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Correlation between Apolipoprotein E genotype and brain metabolism in amyotrophic lateral sclerosis

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The Metabolic Signature of *ApoE* Genotype in ALS

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Author Disclosures

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Abstract

Objective: To evaluate the metabolic correlates of the *ApoE* genotype in ALS patients and to investigate the role of *ApoE* ε2 allele as a risk factor for cognitive impairment.

Methods: A total of 159 ALS cases enrolled at the ALS Centre of Turin underwent *ApoE* and ALS-related genes analysis, neuropsychological assessment and cerebral ^{18}F-FDG-PET. The *ApoE* genotype was regressed against whole brain metabolism as assessed by ^{18}F-FDG-PET, with age, sex, education, type of onset and *C9orf72* status as covariates.

Results: Brain metabolism significantly positively correlated with the *ApoE* genotype from ε2/ε2 to ε3/ε4 in left prefrontal (BA 10), orbitofrontal (BAs 11, 45, 47) and anterior cingulate (BA 32) cortices. Relative metabolism in patients carrying the ε2/ε2 genotype was the lowest and metabolism in carriers of the ε3/ε4 genotype had the highest value. No significant negative correlation was found between the *ApoE* genotypes and metabolism.

Conclusions: In a sample of 159 ALS patients we have found a highly significant, relatively lower metabolism in association with the ε2 allele in extramotor areas tipically affected in FTD, such as left prefrontal, orbitofrontal and anterior cingulate cortices, strengthening the finding of a role of ε2 as a risk factor for cognitive impairment in ALS as compared to the other alleles. Our data support the hypothesis of a link between cholesterol homeostasis and neurodegeneration based on the influence of *ApoE* ε2 allele. We suggest that neuroinflammation may play a role as underlying mechanism of such relationship, but this hypothesis needs to be confirmed.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting upper and lower motor neurons, leading to death due to respiratory failure within ~3 years. An increasing body of literature has highlighted the overlap between ALS and frontotemporal dementia (FTD) from the clinical, genetic and neuropathological point of view. Two population-based studies have reported that approximately 15% of ALS patients also display an overt FTD, while 35% show milder frontotemporal syndromes.[1,2] Several genes have been related to both ALS and FTD, including *C9orf72*, *VCP*, *SQSTM1*, *OPTN* and *UBQLN2*.[3] A recent population-based study confirmed that *C9orf72* repeat expansions have a primary role in increasing the risk of cognitive impairment in patients with ALS, but also showed that *Apolipoprotein E* (*ApoE*) ε2 allele independently increased the risk of FTD.[4] The aim of the present study was to evaluate the metabolic correlates of the *ApoE* genotype in ALS patients, particularly focusing on extramotor cerebral areas, to substantiate the metabolic correlates of the role of *ApoE* ε2 allele as a risk factor for cognitive impairment.

Materials and methods

Patients

In our recent survey about the influence of *ApoE* genotype on the risk of FTD in ALS[4] we analysed 357 patients fulfilling El Escorial revised diagnostic criteria[5] for definite, probable, and probable laboratory-supported ALS. These were incident cases resident in the provinces of Torino and Cuneo of Piemonte region, Italy, diagnosed between January 1, 2009, and December 31, 2013, and were identified through the Piemonte and Valle d'Aosta register for ALS (PARALS).[6] A subset of 159 patients of this cohort underwent ^{18}F-2-fluoro-2-deoxy-D-glucose-PET (^{18}F-FDG-PET) and were included in the present study. The remaining patients were not assessed for the following reasons:

denial to undergo the examination, impossibility to complete the acquisition because of severe orthopnoea or the difficulty going to the PET Centre due to motor disability.

ApoE and ALS-related Genes Analysis

The analysis of *ApoE, SOD1, TARDBP, FUS/TLS, C9orf72* and *ANG* was performed in the whole sample, while *OPTN* and *MATR3* were analysed only in familial cases. Detailed methods were reported elsewhere.[4,7]

Neuropsychological Assessment

The neuropsychological battery, evaluating executive function, memory, visuospatial function, and language, was built according to the Clinical Diagnostic Criteria for Frontotemporal Lobar Degeneration[8] and the ALS-FTD Consensus Criteria.[9] Information about the neuropsychological battery, the testing procedure and the cognitive classification has been provided in previous papers.[2,4]

^{18}F-FDG-PET

PET was performed by ^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG-PET) according to previously published Guidelines.[10] Briefly, subjects fasted for a minimum of six hours before the exam and blood glucose was checked before starting the procedure (<7.2 mmol/l in all cases). After a 20-minute rest in a silent dark room, with eyes closed and ears unplugged, approximately 185 MBq of ^{18}F-FDG were injected *via* a venous cannula. The PET acquisition procedure started approximately 60 minutes after the injection. A polycarbonate head holder was used to reduce head movements during the scan.

PET/CT scans were performed by a Discovery ST-E System (General Electric). CT scan of the brain (thickness of 3.75 millimetres, 140 kVolt, 60-80 mA/sec) and PET brain scan (1 FOV of 30 transaxial centimetres) were sequentially acquired, being the former used for attenuation correction

of the PET data. Data were collected in 128×128 matrices with a reconstructed voxel of 2.34 x 2.34 x 2.00 mm.

Statistical analysis

Detailed methods have been described previously.[11] Briefly, SPM8 implemented in Matlab, version 7.10.0 (MathWorks) was used for image normalization. A customized brain ^{18}F-FDG-PET template, obtained from scans performed at the same centre, was utilized for spatial normalization. Intensity normalization was performed using the 0.8 default SPM value of grey matter threshold and images were subsequently smoothed with an 8 mm filter and submitted to statistical analysis. According to *ApoE* genotype, 1 subject was ε2/ε2, 16 ε2/ε3, 2 ε2/ε4, 123 ε3/ε3, 17 ε3/ε4.
In our sample of 159 ALS patients *ApoE* genotype was regressed against whole brain metabolism. The correlation procedure was made possible by the transformation of categorical *ApoE* genotype into a rank variable as follows: ε2/ε2 = 1, ε2/ε3 = 2, ε2/ε4 = 3, ε3/ε3 = 4, ε3/ε4 = 5.[12] SPM8 Multiple Regression routine was implemented with age, sex, education, type of onset and *C9orf72* status as covariates. One of the working hypotheses of the study was that *ApoE* genotype could be a risk factor for cognitive impairment. For this reason, we chose to covary for education and *C9orf72* status, both possibly influencing cognition, in order to regress out the impact of such variables on the results. The height threshold was set at $p<0.005$ ($p<0.05$ $FDR_{corrected}$ at cluster level) and only clusters containing more than 64 contiguous voxels were considered significant. Brodmann areas (BAs) were identified at a 0-2 mm range from the Talairach coordinates of the SPM output isocentres corrected by Talairach Client (http://www.talairach.org/index.html).

Standard protocol approvals, registrations, and patient consents

The study was approved by the local ethical committee (Comitato Etico Interaziendale Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino). Patients signed a written

informed consent and databases were treated according the Italian privacy regulations, including deidentification of patients.

Results

Patients

The study sample included 159 cases, with mean age at onset of 63.6 years (SD 10.8), mean age at diagnosis of 64.3 years (11.7), and mean age at PET of 64.9 years (10.6). The mean disease duration at time of PET examination was 15 months (11.4). The mean ALSFRS–R score at PET was 39.3 (5.8). 18 subjects carried a *C9orf72* expansion, 9 a missense mutation of *TARDBP*, 3 of *SOD1*, 2 of *FUS* and 1 of *MATR3*. Among *C9orf72* expansion carriers, the *ApoE* genotype was ε2/ε3 in 1 subject and ε3/ε3 in 17. 97 patients resulted cognitively normal (ALS-Cn), 19 had FTD (ALS-FTD), 35 showed cognitive impairment not fulfilling FTD criteria (ALS-Ci), and 8 had prevalent behavioural impairment (ALS-Bi). The mean education was 9 years (3.8).

^{18}F-FDG-PET

Brain metabolism correlated positively with the *ApoE* genotype as transformed into rank variable: patients carrying the ε2/ε2 genotype showed the lowest relative metabolism, the ones with ε3/ε4 the highest. Such correlation was found in left prefrontal (BA 10), orbitofrontal (BAs 11, 45, 47) and anterior cingulate (BA 32) cortices (Figure 1, Table 1). No significant negative correlation was found between the *ApoE* genotypes and metabolism.

Discussion

In our series of ALS patients, we have found a significantly lower relative metabolism in association with the ε2 allele in extramotor areas typically affected in FTD,[13] such as left prefrontal,

orbitofrontal and anterior cingulate cortices, strengthening the finding of a role of ε2 as a risk factor for cognitive impairment in ALS as compared to the other alleles.[4]

We previously evaluated the metabolic correlates of the different degrees of cognitive impairment in patients with ALS employing ^{18}F-FDG-PET.[14] We found a significant relative hypometabolism in frontal and prefrontal cortex in the ALS-FTD group as compared to patients with normal cognitive status (ALS-Cn). Moreover, patients with intermediate cognitive deficit (ALS-Ci) showed an intermediate metabolic behaviour in frontal cortex, being hypometabolic as compared to ALS-Cn subjects, and demonstrating a cluster of higher relative metabolism as compared to ALS-FTD. Such cluster was included in the same left frontal regions found to be more severely hypometabolic in ALS-FTD as compared to ALS-Cn, suggesting a continuum between cognitive decline and metabolic activity in these areas. Strikingly, the present study has identified a significant relative hypometabolism in association with *ApoE* ε2 allele in left frontal clusters, largely overlapping with those reported in the study mentioned above. This concordance of results strengthens the hypothesis of a role of *ApoE* ε2 allele as risk factor for cognitive deterioration in ALS.

The *ApoE* gene has three alleles, occurring at different frequencies in humans: ε2 (5-10%), ε3 (65-70%) and ε4 (15-20%), corresponding to three homozygous (ApoE2/E2, ApoE3/E3 and ApoE4/E4) and three heterozygous (ApoE2/E3, ApoE2/E4 and ApoE3/E4) phenotypes.[15] ApoE is a key lipoprotein involved in metabolism, transport, delivery and distribution of lipids among tissues.[16]

The role of *ApoE* genotype in AD is well known. The ε2 allele reduces the risk and delays the onset of dementia, while the ε4 isoform leads to increased risk of dementia by ~3-fold in heterozygous carriers and 12-fold in homozygous carriers.[17]

The influence of *ApoE* genotype on the risk of FTD has been evaluated in various studies with conflicting results. A possible explanation could be provided considering the frequent limitations of surveys about this issue. They usually include relatively small samples and clinic-based series

instead of population-based series. Besides, the diagnostic criteria vary across studies. An association study and meta-analysis published in 2002 by Verpillat and colleagues reported a detrimental role for the ε2 allele.[18] Conversely, more recent studies,[19] including a meta-analysis,[20] suggest that the ε4 allele is a risk factor for FTD.

The correlation of *ApoE* genotype with ALS motor phenotype has been investigated in several surveys. A study by Li and colleagues[21] reported a protective role of the ε2 allele on age at ALS onset, with carriers displaying symptoms three years later than non-carriers. Some papers suggested a possible detrimental effect of the ε4 allele in terms of lower age at onset,[22] increased risk of bulbar onset[23] and shorter survival.[24] Conversely, a study on a clinic-based series of 852 ALS patients failed to show any correlation between *ApoE* genotype and ALS clinical expression in terms of age of onset, rate of progression, and survival.[25] This study reported that *ApoE* genotype was not correlated with cognitive impairment, but it did not correct for *C9ORF72* status. Such inconstant results may be due almost in part to the fact that the study cohorts are not population-based and have different geographic origin.

We recently reported that the ε2 allele increased the risk of FTD in a population-based ALS series.[4] Noteworthy, our previous findings are strengthened by the present study, demonstrating that the presence of the ε2 allele is associated with relatively reduced metabolism in brain regions typically affected in FTD.[13]

The mechanisms of the possible detrimental effect of the ε2 allele are unclear. ~75% of the body's production of ApoE is provided by the liver. The brain is the second most important producing site, where it is synthetized mainly by astrocytes but also by oligodendrocytes, microglia and neurons, particularly in case of cell injury or stress.[15] ApoE is involved in cholesterol transport from astrocytes to neurons. ApoE2 defectively binds to the LDL receptor (LDL-R), i.e. ~2 % of normal activity.[15] The main pathway of cholesterol metabolism in neurons leads to products binding to the β-isoform of Liver X Receptor (LXRβ).[26] Noteworthy, mice knock out (KO) for *LXRβ* manifest an

adult-onset motor neuron degeneration,[27] associated with upregulation of ApoE expression, accumulation of cholesterol in ventral horns neurons, gliosis and increased expression of proinflammatory cytokines and monocyte chemotactic protein 1 in the spinal cord.[28] A possible mechanism underlying motor neuron degeneration in KO mice may be the loss of LXRβ function of attenuating the inflammatory response.[29] Interestingly, LXRβ$^{-/-}$ mice also display ubiquitin-positive and TDP-43-positive cytoplasmic inclusions in spinal cord motor neurons resembling the pathologic hallmark of ALS in humans.[30] Data from *in vitro* studies and animal models suggest that specific cholestenoic acids, intermediates in the conversion of cholesterol into bile acids, can activate LXRs, thus helping motor neurons survival.[31] In a cohort of 438 ALS patients and 330 healthy controls the *LXRβ* SNP rs2695121 was associated with a 30% increase of ALS duration.[32] Taken together, these data suggest that LXRs might constitute a link between cholesterol homeostasis and neurodegeneration involving neuroinflammatory mechanisms.[33]
We can hypothesize that the low binding-activity of ApoE2 for LDL-R impairs cholesterol transport from astrocytes to neurons, leading to defective cholesterol metabolism and finally to a decrease in LXRβ ligands in neurons.

A recent neuropathologic staging model of ALS has proposed that phosphorylated TDP-43 necessarily tends to spread with disease progression from the primary motor cortex to the prefrontal areas, suggesting that all patients are susceptible to develop frontal cognitive impairment over time.[34] Our findings suggest that the *ApoE* ε2 allele makes neurons more vulnerable to degeneration, enhancing the spreading of the pathological process to brain areas involved in cognitive functions.

The present study demonstrates that in ALS patients the presence of the ε2 allele of *ApoE* is associated with a relative hypometabolism in frontal regions as compared to other alleles independently from the *C9orf72* status, strengthening our previous finding about its role as a risk factor for cognitive impairment in ALS. Our data also support the hypothesis of a link between

cholesterol homeostasis and neurodegeneration based on the influence of *ApoE* ε2 allele. Further studies are necessary to confirm the possible role of neuroinflammation as the underlying mechanism of such relationship, since it could provide novel therapeutic targets.

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Table 1. Results of the positive correlation between whole brain metabolism and *ApoE* genotypes. Abbreviations: BA=Brodmann Area.

Cluster Extent	p(FDR-corr)	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
774	0.05	4,270	-20	56	25	Frontal	Left Superior Frontal Gyrus	10
		3,087	-16	58	4	Frontal	Left Medial Frontal Gyrus	10
		2,943	-8	41	9	Frontal	Left Anterior Cingulate	32
698	0.05	3,589	-30	36	-20	Frontal	Left Middle Frontal Gyrus	11
		3,315	-48	32	-12	Frontal	Left Inferior Frontal Gyrus	47
		3,286	-55	24	6	Frontal	Left Inferior Frontal Gyrus	45

Figure Legend

Figure 1

Positive correlation between metabolism and *ApoE* genotypes. The clusters showing a statistically significant correlation are projected on brain surface. Top left: frontal view; top right posterior view; middle left: right view; middle right: left view; bottom left: view from below; bottom right view from above.