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APOE Genotype Modulates Proton Magnetic Resonance Spectroscopy Metabolites in the Aging Brain

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Abstract

Background—Proton magnetic resonance spectroscopy (^{1}H-MRS) studies on healthy aging have reported inconsistent findings and have not systematically taken into account the possible modulatory effect of *APOE* genotype. We aimed to quantify brain metabolite changes in healthy subjects in relation to age and the presence of the *APOE* E4 genetic risk factor for Alzheimer's disease. Additionally, we examined these measures in relation to cognition.

Methods—We studied a cohort of 112 normal adults between 50 and 86 years old who were genotyped for *APOE* genetic polymorphism. Measurements of ^{1}H-MRS metabolites were obtained in the posterior cingulate and precuneus region. Measures of general cognitive functioning, memory, executive function, semantic fluency, and speed of processing were also obtained.

Results—General linear model analysis demonstrated that older *APOE* E4 carriers had significantly higher choline/creatine and myoinositol/creatine ratios than *APOE* E3 homozygotes. Structural equation modeling resulted in a model with an excellent goodness of fit and in which the *APOE* × age interaction and *APOE* status each had a significant effect on ^{1}H-MRS metabolites (choline/creatine and myo-inositol/creatine). Furthermore, the *APOE* × age variable modulation of cognition was mediated by ^{1}H-MRS metabolites.

Conclusions—In a healthy aging normal population, choline/creatine and myo-inositol/creatine ratios were significantly increased in *APOE* E4 carriers, suggesting the presence of neuroinflammatory processes and greater membrane turnover in older carriers. Structural equation

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modeling analysis confirmed these possible neurodegenerative markers and also indicated the mediator role of these metabolites on cognitive performance among older *APOE* E4 carriers.

Keywords

Aging; APOE genotype; cognitive function; neurodegeneration; proton magnetic resonance spectroscopy; structural equation modeling

Proton magnetic resonance spectroscopy (^{1}H-MRS) has become a useful technique to measure the concentration of brain metabolites in vivo. Numerous studies have examined ^{1}H-MRS metabolite ratios in patients with Alzheimer's disease (AD). There is strong evidence that abnormalities in myo-inositol (mI)/creatine (Cr) and choline (Cho)/Cr ratios in the posterior cingulate/precuneus region are present in AD and mild cognitive impairment (MCI) (mean effect size [ES] of .91 in four studies and mean ES of .71 in three studies, respectively) (1–4). *N*-acetylaspartate (NAA)/Cr ratios have been found to be reduced in subjects with AD and MCI (mean ES of −.60 in four studies). (For more details, see Table S1 in Supplement 1.) The precuneus and posterior cingulate region is affected relatively early in AD, as reflected by reduced cortical thickness (5), amyloid deposition (6), and functional imaging and metabolic alterations (7).

To the best of our knowledge, there has been only a single ^{1}H-MRS study of the impact of *APOE* genotype in otherwise healthy individuals at risk for AD by virtue of carrying the *APOE* E4 allele, the strongest genetic risk factor for late-onset AD (8). In a sample that included only a small number of E4 carriers (n = 8), *APOE* genotype did not have significant effects on ^{1}H-MRS metabolites or on general cognitive function and memory performance (3).

Nevertheless, given *APOE* effects on multiple AD-related biomarkers, including fluorodeoxyglucose-positron emission tomography (9), β-amyloid measures (10), and medial temporal atrophy (11), we posited that *APOE* effects on ^{1}H-MRS metabolites may be observable in a larger sample of healthy individuals using advanced statistical approaches. Thus, we tested the prediction that *APOE* E4 status would interact with age such that differentially higher Cho/Cr and mI/Cr ratios and lower NAA/Cr ratios would be found in older E4 subjects. Specifically, we wanted to examine *APOE* genotype × age interactions because of the possibility that genotypic differences might become increasingly larger with age due to diminished cognitive reserve, redundancy in neural systems, and/or compensatory potential (12).

We also sought to determine relationships among *APOE* genotype, age, ^{1}H-MRS metabolites, and cognition by means of structural equation modeling, a pathway-based statistical technique. We predicted that *APOE* genotype and/or its interaction with age would impact cognition when mediated by ^{1}H-MRS measures. The construction of the model specified simultaneous causal paths among *APOE* genotype, age, and their interaction through ^{1}H-MRS metabolites to cognitive measures. In this way, we were able to determine that *APOE* genotype directly or in interaction with age significantly modulated Cho/Cr and mI/Cr ratios, and furthermore, from our structural equation model, that mI/Cr ratios appeared to mediate the relationship between genotype and cognition.

Methods and Materials

Participants

Participants (n = 112) were cognitively healthy subjects recruited at the Litwin-Zucker Research Center for the Study of Alzheimer's Disease and Memory Related Disorders. All potential participants underwent examination by a neurologist or a geriatric psychiatrist to determine study eligibility. All subjects were between the ages of 50 and 86 years, had Mini-Mental State Examination score ≥24, and did not meet Petersen's criteria for MCI (see Supplement 1 for a detailed description of exclusionary criteria).

All procedures were approved by the North Shore-Long Island Jewish Health System Institutional Review Board. All the subjects included in the study signed written informed consent for participation.

Genotyping

Genotyping was conducted by Polymorphic DNA at two loci in *APOE* exon 4 by amplicon sequencing methods to produce small polymerase chain reaction products that serve as templates for bidirectional sequencing. There were 89 *APOE* E3 homozygotes and 23 E4 carriers (4 of whom were *APOE* E4 homozygotes). *APOE* E2 carriers were excluded because of the low number of E2 carriers in our dataset and the putative neuroprotective effects of the E2 allele, which could confound these analyses.

Proton Magnetic Resonance Spectroscopy

A mid-sagittal oblique 10.8 cm^3 (2 × 2 × 2.7 cm) voxel was prescribed in the region of the precuneus and posterior cingulate cortex (Figure S1 in Supplement 1). The ^{1}H-MRS voxel was placed midway between the parieto-occipital sulcus and the marginal branch of the cingulate sulcus, with the long axis parallel to these sulci, covering the precuneus and posterior cingulate region. Double spin-echo point resolved spectroscopy spectra were acquired on a GE Twinspeed 3T magnetic resonance imaging scanner with HDx technology, using an 8-channel phased array head coil (GE Healthcare, Milwaukee, Wisconsin), with 128 excitations acquired at repetition time = 1600 msec and echo time = 30 msec (see Supplement 1 for a detailed description of the procedure).

We elected to measure metabolites in the precuneus/posterior cingulate region, because it is known to demonstrate early changes in the default mode network (13), increases in Pittsburgh Compound B binding (14), an abnormal mitochondrial transcriptional profile (15), positron emission tomography glucose hypometabolism in younger E4 carriers (9), and AD type histopathology (16) at early stages of the disease. These features are considered important biomarkers in the transition from normal aging to MCI and MCI to AD.

Proton magnetic resonance spectroscopy metabolite ratios were determined for NAA/Cr, Cho/Cr, and mI/Cr. Ratios were quantified automatically with vendor-supplied software. In ^{1}H-MRS, the measured Cr peak includes both the metabolites Cr and phosphocreatine and is thought to be a reliable marker of brain energy metabolism, often used as a relatively stable reference level. The measured NAA peak includes NAA and *N*-

acetylaspartylglutamate. *N*-acetylaspartate is an amino acid thought to reflect general neuronal integrity/viability (17) and may also reflect brain mitochondrial function (18). The mI metabolite is located in glial cells and is a physiologically important osmolyte in the brain (19). It is generally considered to be a marker of microglial and astrocyte activation, suggesting that increased mI could be an indicator of neuroinflammation. Among the pathways involved in neurodegeneration in AD, neuroinflammation has been consistently identified (20). The measured Cho is a sum of phosphorylcholine, glycerophosphorylcholine, and a small percentage of free choline. Hence, Cho is considered to be a cell membrane marker. There is also evidence (21) that Cho may generally reflect not only membrane turnover generally, but in the context of Alzheimer's disease, Cho may also specifically reflect membrane turnover in cholinergic (acetylcholine) neurons. Acetylcholine neurons may not only have greater amounts of Cho than noncholinergic neurons, but may also be selectively vulnerable to early AD pathology.

Cognitive Assessment

The Mini-Mental State Examination (22) was used as a screening inclusion criterion and as a measure of general cognitive/ intellectual performance for further analyses. Memory was assessed through a verbal list learning test (Rey Auditory Verbal Learning Test) (23). Attention/executive function was assessed through the accuracy performance on a visual-spatial version of the 1-back task (24) and Trail Making Test part B (25). Semantic fluency was measured through the animal naming version test. Finally, speed of processing was measured by the Digit-Symbol Coding Test. These measures were combined into a single global cognitive score. Factor analyses techniques have demonstrated the feasibility and reliability of the use of a g or composite factor as representative of cognitive architecture (26,27).

Statistical Analyses

General linear models (GLM) were fitted to estimate the effect of *APOE* status (E3/E3 homozygotes vs. E4 carriers), age (as a continuous variable), and the interaction of *APOE* × age on the ^{1}H-MRS metabolite ratios. Gender was also considered in the models.

Next, we sought to fit a structural equation model for the causal relationships between *APOE* polymorphism, age, and the interaction between them on cognitive performance, with both direct effects on cognition, and indirect effects through brain metabolites measured by ^{1}H-MRS. Structural equation modeling is a statistical method that can estimate the causal effect of paths between one or more exogenous variables (independent variables) and one or more endogenous variables (dependent variables), directly, or mediated by other variables (28). In our modeling, *APOE* genotype, age, and the interaction between them were considered as exogenous variables. A latent general cognitive ability factor was created from the loadings of the specific individual neuropsychological tests; this factor was considered as an endogenous variable in the model. Additionally, a latent ^{1}H-MRS factor was created from the loadings of individual brain metabolite ratios; this factor was considered as mediator between the effect of *APOE*, age, and *APOE* × age interaction on the cognitive factor. To validate the proposed model, we first fitted a model without preliminary assumptions about the relative causal effects of paths among the endogenous, exogenous,

and mediating variables. To obtain a more parsimonious model, progressive steps were taken to remove nonsignificant paths from the initial model that additionally improved the overall goodness of fit. For that purpose, the Wald test was used to identify paths that might be dropped, improving the model chi-square overall fit. The chi-square/degrees of freedom ratio, the Bentler's comparative fit index (CFI), and the root mean square error of approximation (RMSEA) were used to assess overall goodness of fit of the covariance structure of the models. Models were fitted using a maximum likelihood parameter estimation method.

All the statistical analyses were performed with SAS software version 9.3 (SAS Institute Inc., Cary, North Carolina) using GLM and CALIS procedures.

Results

Demographic and Cognitive Characteristics

Demographic characteristics and cognitive composite scores stratified by *APOE* status (carriers of the *APOE* E4 allele vs. E3 homozygotes) and age as based on a median split of the sample (<65 years vs. ≥65 years) are provided in Table 1 (age was split for presentation purposes in this table). Age range was 50 to 64 years (SD = 4.66) for the subjects younger than 65 years and 65 to 86 years (SD = 5.90) for the subjects older than 65 years. Gender distribution showed an imbalance in the *APOE* E4 subgroup. There was no influence of *APOE* genotype or age on years of education. Regarding cognition, an effect of age was detected; as expected, older subjects performed worse than younger subjects, and older *APOE* E4 carriers showed the worst neurocognitive performance. No influence of *APOE* or the interaction between *APOE* and age on cognition was found. Gender did not show an effect on cognition.

^{1}H-MRS Metabolite Ratios

Proton magnetic resonance spectroscopy metabolite ratios to Cr in relation to age and stratified by *APOE* genotype are plotted in Figure 1. As is apparent, older E4 carriers had higher Cho/Cr and mI/Cr ratios. Individual GLM analysis examining the effect of *APOE* polymorphism, age (as a continuous variable), and *APOE* × age interaction on the ^{1}H-MRS metabolite ratios are detailed in the Figure 1 legend. The ES for the comparison between older carriers of *APOE* E4 and younger *APOE* E3 homozygotes was medium (.66) for mI and medium (approaching large) .76 for Cho (age was dichotomized after median split for calculation of ES) (Figure 2). Given the imbalance in gender, we also conducted the above analyses adjusting by gender. Gender did not show an interaction with *APOE* or age and hence was not considered further.

Structural Equation Modeling

To estimate the model, two latent factors were created: a ^{1}H-MRS metabolites factor and a cognitive factor. Choline/Cr and mI/Cr were included in the ^{1}H-MRS factor, as they showed an effect on the previous GLM analysis (effect of *APOE* and *APOE* × age on Cho/Cr; effect of age and *APOE* × age on mI/Cr). The cognitive latent factor was comprised of the individual cognitive test scores.

An initial general model, without any prior assumptions about the relations between exogenous variables (*APOE*, age, and their interaction), including direct effects on both the latent ^{1}H-MRS factor and the latent general cognition factor, as well as mediator effects on cognition through ^{1}H-MRS metabolites, fit the data well, as indicated by goodness of fit statistics (chi-square = 43.28/*df* = 35/*p* = .16; CFI = .99; RMSEA = .05). Specifically, this model included direct effects of *APOE*, age, and *APOE* × age on cognition and indirect effects of the same variables through ^{1}H-MRS metabolites. However, inspection of the individual paths of this general model showed that multiple and critical path weights were not significant.

Therefore, we next attempted to establish that by progressively deleting statistically nonsignificant paths, we would improve global goodness of fit and increase path weights and model parsimony. In this iterative process, with respect to cognition, we found no significant direct effect of *APOE* × age interaction. With respect to the ^{1}H-MRS factor, we found no significant effect of age independent from the effect of *APOE*..

In the resultant more parsimonious final model (chi-square = 44.25/*df* = 38/*p* = .22; CFI = .99; RMSEA = .04), the interaction between *APOE* and age had a significant effect on the ^{1}H-MRS factor as shown in Figure 3. Additionally, the path from the ^{1}H-MRS factor (including Cho/Cr and mI/Cr) to the cognitive factor demonstrated a significant effect ($p < .01$). This indicated that the ^{1}H-MRS metabolites were in a mediating relationship between the *APOE* × age interaction variable and the cognition factor. The direction of the paths were as expected, older *APOE* E4 carriers had higher ^{1}H-MRS metabolite ratios (Cho/Cr and mI/Cr), and higher ^{1}H-MRS ratios of these metabolites correlated with lower cognitive performance. As expected, age also had a highly significant and negative effect on cognition. *APOE* genotype also showed a direct effect on ^{1}H-MRS and an indirect effect on cognition. The final model with the standardized path coefficients is shown in Figure 3. The successive steps taken to obtain the final model are summarized in Table S3 in Supplement 1.

Discussion

This is the first study to find significant *APOE* genotype effects on ^{1}H-MRS metabolites in a healthy aging population. Specifically, GLM analyses indicated that Cho/Cr and mI/Cr ratios were influenced by an *APOE* × age interaction (such that older *APOE* E4 carriers had higher ratios than other subgroups). In addition, Cho/ Cr ratio was independently modulated by a main effect of *APOE* (E4 carriers had higher ratios than E3/E3 homozygotes), and mI/Cr ratio was independently modulated by a main effect of age (older subjects had higher ratios than younger subjects).

Structural equation modeling was used to integrate causal relationships between *APOE*, age, and the interaction between *APOE* and age on cognition through ^{1}H-MRS metabolites. In the final optimized structural equation model cognition was modulated by *APOE* indirectly through an intermediary neurochemical measure (including both Cho/Cr and mI/Cr). Interestingly, no direct paths from *APOE* to cognition were present, in keeping with notions of intermediate phenotype (29) and the logic of our model. Effects of the interaction

between *APOE* and age, as well as the effect of *APOE* alone on ^{1}H-MRS were also significant. Brain metabolite ratios Cho/Cr and mI/Cr were thus sensitive to a variety of inputs in our sample (older subjects and carriers of the *APOE* E4 allele). As expected, our results also suggest that older age had a detrimental effect on cognition.

Our structural equation model had several salient features. First and foremost, it demonstrated a strong and significant path relationship between the interaction variable of *APOE* × age and the composite ^{1}H-MRS metabolite variable. This was in keeping with our overarching conceptual notion that genotypic effects would become larger with age as compensatory systems attenuate. Second, the model demonstrated a significant path ($p < .01$) from the ^{1}H-MRS variable to cognition, in the predicted neurobiologically plausible direction (higher Cho and mI predicted lower cognition). No direct effect of *APOE* on cognition was found, suggesting that ^{1}H-MRS was an important mediating variable. Last, we found an effect of age on cognition but not on ^{1}H-MRS metabolites, in keeping with the literature (see below). We note that our model was not fully overlapping with our GLM results. This was due to several factors. Our model was, in part, driven by parsimony to most clearly define key aspects of our data structure. Moreover, the structural equation model's path weights were based on multiple covariance structures, mediating variables, a ^{1}H-MRS latent variable that included both Cho/Cr and mI/Cr ratios, and a latent cognitive variable, considered simultaneously in an interactive causal assumption modeling. Resultant path weights were dependent on all other paths and structured by the logic of the designated model to maximize goodness of fit. The GLM approach, instead, took into account effects of independent variables on individual brain metabolite ratios.

Interactive associations between aging and *APOE* status on ^{1}H-MRS metabolites and cognition have not been previously reported. Generally, increases in Cho/Cr found in older *APOE* E4 carriers suggest greater membrane turnover or cycling, in accord with degradation and demands for repair (30,31). Little is known about *APOE* interactions with cholinergic neurons, another possible source of Cho. Speculatively, because *APOE* E4 is associated with earlier age of AD onset (32), it may consequently compromise cholinergic neurons known to be vulnerable to early AD pathology.

It has been previously suggested (2,30) that the initial ^{1}H-MRS change in the pathologic progression of AD is an increase in mI/Cr ratio. Myo-inositol is a marker predominantly present in astrocytes that may reflect glial proliferation or inflammatory activity. More specifically, microglial activation as an indicator of neuroinflammation has been considered an early event in the pathogenesis of AD, as demonstrated by the positron emission tomography ligand [^{11}C](R)-PK11195, specific for binding activated microglia (33). Likewise, it has been suggested that *APOE* E4 may confer some of its risk for AD by modulating immune responses (34,35). In keeping with this line of evidence, a recent review has suggested that chronic inflammation may cause dysregulation of mechanisms to clear damaged and misfolded proteins (mislocalization and hyperphosphorylation of tau) that accumulate with age (36), a process that could start years before AD symptoms first manifest.

Several other points about our study may be worth considering. One potential concern in the quantification of ^{1}H-MRS metabolites is that age-dependent atrophy of the precuneus/posterior cingulate voxel might affect the measurements. We did not expect atrophic changes to be large, as our sample was comprised of cognitively healthy individuals who did not meet criteria for MCI or dementia. Furthermore, in principle, the metabolite ratios should not be affected by the total signal decrease due to reduced tissue and increment of cerebrospinal fluid in the voxel as a consequence of normal brain aging. Nevertheless, we also performed a cortical thickness analysis of the posterior cingulate/precuneus area using Freesurfer software (Martinos Center for Biomedical Imaging, Boston, Massachusetts), and found no effect of *APOE*, age, or *APOE* × age interaction.

Age effects on ^{1}H-MRS metabolites have been inconsistently described, have been small, and have been dependent on metabolite and voxel placement [see Haga et al. (37) for a systematic review]. In keeping with this view, we found that age had inconsistent effects on ^{1}H-MRS metabolites in an analysis-dependent manner. In our sample, NAA/Cr ratios were found not to be influenced by APOE, age, or their interaction. This result may be viewed as counterintuitive, as NAA is considered a marker of neuronal viability that is likely to be affected in normal brain aging. Nevertheless, NAA levels have been found to be relatively stable in cognitively normal older adults during longitudinal studies in different cohorts (4,38). Additionally, our negative findings of the influence of NAA on aging and cognition are in line with those of a very recent study (39) that concluded that whole-brain NAA concentrations are conserved throughout normal aging and are not associated with cognition.

Ratios of ^{1}H-MRS metabolites to creatine are commonly reported in the literature because brain creatine is relatively stable over the course of months within an individual (40), and unaffected by most neurological diseases (31), making the Cr peak suitable as an internal reference. We used proton brain exam (PROBE) as an automated metabolite quantification method, which provides ratio-based (to Cr) quantifications. Quantifying metabolite intensities by referencing to an internal standard is preferred in clinical ^{1}H-MRS (30). Proton brain exam is considered a well-suited method for the analysis of large datasets in that it allows direct comparison of our results with the majority of previous studies in the field (38). However, it is important to note that the proton brain exam metabolite quantification method has the limitation of not providing specific goodness-of-fit measures of the spectral data. In addition, we acknowledge that increased creatine levels with age have also been reported (41). In our study, increased creatine levels would have caused decreased ratios of mI and Cho (by having a greater denominator), which are the opposite of our findings (mI and Cho increased with age).

As a cross-sectional study, our results may have been subject to sample acquisition biases. Particularly, we cannot rule out the possibility that there might have been a survivor effect related to APOE status, since life expectancy in APOE E4 carriers is reduced due to associated increased risk of cardiovascular disease and Alzheimer's disease. In effect, we might have been assaying a relatively healthy, surviving older E4 population. Importantly, this would work against our main hypothesis, i.e., this subsample would be less likely to demonstrate magnetic resonance spectroscopy abnormalities, and thus our results would

appear to be robust. Nevertheless, we acknowledge that as a cross-sectional study, inferences are limited and may be affected by sample ascertainment biases.

Finally, it is important to note that this is not intended to be a clinical biomarker study, as we were not utilizing ^{1}H-MRS measures to characterize MCI, predict AD, or track progression of AD. Rather, we sought to understand how the APOE E4 variant might adversely impact the neurobiology of the aging brain.

In summary, we found that APOE genotype and its interaction with age directly and significantly, modulated brain metabolites (Cho/Cr and mI/Cr). Both Cho/Cr and mI/Cr ratios were increased in older APOE E4 carriers, suggesting increased membrane turnover and inflammatory changes. Furthermore, these ^{1}H-MRS ratios impacted a composite measure of cognition in our structural equation model. Thus, brain metabolites appeared to have a role in mediating the relationship between APOE and cognition. Finally, we suggest that this set of findings provides a window on brain changes in the posterior precuneus/cingulate region, associated with APOE E4, that may precede the development of clinically significant impairments in cognition and everyday function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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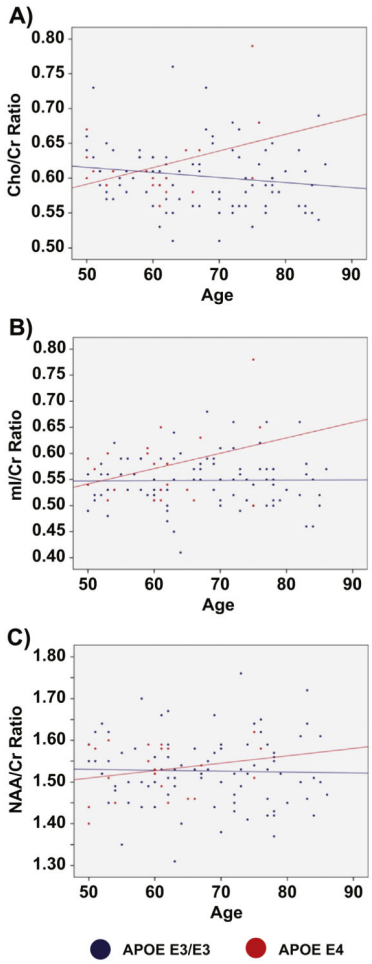

Figure 1.

Proton magnetic resonance spectroscopy metabolite ratios of the subjects and their relation to *APOE* genotype and age. Scatter plots display proton magnetic resonance spectroscopy metabolite ratios for *APOE* E3/E3 homozygotes and *APOE* E4 carriers. Choline (Cho)/creatine (Cr) and myoinositol (mI)/Cr are significantly elevated in older *APOE* E4 carriers (**A, B**). *N*-acetylaspartate (NAA)/Cr is similar across groups (C). For Cho/Cr, general linear model analysis shows an overall model fit near significance ($F_{3,106} = 2.41$, $p = .07$; $R^2 = .06$), with *APOE* genotype having a significant modulatory effect ($F_{1,108} = 4.55$, $p = .03$), as does *APOE* × age interaction term ($F_{1,108} = 5.23$, $p = .02$); age effect is not significant ($F_{1,108} = 1.48$, $p = .23$). Myo-inositol/Cr ratios show a significant overall model ($F_{3,106} = 2.72$, $p = .05$; $R^2 = .07$), with an independent effect of age ($F_{1,108} = 3.85$, $p = .05$) and a near significant effect of the *APOE* × age interaction ($F_{1,108} = 3.63$, $p = .06$); *APOE* is not significant in the model ($F_{1,108} = 2.55$, $p = .11$). For NAA/Cr ratios (overall model fit $F_{3,106} = .24$, $p = .86$, $R^2 = .01$), no effects of *APOE* ($F_{1,108} = .69$, $p = .41$), age ($F_{1,108} = .45$, $p = .51$), or the interaction between *APOE* and age ($F_{1,108} = .72$, $p = .40$) are found.

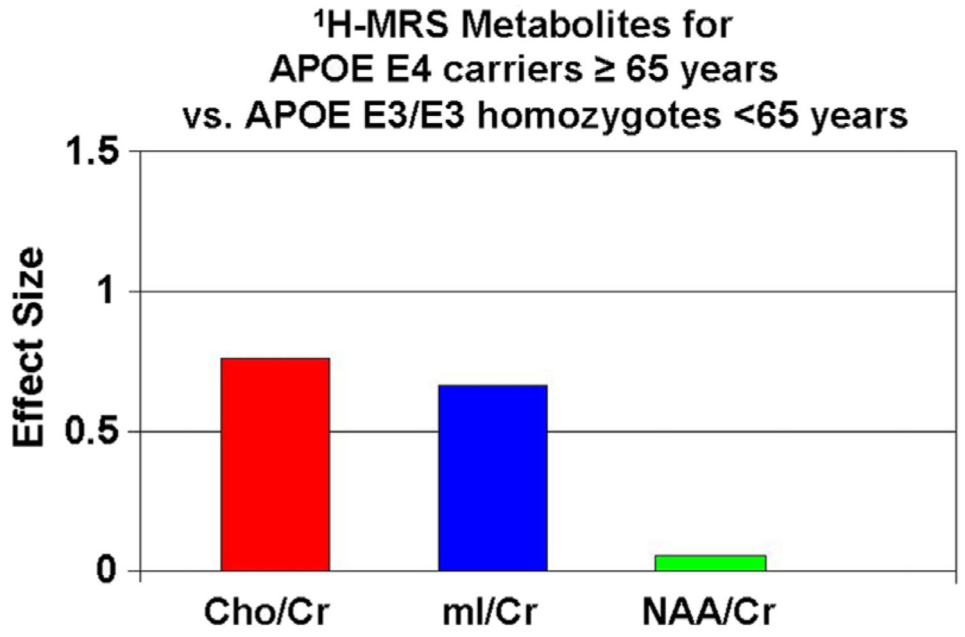

Figure 2.

Effect sizes (ES) for the comparison between older E4 carriers and younger E3 homozygotes on proton magnetic resonance spectroscopy (^{1}H-MRS) metabolite ratios. Bars represent ES for the difference between older E4 carriers and younger E3 homozygotes on choline (Cho)/creatine (Cr) (ES = .76), myo-inositol (mI)/Cr (ES = .66), and *N*-acetylaspartate (NAA)/Cr (ES = .06).

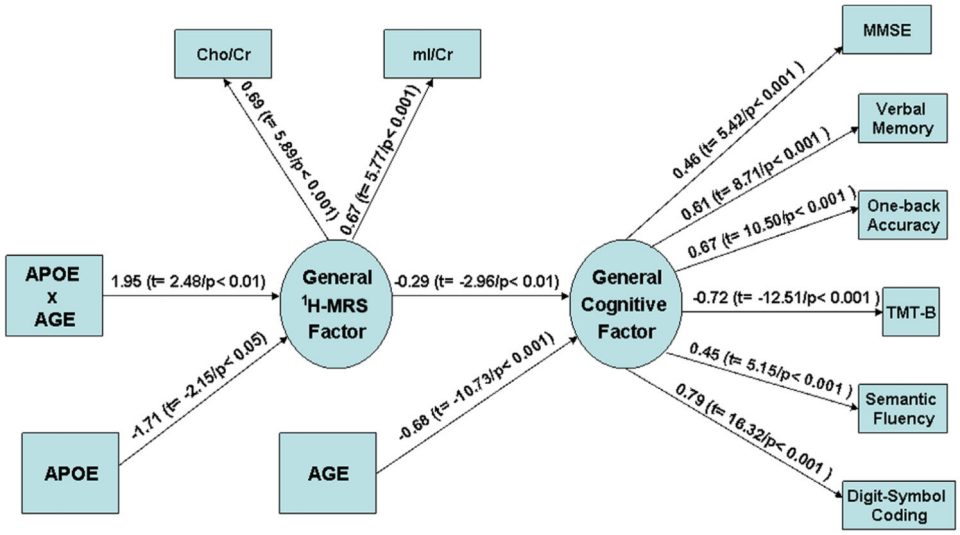

Figure 3.

Final structural equation model with standardized coefficients. Figure shows the direct effect of the interaction between APOE and age and APOE alone on the proton magnetic resonance spectroscopy factor (comprising choline [Cho]/creatine [Cr] and myo-inositol [mI]/Cr), the mediator effect of the interaction between APOE and age on cognition through proton magnetic resonance spectroscopy metabolites, and the direct negative effect of age on cognition. Older *APOE* E4 carriers have higher Cho/Cr and mI/Cr levels, and higher Cho/Cr and mI/Cr levels are associated with worse cognitive performance. Path weights and their significance are shown in this figure. Goodness-of-fit indexes are described in the text. MMSE, Mini-Mental State Examination; ^{1}H-MRS, proton magnetic resonance spectroscopy; TMT-B, Trail Making Test part B.

Table 1

Demographic Characteristics and Cognitive Scores of the Subjects

	***APOE* E3/E3 (*n* = 89)**		***APOE* E4 Carriers (*n* = 23)**		
	<65 Years (*n* = 39)	**≥65 Years (*n* = 50)**	**<65 Years (*n* = 17)**	**≥65 Years (*n* = 6)**	**Statistical Test**
Gender (Male/Female)	11/27	17/33	4/12	5/1	*APOE* E3 X = .25/p = .61 *APOE* E4 X = 6.14/p = .01
Mean Education (SD), Years	16.16 (2.36)	16.17 (3.16)	17.37 (1.50)	17.17 (4.12)	$F_{4,100}$ = 1.01/p = .41; no effect of *APOE*, age, or *APOE* × age
Mean Global Cognition Z Score (SD)	.28 (.36)	−.23(.48)	.20 (.41)	−.37 (.58)	$F_{4,105}$ = 11.62/p < .001 (significant effect of age F = 22.05/p < .001)