ApoE and cholesterol in schizophrenia and bipolar disorder: comparison of grey and white matter and relation with APOE genotype

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Background: Apolipoprotein E (apoE) and cholesterol play a critical role in synapse and myelin maintenance and integrity and are thus appealing candidates in the pathogenesis of schizophrenia and bipolar disorder. To explore the role of these 2 molecules, we quantified cholesterol and apoE levels in prefrontal grey and white matter in patients with schizophrenia, bipolar disorder and healthy controls. Furthermore, we investigated the relations between apoE and cholesterol levels and the APOE genotype.

Methods: We obtained dorsolateral prefrontal grey and white matter from the Stanley Medical Research Institute Brain Collection (schizophrenia n = 35, bipolar disorder n = 35 and controls n = 35). Cholesterol levels were quantified using high-pressure liquid chromatography, whereas apoE was measured by enzyme-linked immunosorbent assay.

Results: We found no significant differences in cholesterol or apoE levels among the groups. ApoE levels were higher in grey matter than in white matter in all groups; conversely, levels of cholesterol were higher in white matter than in grey matter. We observed a significant inverse correlation between apoE and cholesterol levels in both grey and white matter. Furthermore, in grey matter, apoE levels were significantly higher in APOE ε2 carriers compared with APOE ε3 or ε4 carriers, with cholesterol levels following the opposite trend.

Limitations: Limitations of our study include our inability to control for potential confounding variables and the small numbers of APOE ε2 and ε4 carriers in each group.

Conclusion: Although large amounts of cholesterol are present in white matter, apoE expression is limited. The APOE genotype may play a role in the regulation of both cholesterol and apoE levels in grey matter. The impact of APOE polymorphisms on lipid homeostasis in people with psychiatric disorders warrants further investigation.
among neurons and their supporting cells.14 Both apoE and cholesterol play a critical role in synaptogenesis, neurite outgrowth and membrane repair and maintenance, including that of the myelin sheath.9–15 Specifically, when cocultured with glial cells, neurons develop more synapses, and those synapses are more efficient compared with neurons cultured without glial cells.8 The factors implicated in this process were determined to be apoE and cholesterol. Cholesterol and apoE also play a role in the regulation of neurite outgrowth,16–19 with apoE3 increasing neurite outgrowth but apoE4 having the opposite effect.8 In addition, apoE plays a critical role in collecting freed cholesterol from damaged myelin and recycling that cholesterol by incorporating it back into repaired membranes.15 Whereas altered lipid levels have been identified in blood and skin fibroblasts in people with schizophrenia and bipolar disorder,20–23 to date there have been few studies in brain tissue. We previously reported reduced cholesterol levels in visual association cortex in people with major depressive disorder, with a similar trend in people with bipolar disorder.12 Levels in visual association cortex in people with major depressive disorder were lower in Brodmann areas (BAs) 11 and 47 in individuals who had committed suicide by violent means.12 Evidence also suggests that brain apoE levels are altered in people with schizophrenia and bipolar disorder. Specifically, Dean and colleagues20 found increased apoE in BA 9 and 46, with no difference in BA 10 or the striatum in people with schizophrenia, whereas apoE was lower in BA 10 but higher in BA 9 and in the striatum in people with bipolar disorder.21 Finally, a recent meta-analysis identified the APOE ε4 polymorphism as significantly associated with schizophrenia,22 whereas the ε4 allele has also been linked to early onset bipolar disorder with psychotic symptoms.22

Whereas cholesterol and apoE appear to be good candidates for a role in the pathophysiology of schizophrenia and bipolar disorder, few studies have assessed cholesterol and apoE abundance in postmortem brain tissues from people with these disorders. Furthermore, despite differences in lipid composition between grey and white matter, neither apoE nor cholesterol levels have yet been quantified in white matter from people with schizophrenia or bipolar disorder. In light of the reported association between the presence of APOE ε4 alleles and schizophrenia and bipolar disorder, investigation of the relation between APOE genotype, apoE protein levels and cholesterol concentrations is warranted. Whereas studies involving people with Alzheimer disease24–30 and transgenic mice7 have reported a relation between apoE protein levels and genotype (ε2/2 > ε3/3 > ε4/4), this has not been investigated in populations with other disorders. Based on findings of reduced synaptic and neuritic density and altered myelin in people with schizophrenia and bipolar disorder, we propose that levels of cholesterol and its transporter apoE are reduced in grey and white matter in these populations. In addition, we suggest that the APOE genotype may play a role in the regulation of cholesterol and apoE levels in both brain regions. The aim of this study was to quantify cholesterol and apoE levels in dorsolateral prefrontal grey and white matter in patients with schizophrenia, patients with bipolar disorder and healthy controls. Furthermore, we investigated the relation between apoE, cholesterol and the APOE genotype.

Methods

Brain tissue

We obtained frozen samples of the dorsolateral prefrontal region (BA 9) from the Stanley Foundation Neuropathology Consortium. Tissue was available from 1 hemisphere of each brain, with about equal numbers sampled in a random manner for each side, and was carefully dissected out into grey matter and adjacent white matter. Our sample consisted of tissue from 105 brains (35 from controls with no known psychiatric or neurologic disorder, 35 from patients with schizophrenia and 35 from patients with bipolar disorder). Diagnoses were made according to DSM-IV criteria.25 All brains underwent clinical neuropathologic examination, and none demonstrated evidence of neurodegenerative changes or other pathological lesions. We excluded the tissue sample from 1 patient with bipolar disorder from this study owing to a change in diagnosis. Investigators were blind to diagnosis and genotype when measuring cholesterol and apoE levels. This study was approved by the University of British Columbia Clinical Research Ethics Board.

Free cholesterol quantification: high-performance liquid chromatography

Cholesterol was separated from other lipids and quantified using a Waters Alliance 2695 high-performance liquid chromatography (HPLC) system equipped with an autosampler and evaporative light scattering mass detector (ESLD) as previously described.27 Briefly, tissue was homogenized in 15 volumes of ice-cold tris buffered saline (TBS) and protein quantified using a Lowry-based method (DC assay; Bio-Rad). Total lipids were extracted from grey and white matter (wet weight, 13.33 and 6.67 mg, respectively) using a modification of the Folch method.28 Sample homogenate was made up to a total of 2.5 mL with NaCl/EDTA in water (9 g/L/1.14 g/L), 3 mL methanol was added and the sample vortexed. A further 6 mL chloroform was added, and the sample vortexed and centrifuged to enable separation of the organic and inorganic phases. The organic phase was then recovered and transferred to a clean tube. The remaining inorganic phase was extracted twice more to ensure complete recovery of all lipids, with organic phases combined. The solvent was then evaporated under nitrogen and resuspended in 50 mL of hexane/acetone/methanol/chloroform, 1/1/6/4 by volume, containing 75 μg of betulin as the internal standard. Lipids classes, including unesterified cholesterol, were separated using a YMC diol 4.6 mm × 250 mm column at 35°C with a quaternary gradient of hexane, methanol, 1.7% triethylamine in acetone and 0.5% acetic acid in isopropanol. The quantity of cholesterol was determined from the area ratio of cholesterol
to betulin, which was constant in all sample injections. The detector response is linear for unesterified cholesterol over the range of 0.1–2.4 g/L with a relative response of cholesterol to betulin of 1.2±1. Data were expressed as μg cholesterol per mg protein.

ApoE quantification: enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assays (ELISA) were performed as previously described, with slight modifications. Briefly, tissue homogenates were diluted to 240 μg protein/mL in TBS. Duplicate samples were then serially diluted over a 128-fold range and incubated overnight at 4°C in 384 well Maxisorp plates (Nunc). Nonspecific binding was blocked using TBS containing 5% milk. Plates were then incubated with primary antibody (WU E-14, obtained from ATCC, 1:10) overnight at 4°C. Each plate also contained negative control wells in which tissue culture-conditioned media was substituted for the primary antibody. The plates were further incubated with peroxidase-conjugated secondary antibody (goat anti-mouse 1:1000, Jackson Immunoresearch Laboratories). Finally, 3,3′,5,5′-tetramentylbenzidine (KPL) was added, and after 30 minutes the reaction was stopped with 1 M phosphoric acid and the optical density determined at 450 nm. The optical density of each well was plotted against the protein concentration and the linear portion of the curve assessed for each sample. The assay was linear over an average 22- and 14-fold-range for grey and white matter, respectively. Samples were run twice, on different days, and mean values used for analyses. Between-run correlations were greater than 0.90. A serial dilution of a reference brain sample was run on each plate to compute a between-plate coefficient of variation. This coefficient of variation was calculated to be 5.1% and 5.7%. To compare immunoreactivity between samples and regions, the amount of protein required to give an optical density reading of 0.4 was used for both grey and white matter. We excluded 2 tissue samples from the white matter analysis and 1 from the grey matter analysis because an optical density reading of 0.4 did not fall within the linear range. The amount of protein required to give an optical density of 0.4 is inversely related to the amount of target antigen present; therefore, low values indicate high amounts of apoE.

ApoE Western blot

We conducted an immunoblot experiment to confirm the specificity of the antibody. Briefly, 20 μg of brain homogenate from 3 samples (ε2/ε3, ε3/ε3, ε4/ε3) was separated on a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel. After transfer to polyvinylidine fluoride (PVDF) membrane (Bio-Rad), the blot was incubated with primary antibody (WU E-14, 1:10) overnight. The blot was further incubated with peroxidase-conjugated secondary antibody (goat anti-mouse 1:2000, Jackson Immunoresearch Laboratories) for 1 hour. Then ECL reagent (GE Healthcare) was added, and blots imaged using a LAS-3000 imager (Fujifilm). Using this antibody, we were able to detect a doublet at the expected molecular weight of about 36 kDa (Fig. 1), as well as a heavier band at about 80kDa. Doublets at 36kDa have been described previously and are thought to result from apoE sialylation. In addition, apoE complexes that are not dissociated by SDS have been reported at a molecular weight of about 80kDa.

APOE genotyping: polymerase chain reaction–restriction fragment length polymorphism

We performed APOE genotyping using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), as described previously, but with slight modifications. Genomic DNA was isolated from 15–25 mg of brain tissue using a DNA purification kit (DNeasy Blood and Tissue Kit; Qiagen). A 318-bp fragment from the APOE gene was PCR-amplified in 50 μL containing 0.1–0.4 ng purified genomic DNA, 1× Qagen PCR buffer, 0.35 μM each primer, 200 μM each dNTP, 2x Q-Solution and 1.5 U Qiagen Taq DNA polymerase. Two primers were used in the amplification: upstream primer E2mut (5’ ACT GAC CCC GGT GGC GGA GGA GAC GCG TGC) and downstream primer E3 (5’ TGT TCC ACC AGG GCC CCC AGG CGC TCG CGG). Primer E2mut differs from the genomic sequence at 1 position, which creates an additional AlfIII recognition site in the

Fig. 1: Western blot of apoE immunoreactivity in tissue samples of human dorsolateral prefrontal cortex (APOE genotypes ε4/ε3, ε2/ε3, ε3/ε3). Homogenates (20 μg protein) were diluted in reducing Laemmli buffer and run on a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel. A doublet can be observed at about 36kDa, in addition to a second band at about 80kDa (arrowheads). It has been suggested that doublets may reflect apoE sialylation, whereas the band at about 80kDa represents an apoE complex not dissociated by SDS. No qualitative differences in staining were observed among the 3 genotypes.
amplified fragment. Reaction mixtures were incubated at 94°C for 3 minutes, subjected to 40 cycles of amplification (95°C, 20 s; 65°C, 40 s; 72°C, 45 s) and incubated at 72°C for 7 minutes. Restriction digests containing 15 µL amplification reaction (2.1 µL H2O, 0.15 µL BSA and either 2 µL Qiagen buffer #3 and 0.75 U Afl III or 2 µL Qiagen buffer #4 and 0.125 U Hae II) were incubated at 37°C overnight. The digested product was run on 4% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. We determined genotype by comparison with standards run on the same gel.

Statistical analysis

We assessed differences in age, postmortem interval (PMI) and brain pH among groups using a 1-way analysis of variance (ANOVA), with a significance level of α = 0.05. We used Shapiro-Wilk tests to determine whether ApoE and cholesterol measures conformed to a normal distribution. As data were not normally distributed (apoE grey matter W103 = 0.67, p < 0.001; apoE white matter W102 = 0.89, p < 0.001; cholesterol grey matter W103 = 0.88, p < 0.001; cholesterol white matter W103 = 0.93, p < 0.001), we compared cholesterol and apoE levels among groups using the nonparametric Kruskal–Wallis H test. We further compared ApoE and cholesterol levels between grey and white matter in the whole-brain series using Wilcoxon signed ranks test. Spearman rank correlations were performed to assess the influence of age, PMI and brain pH on apoE and cholesterol levels, whereas Mann–Whitney tests were used to investigate the relations between apoE and cholesterol levels and sex or brain hemisphere. In addition, in the 2 patient groups, we assessed correlations between apoE and cholesterol levels and age at onset, duration of illness and lifetime antipsychotic dose using Spearman rank analyses. Finally, we used Mann–Whitney or Kruskal–Wallis H tests to examine whether alcohol use (none or social v. past or present moderate or heavy use) or illicit drug use (none or social use) was related to cholesterol or apoE levels, to assess the effect of death by suicide and to compare the effect of different treatment conditions (i.e., typical antipsychotic, atypical antipsychotic, no antipsychotic) on cholesterol and apoE levels.

We assessed differences in APOE genotype among groups using the χ2 test. Additionally, to investigate how apoE and cholesterol levels varied as a function of genotype, we compared cholesterol and apoE levels among an ε2+ group (comprising ε2/ε2 and ε2/ε3 genotypes) and an ε3+ group (comprising ε3/ε3 genotype) and an ε4+ group (comprising ε3/ε4 and ε4/ε4 genotypes) using the nonparametric Kruskal–Wallis H test with post-hoc Mann–Whitney tests. The 1 tissue sample with APOE ε2/ε4 genotype was not included in this analysis. We computed Spearman rank correlations to assess the relation between apoE and cholesterol, both in the whole sample and after stratification by diagnosis and genotype. We performed all statistical analyses using SPSS 17.0.

As the ELISA data values (amount of protein required to give an optical density reading of 0.4) are inversely related to the amount of target antigen present in a sample, for graphing purposes, we employed a simple algebraic transformation to plot the data in the intuitively simpler fashion where greater values represent greater amounts of the target antigen, as previously described.24

Results

Association between apoE and cholesterol levels and clinical and demographic variables

Detailed demographic, postmortem and clinical information is reported in Table 1. Groups did not differ in age or PMI, although there were a higher proportion of tissue samples from women in the bipolar disorder group compared with the schizophrenia and control groups (χ2 = 7.5, p = 0.024, Table 1). Brain pH also differed among groups (F1,103 = 4.174, p = 0.018), being slightly lower in both the bipolar disorder and schizophrenia groups.

The apoE and cholesterol levels are presented in Table 2. We observed no significant differences in either apoE or cholesterol levels among the groups in grey or white matter. Examining the whole series, levels of apoE or cholesterol did not correlate with age or pH, although when stratified by diagnosis, apoE levels in white matter correlated with brain pH in the schizophrenia group (rho = −0.397, p = 0.020) but not in the control or bipolar disorder groups. Postmortem interval correlated weakly with apoE levels in white matter (rho = −0.207, p = 0.037), but not in grey matter. Neither apoE nor cholesterol levels differed as a function of sex or brain hemisphere. However, when stratified by diagnosis, we observed a significant effect of hemisphere on grey matter cholesterol levels in the schizophrenia group (Z = −2.442, p = 0.014) but not in the control or bipolar disorder groups.

In the psychiatric groups, we found no relations between apoE or cholesterol levels and age at illness onset or duration of illness. Death by suicide had no influence on cholesterol or apoE levels, with this finding remaining when cases were stratified into violent and nonviolent suicides. In grey matter, cholesterol concentration did not differ between individuals with a history of no alcohol use or only social alcohol use (n = 29) and those with moderate or heavy past or present alcohol use (n = 39). However, in white matter we found significantly lower white matter cholesterol levels in the moderate/heavy use group (Mann–Whitney Z = −2.139, p = 0.032). Neither cholesterol nor apoE levels differed between individuals with history of no illicit drug use or only social use (n = 33) and those with moderate or heavy past or present drug use (n = 34) in either grey or white matter. With regards to the effect of medication on levels of apoE or cholesterol, lifetime antipsychotic dose did not correlate with either apoE or cholesterol levels in grey or white matter. Furthermore, neither the type of antipsychotic prescribed at the time of death, nor the presence of antipsychotics at death was related to apoE or cholesterol levels.

ApoE and cholesterol levels in grey versus white matter

Taking the 3 study groups as a whole, we found significantly
higher levels of apoE in grey matter compared with that in
white matter of the same region (Wilcoxon Z = –8.741,
p < 0.001). Conversely, cholesterol levels were significantly
higher in white matter compared with grey matter (Wilcoxon
Z = –8.704, p < 0.001). As reported in Table 2, we observed
a grey to white matter ratio of 10:1 for apoE and 0.37:1 for
cholesterol.

**APOE allele effect on apoE and cholesterol levels**

We observed no significant differences in APOE genotypic or
allelic frequency among the 3 groups (Table 3). Stratifying
the whole sample according to presence of APOE alleles (ε2+,
n = 15; ε3+, n = 60; ε4+, n = 24), significant differences in apoE
expression in grey matter were identified among the 3 geno-
types (χ² = 7.880, p = 0.019, Fig. 2A). Post-hoc analyses re-
vealed that levels of apoE were 105% higher in the ε2+ group
compared with the ε3+ group and 52% greater compared
with the ε4+ group (ε2+ v. ε3+ Z = –2.815, p = 0.005; ε2+ v.
ε4+ Z = –1.848, p = 0.066). There were no significant differ-
ences between the ε3+ and ε4+ groups. Conversely, we ob-
served no significant relation between genotype and chole-
sterol levels. Whereas cholesterol levels were 19% lower in the
ε2+ carriers compared with ε4+ carriers, this did not reach
statistical significance (Z = –1.914, p = 0.057, Fig. 2B). In white
matter, no relation was identified between genotype and apoE
or cholesterol levels.

**Correlation between apoE and cholesterol: effect of
diagnosis and APOE allele**

Cholesterol and apoE levels were inversely correlated in grey
matter (rho = –0.500, p < 0.001). Correlation analyses were
computed after stratification by study group and by
APOE allele status. The control group showed the strongest
correlation (rho = –0.625, p < 0.001), followed by the bipolar
disorder group (rho = –0.521, p = 0.002) and the schizophre-
nia group (rho = –0.339, p = 0.05). The APOE ε4 carriers
showed the strongest correlation between levels of chole-
sterol and apoE protein in grey matter (rho = –0.715, p < 0.001),
followed by the APOE ε3/ε3 group (rho = –0.494, p < 0.001). The APOE ε2 carriers showed no statistically signif-
icant correlation. We also observed an inverse correlation
between cholesterol and apoE in white matter (rho = –0.269,
p = 0.006). This did not reach statistical significance for any individual psychiatric group. We observed significant correlations in the APOE ε3/ε3 (rho = -0.321, p = 0.013) and the APOE ε4 carriers (rho = -0.391, p = 0.059), but again the APOE ε2 carriers showed no statistically significant relation between cholesterol and apoE levels.

**Discussion**

**ApoE and cholesterol levels in people with schizophrenia and bipolar disorder**

Median levels of apoE and cholesterol did not differ significantly in the schizophrenia or bipolar disorder groups relative to the control group in either grey or white matter, although we do report a 15% decrease in apoE levels in grey matter in the schizophrenia group and a 19% decrease in apoE in white matter in the bipolar disorder group. In 2 previous studies of apoE expression in the major psychiatric disorders, apoE levels were higher in the prefrontal cortex (BA 9 and 46) in people with schizophrenia, whereas apoE levels were increased in BA9 but decreased in BA 10 in a small series involving people with bipolar disorder. Several possible reasons might account for the discrepancy between our data and that of the 2 previous studies. First, clinical and demographic characteristics were different; for example, our tissue samples came from younger individuals and included both bipolar I and II disorders. Furthermore, in the previous studies, apoE was only measured in the left hemisphere, thus raising questions about the presence of a laterality effect. In addition, the methodology used to measure apoE differed between the studies. We used an ELISA assay, which targets the protein of interest in its native conformational state, whereas the previous studies used Western blotting, which targets the protein’s epitope in an unfolded state. In agreement with our previous study of visual association cortex, we found that cholesterol levels were not significantly different in people with either bipolar disorder or schizophrenia.

**ApoE and cholesterol levels in grey versus white matter**

Our data indicate that cholesterol levels are higher in white matter than in grey matter, whereas apoE levels are higher in grey matter than in white matter. Within the brain, about 70% of cholesterol is estimated to be present within myelin. Therefore, high levels of cholesterol in white matter are to be expected. We found a grey to white matter ratio of about 0.37:1 for cholesterol and 10:1 for apoE. Previous studies have reported grey to white matter cholesterol ratios of about 0.44:1.

To our knowledge, this is the first study to assess the relation between cholesterol and apoE in the major psychiatric disorders. The brain depends on both cholesterol and apoE. ApoE-dependent recycling mechanism to maintain cholesterol homeostasis. Our results show that apoE levels are substantially lower in white matter, which may be explained by the fact that cholesterol turnover in the myelin pool is extremely low in the nonpathologic adult brain. On the other hand, high levels of apoE in grey matter may point to a more dynamic pool of cholesterol. This is consistent with the wide array of dynamic cellular events occurring in grey matter that require cholesterol trafficking such as synaptic transmission, synaptic plasticity or neuronal maintenance. Alternatively, other apolipoproteins may be involved in cholesterol transport in white matter. In grey matter, we observed an inverse relation between apoE and cholesterol levels. The inverse association may seem counterintuitive. However, we measured free cholesterol (i.e., that bound to membranes), whereas cholesterol is transformed into the esterified form once loaded into the core of lipoproteins. Therefore it seems plausible that when apoE levels are high, more cholesterol is in the esterified form rather than the free form. Nonetheless, this remains to be resolved. Of note, the control and bipolar disorder groups showed a strong correlation between grey matter cholesterol and apoE levels, whereas a much weaker correlation was observed in the schizophrenia group. Although the exploratory nature of the analysis prevents any causal interpretation, this finding may reflect a pathological process occurring in people with schizophrenia that interferes with the lipidation of apoE. Alterations in levels of ApoA1, ApoL and ApoD have been reported to be pathologic, and our data may indicate a general abnormality of lipid transport in people with this disorder.

**APOE allele effect on apoE and cholesterol levels**

We also observed a relation between genotype, apoE and cholesterol levels in grey matter. In this study, apoE levels were higher in APOE ε2 carriers compared with APOE ε3 carriers and to a lesser extent to APOE ε4 carriers. Conversely, cholesterol levels were lower in APOE ε2 carriers compared

Table 3: Summary of genotype and allele frequencies in the control and psychiatric groups

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<th>Genotype</th>
<th>Total no.</th>
<th>Allele frequency: no. (%)</th>
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*Three genotypes are missing owing to unclear resolution of amplified DNA fragments.
with APOE ε4 carriers. This result points to a genotypic regulation of apoE and cholesterol levels and is consistent with a recent animal study reporting that apoE levels in the brain differ depending on genotype, with ε2/2 > ε3/3 > ε4/4. It is not clear what mechanisms may be behind this, although apoE is more prone to remain in a partially unfolded tertiary conformation, making it more susceptible to protease digestion.

In addition, genotype also influenced the strength of the relation between cholesterol and apoE levels. The APOE ε4 carriers had the strongest negative correlation followed by APOE ε3 carriers, with APOE ε2 carriers having no statistically significant correlation. This differential strength of association may be related to the different lipidation capacity of the different isoforms.

**APOE ε4 genotype and risk for schizophrenia and bipolar disorder**

Our study found allele and genotype frequencies similar to those present in the general population. Whereas the number of ε2 carriers was low in the bipolar disorder group, this was not statistically significant, although we may be underpowered to detect such an effect. Whereas initial reports identified an increase in ε4 allele frequency in patients with schizophrenia, later studies have failed to replicate this. However, a recent meta-analysis did find that the APOE ε4 genotype is a risk factor for schizophrenia, albeit of small effect. The ε4 allele has also been associated with early onset bipolar disorder with psychotic symptoms. Although, APOE ε4 is a major genetic risk factor for Alzheimer disease, the mechanism by which carrying the APOE ε4 allele translates to neuropathology remains elusive. It has been suggested that the APOE ε4 allele results in a lower protein expression, which in turn could impair cholesterol homeostasis and synaptic plasticity. Since abnormalities in synaptic proteins have been reported in people with schizophrenia and bipolar disorder, further investigation of the relations between synaptic plasticity, genotype and cholesterol homeostasis is warranted.

![Fig. 2: Levels of apoE and cholesterol in grey matter: effect of APOE allele. The ε2+ group comprised ε2/2 and ε2/3 genotypes; ε3+ group comprised ε3/3 genotype; ε4+ group comprised ε4/ε4 and ε4/ε3 genotypes; ε2/ε4 genotype was excluded. (A) ApoE levels (arbitrary units) stratified by APOE allele status (ε2+, n = 15; ε3+, n = 60; ε4+, n = 24) in grey matter in the total sample series. Bars represent median values. Results of the Kruskal–Wallis H test indicate that apoE levels differ among genotypes (χ² = 7.880, p = 0.005), with higher levels in the ε2+ group compared with ε3+ or ε4+ groups (ε2+ v. ε3+ Z = –2.154, p = 0.000, as indicated by the asterisk; ε2+ v. ε4+ Z = –1.848, p = 0.07). Note: As the enzyme-linked immunosorbent assay data values (amount of protein required to give an optical density reading of 0.4) are inversely related to the amount of target antigen present in a sample, for graphing purposes we employed a simple algebraic transformation (raw value × [-1] + constant) to plot the data in the intuitively simpler fashion where greater values represent greater amounts of the target antigen, as previously described. (B) Cholesterol levels (µg/mg of brain protein) stratified by APOE allele status (ε2+, n = 15; ε3+, n = 60; ε4+, n = 25) in grey matter in the total sample. Bars represent median values. Results of Kruskal–Wallis H tests indicate that cholesterol does not differ significantly between genotypes.

![Fig. 3: Correlation between apoE (arbitrary units) and cholesterol levels (µg/mg of protein) in grey matter in the total sample (n = 103). As the enzyme-linked immunosorbent assay data values (amount of protein required to give an optical density reading of 0.4) are inversely related to the amount of target antigen present in a sample, for graphing purposes we employed a simple algebraic transformation to plot the data in the intuitively simpler fashion where greater values represent greater amounts of the target antigen, as previously described.](image-url)
Limitations

Several limitations of the present study need to be addressed. First, as our primary variables of interest were not normally distributed, our statistical analyses relied on nonparametric tests. Thus, it was not possible to control for potential confounding variables. One such important confounding factor is exposure to antipsychotic medication. We observed no significant correlation between apoE or cholesterol levels and lifetime antipsychotic dose, and there were no differences in apoE or cholesterol levels among patients treated with atypical antipsychotics, typical antipsychotics or no antipsychotics before death. Our data do not support those of a previous study that reported a significant reduction in apoE levels in grey matter in rats treated with haloperidol. Although animal studies are a necessary strategy, it is important to bear in mind differences to human physiology when translating research results. Specifically, rodents do not have the 3 common human allele variants for APOE (i.e., e2, e3, e4), and this difference has an impact on apoE and cholesterol metabolism in rats. The effect of antipsychotic treatment on apoE and cholesterol in the human brain remains open to further research.

A second potentially important factor is the presence of lipid-lowering medications in these patients. Risk for cardiovascular disease and dyslipidemia is elevated in patients with schizophrenia relative to the general population. Unfortunately, information on whether patients were prescribed statins or other lipid-lowering drugs before death is not available to us. However, animal studies indicate that administration of high doses of simvastatin or pravastatin does not change total brain cholesterol levels. Furthermore, a study of human volunteers showed that administration of a high-dose of either simvastatin or pravastatin did not change plasma 24(S)-hydroxycholesterol to cholesterol ratio, a surrogate marker of brain cholesterol homeostasis. In addition, moderate or heavy past or present alcohol use was reported in a significant proportion of the psychiatric patients. In this group of patients, we found a significant deficit in cholesterol levels in white matter but not in grey matter relative to patients reported to have no or only social alcohol use. This finding is contrary to that of Olsson and colleagues, who reported no change in cholesterol levels in either grey or white matter in a small series of individuals with alcoholism. Finally, the number of APOE e2 and e4 carriers was relatively small and precluded examination of the relation between apoE and cholesterol levels between psychiatric groups stratified by genotype.

Conclusion

To our knowledge, our study provides for the first time apoE and cholesterol measurements in white matter in people with schizophrenia and bipolar disorder. Whereas diagnostic effects were not obvious, our data indicate that white matter is rich in cholesterol but apoE is rather scarce, whereas, conversely, apoE is abundant in grey matter but cholesterol is present at substantially lower amounts. In addition, we identified an inverse relation between these molecules in both grey and white matter. We also provide evidence for genotype-dependent regulation of apoE and cholesterol in human grey matter. The impact of APOE polymorphisms on lipid homeostasis in people with psychiatric disorders requires further investigation.

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References


