Author's response to reviews

Title: Plasma 24S-hydroxycholesterol levels in elderly subjects with late onset Alzheimer's disease or vascular dementia: a case-control study

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Author's response to reviews: see over
ANSWER TO THE EDITOR

1. Discussion: we modified the discussion following both the suggestions of the three Referees and of the Editor

2. apo E4: the Editor is right, apo E phenotype might influence plasma levels of 24S-OH-Cholesterol. We evaluated apo E genotype in DNA from 84 patients out of 120 (70%). We found no differences in mean/median 24S-OH-Chol levels when comparing patients bearing or not the ε4 allele (ANOVA / Mann-Whitney test p: 0.86 and 0.96, respectively. This has been added at the end of discussion.

3. criteria for CIND: we clearly specified the criteria we used for the definition of CIND in our study.

4. CSF biomarkers: although the recent guidelines by NIA and AA workgroup on AD (2011) suggest the use of CSF biomarkers for diagnosis of AD and AD-related MCI, in our study (2006-2009) we followed the current diagnostic criteria by NINCDS-ADRDA for LOAD, and NINDS-AIREN for VD. In the “oldest” guidelines, the use of CSF markers was not suggested. We clearly stated in the text that “No Cerebrospinal fluid (CSF) biomarkers were available for all the patients (LOAD, VD, and CIND) enrolled into this study”.

5. ANOVA: as suggested, we re-calculated the possible differences in plasma 24S-OH-Chol levels by ANOVA, after log-transformation of (not-normally distributed) 24S-OH-Chol values. Fisher’s least significant difference - LSD - was used for post-hoc test analysis.

6. the title was also modified according to the changes of the discussion
ANSWER TO REFEREE 1 (DR. LEONI)

1. CT scan and brain atrophy: effectively, this is an important limitation of the study, and this has been clearly stated at the end of discussion.
2. VD and brain atrophy: VD was not considered as a late stage of LOAD, and brain atrophy was not the main outcome of the study. However, data about qualitative evaluation of brain atrophy as well as other CT scan findings have been now reported in Table 1.
3. CSF markers: it has been clearly stated that CSF markers were not available for this study, and it has this has been included in the limitations of the study at the end of discussion.
4. Data about brain atrophy as well as other CT scan findings have been reported in Table 1 as suggested and compared by chi-square test (prevalence).
5. CIND: the definition of CIND was been reported in the methods section as suggested.
6. page 3 line 8: “degraded” has been changed with “eliminated”
7. the sentence “it has been previously reported that an increase…” has been changed with “it has been previously reported that a decrease …” following the indications of the Referee.
8. page 10 line 3: “older” has been changed in “aging” as suggested
9. page 11 line 1: this sentence has been eliminated
10. the sentence “however, our data support the concept…” has been changed as suggested by the Referee.
11. page 11: the sentence about CIND has been eliminated
12. “good” has been eliminated before “agreement” from the sentence as suggested
13. the sentence “third, it has been previously noted that …” has been removed as suggested by th Referee, since it is not supported by literature.
ANSWER TO REFEREE 2 (DR. WOLOZIN)

1. The study was essentially based on the evaluation of plasma 24S-OH-Chol levels in different groups of subjects, as reported in Table 1; data were compared by ANOVA. The 24S-OH-Chol/TC ratio has been eliminated from Table 1.

2. The ratio 24S-OH-Chol/TC was only used in order to test the correlations with other variables. This was based on the suggestions from a previous review by Björkhem and Meaney (Brain Cholesterol: Long secret life behind a barrier. Atheroscler Thromb Vasc Biol 2004; 24: 806-815). These Authors underlined that 24S-OH-Chol and TC plasma levels are significantly correlated in humans (in our sample r: 0.28, p: 0.005) since they are both transported by LDL particles. Of consequence, although the two pools of molecules are independently regulated, the levels of 24S-OH-Chol are significantly influenced by TC levels. Björkhem and Meaney clearly suggested that the 24S-OH-Chol/TC ratio may better reflect brain cholesterol homeostasis than 24S-OH-Chol absolute level.

3. Female gender: it has been reported that the prevalence of female gender was higher in LOAD and lower in VD; this is a typical finding in AD

4. page 12: the Referee is right: we changed “24S-OH-Chol/TC ratio” with 24S-OH-Chol
ANSWER TO REFEREE 3 (DR. ATTI)

1. ANOVA: first, the sentence in the abstract reported by the Referee was really wrong; indeed, the independent correlation between 24S-OH-Chol/TC ratio and plasma CRP was tested by multivariate regression analysis and not by ANOVA (this has been changed). Second, we eliminated the non-parametric analysis (Kruskal-Wallis) and re-calculated means differences by ANOVA, after log-transformation of 24S-OH-Chol values. The Fisher's least significant difference - LSD - was used as post-hoc test.

2. Possible impact of the findings: it is really difficult for us to evaluate the possible impact of our findings; we think our data could be interesting for the better understanding of cholesterol metabolism inside CNS

3. Definition of CIND: the criteria for the diagnosis of CIND have been clearly reported in methods, as suggested by the Referee

4. Definition of controls: we better specified the characteristics of controls

5. Time of the study: it has been reported in the Methods (2006-2009), as suggested

6. Sample: the whole sample was made by 160 individuals; of these, 120 (and not 115 – this has been corrected) were “patients” (i.e. LOAD, VD, and CIND evaluated in the Day Hospital) and 40 were controls. Furthermore, 104 healthy older subjects were “added” to controls for genetic analysis only (total: 144)

7. abstract: the word “in” has been added as suggested

8. page 7: “IS” has been eliminated since it was a mistake

9. Although we acknowledge the paragraph about 24S-OH-Chol measurement might be long, we think it is really necessary since the methodology is really complex. It might be transferred to an Appendix, if the Editor agrees