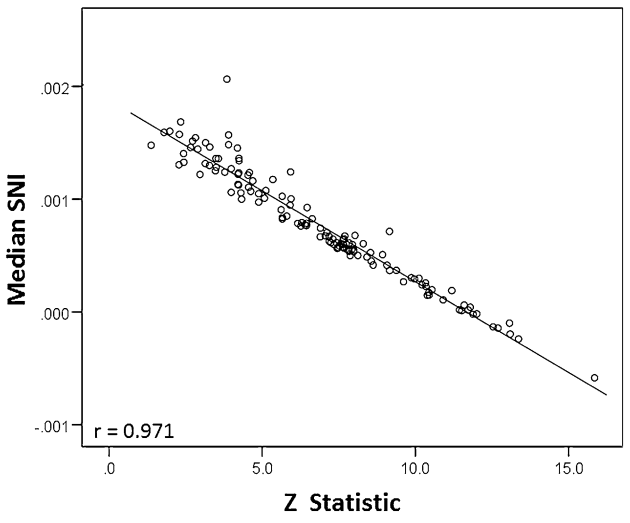

Fig. 8 The SNI median of 129 women is plotted against the KS Z-statistic of the comparison of each SNI distribution with the E2/2 SNI distribution

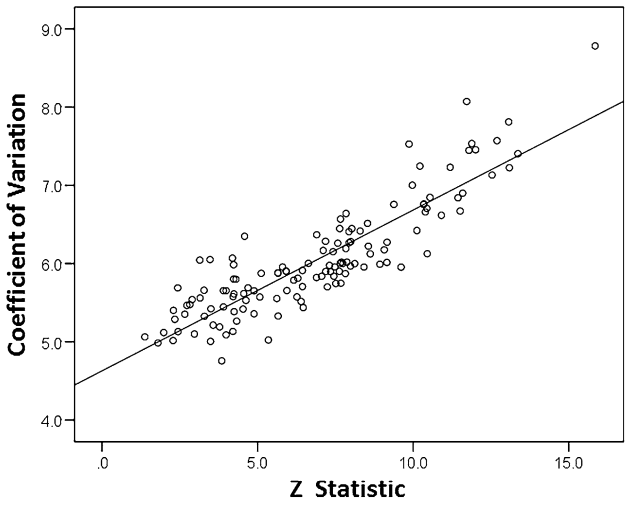

Fig. 9 The SNI coefficient of variation of 129 women is plotted against the KS Z-statistic of the comparison of each SNI distribution with the E2/2 SNI distribution

Discussion

Here, we show for the first time that the apoE genotype affects neural communication in cognitively healthy women, as measured by MEG-SNIs comprising >30,000 partial correlations between 248 MEG sensors per brain. ApoE affected the bell-shaped SNI distribution in a systematic and graded fashion, according to the number of CysR/mole in the apoE molecule. At the one end of the CysR/mole range, the 0-CysR/mole (E4/4) SNI distribution had the lowest mean, highest variance, highest range, and highest coefficient of variation, whereas at the other end, the 4-CysR/mole (E2/2) SNI distribution had the highest mean, lowest variance, lowest range, and lowest coefficient of variation. The special status of the 4-CysR/mole distribution was reinforced by the results of a hierarchical tree analysis where 4-CysR/mole (E2/2) occupied a separate branch by itself and the remaining CysR/mole SNI distributions were placed at increasing distances from the 4-CysR/mole distribution, according to their number of CysR/mole, with the 0-CysR/mole (E4/4) being farthest away. These findings suggested that the 4-CysR/mole (E2/2) SNI distribution could serve as a reference distribution. When the SNI distributions of individual women were expressed as distances from this reference distribution, there was a substantial overlap among women of various CysR/mole. This refocuses the placement of individual women along a CysR/mole continuum, in contrast to the common categorical assignment to a specific apoE genotype. In general, the orderly variation of SNI with the number of CysR/mole is in keeping with recent advances and ideas regarding the molecular mechanisms underlying the differential effects of apoE in the brain which emphasize the healthier stability conferred on the apoE molecule by the increasing number of cysteine–arginine interchanges, with 4-CysR/mole (E2/2) being the best case, as opposed to the instability and increased chance of toxic fragmentation of the apoE molecule with lower number of CysR/mole, with 0-CysR/mole (E4/4) as the worst case (Mahley and Huang 2012a). However, our results also document the appreciable variation of SNI properties within the various CysR/mole groups and individuals which points to the existence and important role of other factors involved in shaping brain function at the network level. We discuss these issues in more detail below.

SNI and apoE genotypes

Given the emphasis in the literature on the presence or absence of the E4 allele, we first tested the null hypothesis

that grouping of women as E4− or E4+ does not have a significant effect on SNI. This hypothesis was rejected, affirming a highly significant effect of the E4 allele on SNI (see below for a discussion of possible cellular mechanisms mediating this effect). Remarkably, this effect was independent of age and cognitive status. These results establish for the first time a direct link between the E4 allele and SNI. We next extended this analysis to all pairwise comparisons between apoE genotypes with the clear result that the E2/2 SNI distribution differed most significantly from any other genotype (bottom row in Table 1). This placed the E2/2 genotype in a unique position among all other genotypes. This finding was surprising for two reasons. First, based on the common emphasis on the E4/4 genotype, we expected the E4/4 genotype to be the most distinctive one. And second, although it is that the E2 allele is thought to be protective against Alzheimer's disease, it is rarely brought up in the literature in any prominent position or discussion. These considerations, and the gradation of the level of statistical significance of the pairwise comparisons, as exemplified by the value of Smirnov's Z-statistic (Table 1), prompted us to attempt to visualize possibly structured relations between the six apoE genotypes. For that purpose, we performed a multidimensional scaling analysis (Fig. 3) using the KS Z-statistic (Table 1) as a proximity measure. This analysis separated clearly the E2/2 SNI distribution from all the rest (colored aquamarine in Fig. 3), and, for the remaining, it separated the genotypes containing the E4 allele (pink) from those that did not (yellow). These findings suggested that the 4-CysR/mole (E2/2) SNI distribution could serve as a reference distribution.

The number of CysR/mole in apoE as a key parameter for SNI

The multidimensional scaling analysis above also indicated an orderly progression along the vertical dimension of the number of CysR/mole. We further explored this specifically by performing a hierarchical tree modeling based on SNI proximities among CysR/mole groups, generated by fusing the E4/2 and E3/3 SNI distributions (each being 2-CysR/mole) and calculating all pairwise KS *Z*-values (i.e. distances) between 0-CysR/mole, 1-CysR/mole, 2-CysR/mole, 3-CysR/mole, and 4-CysR/mole groups (Table 2). There are three major points in the derived dendrogram (Fig. 4). First, the 4-CysR/mole (E2/2) SNI distribution is in a branch all by itself, placing it firmly at one end of the CysR/mole SNI range. Second, the 0-CysR/mole (E4/4) SNI distribution is at the other end of tree, placing it firmly on the opposite end of the range. And third, the remaining CysR/mole SNI distributions are in an orderly fashion between the 4-CysR/mole and 0-CysR/mole SNI distributions, *according to their number of CysR/mole*. This suggests that the number of CysR/mole in apoE is the key parameter for its effect on SNI. This is an important point, for the use of the number of CysR/mole converts the categorical apoE genotype scale, consisting of 6 distinct genotypes above, to a 5-point, ordered, continuous scale (0–4 cysteine residues per mole), a conversion that allows the use of statistical analyses suitable for continuous variables (e.g. regression) to quantify the relations between various factors and apoE. There are two important points to bring attention to in this context. First, the use of the number of CysR/mole as a parameter rests on solid biochemical grounds, since increasing number of CysR/mole confers stability to the apoE molecule and prevent its breaking into toxic fragments (Mahley and Huang 2012a; see "Discussion" section below). And second, the number of CysR/mole is a key parameter in assessing cardiovascular effects of apoE (see "Discussion" section below).

Finally, it is noteworthy that the placement of components in this tree is the most compact possible (Cherniak 1995; see also Lewis et al. 2012), since the difference between successive components (i.e. number of cysteine residues per mole) is the smallest possible, namely, one; that is, successive placements in the tree differ by only one cysteine residue, the minimum possible. What is remarkable is that this cysteine-related layout was derived from purely functional considerations of cortical synchronicity.

The number of CysR/mole as a key parameter for cardiovascular risk

It is interesting that findings similar to those of the present study have been reported regarding the association between apoE genotypes and cardiovascular disease. In one international study, there was a systematic increase in the odds ratio for myocardial infarction (MI) for different apoE genotypes, from the E2/2 (lowest) to the E4/4 (highest) (Anand et al. 2009). Most interestingly, the odds ratios for E4/2 and E3/3 were almost identical, as predicted by the number of CysR/mole hypothesis of the present study (Fig. 2 in Anand et al. 2009). When we reexpressed these odds ratios as a function of the number of CysR/mole, a highly significant effect of the number of CysR/mole on the MI odds ratio was found (Fig. 10), relative to the odds ratio for E3/3 (we averaged the odds ratios for E4/2 and E3/3 in our plot.). Similar linear relations were observed in a large meta-analysis regarding cholesterol levels (Bennet et al. 2007). In a different, prospective study (Ward et al. 2009), the risk for coronary heart disease for the E2 allele was lower than, but not statistically significantly different from, that for the E4 allele.

Overall, it seems that the presence of the E2 allele is protective for cardiovascular disease, a point known since the 1980s (see Davignon et al. 1988). A rare lipid disorder (type III hyperlipidemia; Mahley et al. 1998; Smelt and de Beer 2004) can occur later in adulthood in E2/2 homozygotes

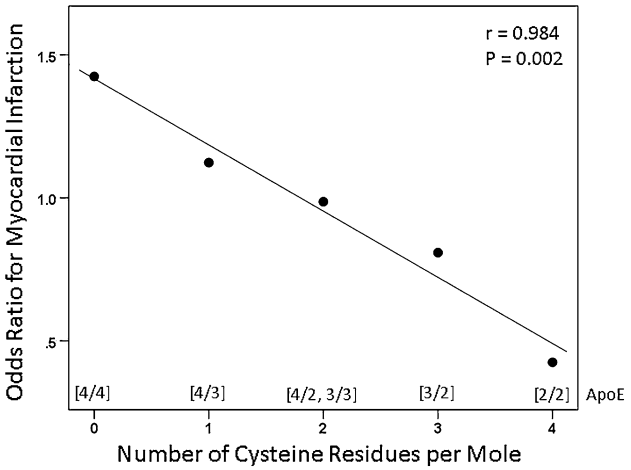

Fig. 10 Odds ratio (risk) for myocardial infarction decreases as the number of CysR/mole increases, using individuals with the E3/3 genotype as the reference group. (Adapted from Fig. 2 in Anand et al. 2009; see text for details.)

(1 in 10,000 overall prevalence; Mahley et al. 1998), but even then, the hyperlipidemia phenotype appears only in the presence of another adverse factors such as obesity, endocrine disease, and other genetic hyperlipidemias. (Davignon et al. 1988; Mahley et al. 1998; Smelt and de Beer 2004). Moreover, proper treatment (e.g. diet, correction of the endocrine disorder, administration of statins) will correct the hyperlipidemia. Thus, like in most cases in medicine, the ultimate effect on health of the presence of the E2 allele will depend on the concomitant presence of, and interplay with, other genetic (e.g. Geisel et al. 2002), environmental and/or medical factors. These considerations hold not only for the cardiovascular system but also for the brain.

The 4-CysR/mole SNI distribution as reference

All the evidence from this work, from the multivariate analyses to the examination of the individual SNI distributions, justifies the employment of the 4-CysR/mole (E2/2) SNI distribution as a reference for the following reasons. First, although this came from one woman, it should be noted that each SNI distribution consists of a large number (>30,000) of partial correlations and not a single value. Second, and more importantly, the 4-CysR/mole (E2/2) SNI distribution was at one end of a continuum which was bracketed by the 0-CysR/mole (E4/4) distribution on the other end, with the remaining distributions in-between in an ordered fashion according to the number of CysR/mole. This arrangement supports the notion that the 4-CysR/mole (E2/2) distribution is representative within a pattern of SNI variation, and not an aberrant one.

The E2/2 genotype is scarce, with a frequency of occurrence of approximately 1 % in the general population. Therefore, our single E2/2 case is in accord with this expectation. Now, it happens that the SNI distribution of this brain had the unique property of possessing the highest mean, lowest variance, and lowest coefficient of variation. Given a brain with such a unique set of properties, one would have expected that, by pure chance, brain would belong to the group with the highest apoE genotype prevalence, namely E3/3 (a probability of $\frac{85}{130} = 0.65$), and not stand out all by itself (a probability of $\frac{1}{130} = 0.008$). Finally, it should be noted that data and results of studies from single subjects have been very useful in brain science, and medicine in general. Examples include the famous case of Paul Broca's patient M. Leborgne suffering from Broca's aphasia (Lorch 2011), patient H.M. (Scoville and Milner 1957) who became the cornerstone of understanding human memory systems, and many other single-case reports that abound in neuropsychology literature. Moreover, the utility of single cases is not limited to brain damage; for example, the most widely used brain atlas (Talairach and Tournoux 1988) is based on the sections of the brain of a single 60-year-old French woman.

The focus on the individual brain

Assuming the assignment of the of 4-CysR/mole SNI distribution as a reference, several considerations follow. First, given its possible function as protective for Alzheimer's disease and dementia, the properties of this distribution acquire special importance, especially its low variability (Figs. 5, 7). For example, it is possible that the neuropathology in Alzheimer's disease induces such a disruption in the functioning of local neural networks that may result in increased SNI variability. Second, it is remarkable that the SNI distributions of other apoE genotypes (and corresponding of CysR/mole) varied substantially in their distance from the 4-CysR/mole (E2/2) distribution (Fig. 6). This indicates that the membership of an individual to an apoE genotype does not determine the "goodness" of their SNI distribution. Instead, a direct comparison of this distribution to the reference one should be the appropriate way to evaluate a specific SNI distribution. This consideration refocuses attention from the group on the individual. The point is that group membership is only a risk (or protection) factor, but the placement of a specific individual's SNI along such a continuum can only be assessed by comparing that distribution to the 4-CysR/mole reference distribution. Finally, it should be emphasized that the SNI distribution is a measure of dynamic brain function at the network level. As such, it obviously depends on many factors related to various aspects of brain function, from membrane channels to neurotransmitters to previous events in life history. Thus, the number of CysR/mole is one of many factors potentially influencing the SNI distribution. In a way, it is because of

these considerations that the orderly variation of SNI properties with the number of CysR/mole is so remarkable.

SNI, apoE genotypes, and neurobiology

The underlying neurobiological mechanisms accounting for the differences in SNI distributions among apoE genotypes (and corresponding CysR/mole) remain to be elucidated. It is likely that there is an effect on calcium channels (Veinbergs et al. 2002; Qiu et al. 2003). For example, the addition of recombinant E4 in a murine hippocampal cell line (HT22) and in primary rat cortical neurons resulted in increased levels of cytosolic calcium associated with cell death in a dose-dependent manner; the application of recombinant E3 had a lesser effect (Veinbergs et al. 2002; unfortunately, E2 was not used in this study). These effects were potentiated by the administration of a calcium ionophore and inhibited by calcium chelators or by blocking calcium channels, but not by inhibitors of intracellular calcium reserves.

In a subsequent study (Qiu et al. 2003), the effects of recombinant human apoE3 and apoE4 on the neuronal calcium response to N-methyl-D-aspartate (NMDA) and cell toxicity were investigated. It was found that apoE4 significantly increased the resting calcium by 70 % and the calcium response to NMDA by 185 %, whereas similar changes were not observed in neurons treated with apoE3. In addition, apoE4, but not apoE3, also significantly increased neurotoxicity. The changes induced by apoE4 in the calcium response to NMDA and associated neurotoxicity were blocked by co-incubation with MK-801, a NMDA antagonist. In addition, both the receptor-associated protein, which inhibits interaction of apoE with members of the LDL receptor family, including the LDL receptor-related protein (LRP), and activated 2-macroglobulin, another LRP ligand, prevented the enhancement of the calcium response to NMDA, resting calcium levels, and neurotoxicity induced by apoE4. Altogether, the results of these studies (Veinbergs et al. 2002; Qiu et al. 2003) documented the differential effects of apoE4 versus apoE3 on calcium homeostasis and NMDA-mediated excitotoxicity. Overall, such effects would be expected to increase the variability in synaptic activity and might underlie the higher variability in the E4/4 SNI distribution that we observed in this study (Figs. 5, 7).

Translational future: toward more apoE2-like

Mahley and Huang (2012a) have recently reviewed thoroughly, succinctly, and lucidly the various neurochemical and molecular biological mechanisms that may account, alone and in combination, for the detrimental effects of the E4 allele on cell function and toxicity, in contrast to the E2 and E3 alleles. These authors draw attention to the fact that "relatively minor changes in apoE sequence yield significant differences in apoE's ability to promote neuronal health versus neuronal damage" (Mahley and Huang 2012a, p. 875). A key factor seems to be "[P]rotein instability and an isoform-specific apoE property called domain interaction are responsible for these neuropathological effects…. [W]hen apoE4 is expressed in neurons, its unique conformation makes it susceptible to proteolysis, resulting in the generation of neurotoxic fragments. These fragments cause pathological mitochondrial dysfunction and cytoskeletal alterations" (Mahley and Huang 2012a, p. 871). The hypothesis is then advanced "that apoE4 (>apoE3 >apoE2) has direct neurotoxic effects and … that blocking domain interactions reverse these detrimental effects" (Mahley and Huang 2012a, p. 871). The inequality signs above are noteworthy, as they encompass a host of results on such gradation of apoE isoform effects on neural structure and function and underscore the quantitative scale of CysR/mole identified in the present work.

In conclusion, in the light of the statements above, our paper can be considered as refocusing the apoE beam from brain disease (0-CysR/mole, apoE4) to brain health (4-CysR/mole, apoE2). In fact, Mahley and Huang (2012a) proposed "that modulation of the abnormal structure of apoE4—by converting it to a more apoE3-like (or apoE2-like) structure—will reverse the apoE-associated detrimental effects in the central nervous system…" (Mahley and Huang 2012a, p. 872). This is a promising strategy (Mahley and Huang 2012b) turning the field toward apoE2 or apoE2-like, a possible new standard of brain health.

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References

Anand SS et al (2009) Genetic variants associated with myocardial infarction risk factors in over 8000 individuals from five ethnic groups: the INTERHEART Genetics Study. Circ Cardiovasc Genet 2:16–25. doi:10.1161/CIRCGENETICS.108.813709

Bellosta S et al (1995) Stable expression and secretion of apolipoproteins E3 and E4 in mouse neuroblastoma cells produces differential effects on neurite outgrowth. J Biol Chem 270:27063–27071

Benjamin R et al (1995) Effects of apolipoprotein E genotype on cortical neuropathology in senile dementia of the Lewy body and Alzheimer's disease. Neurodegeneration 4:443–448

Bennet AM et al (2007) Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 298:1300–1311. doi:10.1001/jama.298.11.1300

Borg I, Groenen PJF (2010) Modern multidimensional scaling: theory and applications, 2nd edn. Springer, New York
Box GEP, Jenkins GW (1970) Time series analysis: forecasting and control. Holden Day, San Francisco, CA
Boyles JK, Pitas RE, Wilson E, Mahley RW, Taylor JM (1985) Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. J Clin Invest 76:1501–15013
Buttini M et al (1999) Expression of human apolipoprotein E3 or E4 in the brains of Apoe−/− mice: isoform-specific effects on neurodegeneration. J Neurosci 19:4867–4880
Buttini M et al (2000) Dominant negative effects of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease. Neuroscience 97:207–210
Cherniak C (1995) Neural component placement. Trends Neurosci 18:522–527
Corder EH et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923
Czech C et al (1994) Apolipoprotein E-4 gene dose in clinically diagnosed Alzheimer's disease: prevalence, plasma cholesterol levels and cerebrovascular change. Eur Arch Psychiatry Clin Neurosci 243:291–292
Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1–21
Deeny SP et al (2008) Exercise, APOE, and working memory: MEG and behavioral evidence for benefit of exercise in epsilon4 carriers. Biol Psychol 78:179–187
Engdahl B et al (2010) Post-traumatic stress disorder: a right temporal lobe syndrome? J Neural Eng 7:066005
Filbey FM, Slack KJ, Sunderland TP, Cohen RM (2006) Functional magnetic resonance imaging and magnetoencephalography differences associated with APOEepsilon4 in young healthy adults. NeuroReport 17:1585–1590
Fisher RA (1958) Statistical methods for research workers, 13th edn. Oliver and Boyd, Edinburgh
Geisel J, Bunte T, Bodis M, Oette K, Herrmann W (2002) Apolipoprotein E2/E2 genotype in combination with mutations in the LDL receptor gene causes type III hyperlipoproteinemia. Clin Chem Lab Med 40:475–479. doi:10.1515/CCLM.2002.082
Georgopoulos AP et al (2007) Synchronous neural interactions assessed by magnetoencephalography: a functional biomarker for brain disorders. J Neural Eng 4:349–355
Georgopoulos AP et al (2010) The synchronous neural interactions test as a functional neuromarker for post-traumatic stress disorder (PTSD): a robust classification method based on the bootstrap. J Neural Eng 7:016011
Gustafson L, Abrahamson M, Grubb A, Nilsson K, Fex G (1997) Apolipoprotein-E genotyping in Alzheimer's disease and frontotemporal dementia. Dement Geriatr Cogn Disord 8:240–243
Han SH et al (1994) Apolipoprotein E is localized to the cytoplasm of human cortical neurons: a light and electron microscopic study. J Neuropathol Exp Neurol 53:535–544
Handelmann GE, Boyles JK, Weisgraber KH, Mahley RW, Pitas RE (1992) Effects of apolipoprotein E, beta-very low density lipoproteins, and cholesterol on the extension of neurites by rabbit dorsal root ganglion neurons in vitro. J Lipid Res 33:1677–1688
Harr SD, Uint L, Hollister R, Hyman BT, Mendez AJ (1996) Brain expression of apolipoproteins E, J, and A-I in Alzheimer's disease. J Neurochem 66:2429–2435
Hesse C, Bogdanovic N, Davidsson P, Blennow K (1999) A quantitative and immunohistochemical study on apolipoprotein E in brain tissue in Alzheimer's disease. Dement Geriatr Cogn Disord 10:452–459
Høgh P et al (2000) Apoliprotein E and multiple sclerosis: impact of the epsilon-4 allele on susceptibility, clinical type and progression rate. Mult Scler 6:226–230
Holtzman DM et al (1995) Low density lipoprotein receptor-related protein mediates apolipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. Proc Natl Acad Sci USA 92:9480–9484
Ignatius MJ et al (1986) Expression of apolipoprotein E during nerve degeneration and regeneration. Proc Natl Acad Sci USA 83:1125–1129
Ignatius MJ, Shooter EM, Pitas RE, Mahley RW (1987) Lipoprotein uptake by neuronal growth cones in vitro. Science 236:959–962
Innerarity TL, Friedlander EJ, Rall SC Jr, Weisgraber KH, Mahley RW (1983) The receptor-binding domain of human apolipoprotein E. Binding of apolipoprotein E fragments. J Biol Chem 258:12341–12347
Innerarity TL, Weisgraber KH, Arnold KS, Rall SC Jr, Mahley RW (1984a) Normalization of receptor binding of apolipoprotein E2. Evidence for modulation of the binding site conformation. J Biol Chem 259:7261–7267
Innerarity TL et al (1984b) Receptor binding activity of high-density lipoproteins containing apoprotein E from abetalipoproteinemic and normal neonate plasma. Metabolism 133:186–195
Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer's disease. Neuron 63:287–303
Lalazar A et al (1988) Site-specific mutagenesis of human apolipoprotein E. Receptor binding activity of variants with single amino acid substitutions. J Biol Chem 263:3542–3545
Langheim FJP, Leuthold AC, Georgopoulos AP (2006) Synchronous dynamic brain networks revealed by magnetoencephalography (MEG). Proc Natl Acad Sci USA 103:455–459
LeBlanc AC, Poduslo JF (1990) Regulation of apolipoprotein E gene expression after injury of the rat sciatic nerve. J Neurosci Res 25:162–171
Leuthold AC, Langheim FJP, Lewis SM, Georgopoulos AP (2005) Time series analysis of magnetoencephalographic (MEG) data during copying. Exp Brain Res 164:211–222
Lewis SM, Christova P, Jerde TA, Georgopoulos AP (2012) An organizational chart of the cerebral cortex derived from prewhitened resting-state fMRI data: Cherniak's adjacency rule, size law and metamodule grouping upheld. Front Neuroanat. doi:10.3389/fnana.2012.00036
Li G et al (2009) GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. Cell Stem Cell 5:634–645
Lorch M (2011) Re-examining Paul Broca's initial presentation of M. Leborgne: understanding the impetus for brain and language research. Cortex 47:1228–1235. doi:10.1016/j.cortex.2011.06.022
Lucotte G, Aouizérate A, Gérard N, Turpin JC, Landais P (1995) Allele doses of apolipoprotein E type epsilon 4 in sporadic late-onset Alzheimer's disease. Am J Med Genet 60:566–569
Mahley RW (1983) Apolipoprotein E and cholesterol metabolism. Klin Wochenschr 61:225–232
Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630
Mahley RW, Huang Y (2012a) Apolipoprotein e sets the stage: response to injury triggers neuropathology. Neuron 76:871–885. doi:10.1016/j.neuron.2012.11.020
Mahley RW, Huang Y (2012b) Small-molecule structure correctors target abnormal protein structure and function: structure corrector rescue of apolipoprotein E4-associated neuropathology. J Med Chem 55:8997–9008. doi:10.1021/jm3008618
Mahley RW, Weisgraber KH, Farese RV Jr (1998) Disorders of lipid metabolism. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (eds) Williams textbook of endocrinology, 9th edn. Saunders, Philadelphia, pp 1099–1153
Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci USA 103:5644–5651

Metzger RE et al (1996) Neurons of the human frontal cortex display apolipoprotein E immunoreactivity: implications for Alzheimer's disease. J Neuropathol Exp Neurol 55:372–380

Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW (2008) Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. Neuroimage 42:42–48

Nalbantoglu J, Gilfix BM, Bertrand P, Robitaille Y, Gauthier S, Rosenblatt DS, Poirier J (1994) Predictive value of apolipoprotein E genotyping in Alzheimer's disease: results of an autopsy series and an analysis of several combined studies. Ann Neurol 36:889–895

Nathan BP et al (1994) Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. Science 264:850–852

Nathan BP, Chang KC, Bellosta S, Brisch E, Ge N, Mahley RW, Pitas RE (1995) The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. J Biol Chem 270:19791–19799

Nicoll JA, Roberts GW, Graham DI (1995) Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. Nat Med 1:135–137

Norberg J et al (2011) Regional differences in effects of APOE ε4 on cognitive impairment in non-demented subjects. Dement Geriatr Cogn Disord 32:135–142

Payton A (2009) The APOE gene and cognitive function in non-demented and Alzheimer's disease patients. Rev Clin Gerontol. doi:10.1017/S0959259809990268

Pitas RE, Boyles JK, Lee SH, Foss D, Mahley RW (1987) Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. Biochim Biophys Acta 917:148–161

Qiu Z, Crutcher KA, Hyman BT, Rebeck GW (2003) ApoE isoforms affect neuronal N-methyl-D-aspartate calcium responses and toxicity via receptor-mediated processes. Neuroscience 122:291–303

Rall SC Jr, Mahley RW (1992) The role of apolipoprotein E genetic variants in lipoprotein disorders. J Intern Med 231:653–659

Rall SC Jr, Weisgraber KH, Mahley RW (1982) Human apolipoprotein E. The complete amino acid sequence. J Biol Chem 257:4171–4178

Reymer WA, Groenemeyer BE, Van de Burg R (1995) Apolipoprotein E genotyping on agarose gels. Clin Chem 41:1046–1047

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11–21

Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. Neuron 6:487–498

Shepard RN (1980) Multidimensional scaling, tree-fitting, and clustering. Science 210:390–398

Shepard RN (1988) George Miller's data and the development of methods for representing cognitive structures. In: Hirst W (ed) The making of cognitive science. Cambridge University Press, Cambridge MA, pp 45–70

Shore VG, Shore B (1973) Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. Biochemistry 12:502–507

Smelt AH, de Beer F (2004) Apolipoprotein E and familial dysbetalipoproteinemia: clinical, biochemical, and genetic aspects. Semin Vasc Med 4:249–257. doi:10.1055/s-2004-861492

Smirnov N (1948) Table for estimating the goodness of fit of empirical distributions. Ann Math Stat 19:279–281

Spampinato MV, Rumboldt Z, Hosker RJ, Mintzer JE (2011) Alzheimer's disease neuroimaging initiative apolipoprotein E and gray matter volume loss in patients with mild cognitive impairment and Alzheimer disease. Radiology 258:843–852

St Clair D et al (1995) Apolipoprotein E epsilon 4 allele is a risk factor for familial and sporadic presenile Alzheimer's disease in both homozygote and heterozygote carriers. J Med Genet 32:642–644

Tagaris GA et al (1998) Functional magnetic resonance imaging of mental rotation and memory scanning: a multidimensional scaling analysis of brain activation patterns. Brain Res Rev 26:106–112

Talairach J, Tournoux P (1988) Co-planar stereotaxic atlas of the human brain. Thieme, New York, NY

Trachtenberg AJ et al (2012a) The effects of APOE on brain activity do not simply reflect the risk of Alzheimer's disease. Neurobiol Aging 33:618.e1–618.e13

Trachtenberg AJ et al (2012b) The effects of APOE on the functional architecture of the resting brain. Neuroimage 59:565–572

Trommer BL et al (2004) ApoE isoform affects LTP in human targeted replacement mice. NeuroReport 15:2655–2658

Tzagarakis C, Jerde TA, Lewis SM, Uğurbil K, Georgopoulos AP (2009) Cerebral cortical mechanisms of copying geometrical shapes: a multidimensional scaling analysis of fMRI patterns of activation. Exp Brain Res 194:369–380

Veinbergs I, Everson A, Sagara Y, Masliah E (2002) Neurotoxic effects of apolipoprotein E4 are mediated via dysregulation of calcium homeostasis. J Neurosci Res 67:379–387

Ward H, Mitrou PN, Bowman R, Luben R, Wareham NJ, Khaw KT, Bingham S (2009) APOE genotype, lipids, and coronary heart disease risk: a prospective population study. Arch Intern Med 169:1424–1429. doi:10.1001/archinternmed.2009.234

Weisgraber KH, Rall SC Jr, Mahley RW (1981) Human E apoprotein heterogeneity Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 256:9077–9083

Weisgraber KH et al (1983) The receptor-binding domain of human apolipoprotein E. Monoclonal antibody inhibition of binding. J Biol Chem 258:12348–12354

Whang KC, Crowe DA, Georgopoulos AP (1999) Multidimensional scaling analysis of two construction-related tasks. Exp Brain Res 125:231–238

Wilson C, Wardell MR, Weisgraber KH, Mahley RW, Agard DA (1991) Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. Science 252:1817–1822

Xu PT et al (1999) Specific regional transcription of apolipoprotein E in human brain neurons. Am J Pathol 154:601–611

Young MP et al (1995) Non-metric multidimensional scaling in the analysis of neuroanatomical connection data and the organization of the primate cortical visual system. Philos Trans R Soc Lond B Biol Sci 348:281–308

Zannis VI et al (1982) Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. J Lipid Res 23:911–914