

## Chorein deficiency and Alzheimer's disease: an intriguing, yet premature speculation

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### To the Editor

Mutations in *VPS13A* cause the rare recessive neurodegenerative disease chorea-acanthocytosis (ChAc) and the VPS13A protein, chorein, is absent in most affected subjects<sup>1,2</sup>. However, little is known about the physiological role of chorein or the pathomechanism of ChAc. At least, previous claims as to possible dominant inheritance of ChAc were laid to rest and based on the currently published evidence it is not very likely that subjects with a mutation in only one of their *VPS13A* alleles show related clinical findings<sup>3</sup>, e.g. parents of ChAc patients typically are fully normal.

All the more interesting is the recent proposal by Lazarczyk et al.<sup>4</sup> of an association of “heterozygous *chorein* deficiency” and Alzheimer's disease (AD). Their patient, a female in her late 50s, had a clinical and neuroimaging pattern compatible with AD, including low CSF Aβ40. In addition, she, her daughter and one son suffered from “psychotic episodes”, which are not further characterized. Genetic studies were performed in the proband and her daughter, including copy number variation (CNV) analysis and exome-wide sequencing. A single *a priori* relevant heterozygous mutation was found, a deletion encompassing exons 70-73 of *VPS13A*. Searching for functional correlates, the authors studied SH-SY5Y cells after siRNA transfection, finding reduced *VPS13A* expression, as assessed by quantitative PCR, and low Aβ40 in culture supernatants.

In essence, Lazarczyk et al. report a heterozygous *VPS13A* mutation in a relatively young lady with findings suggestive of AD. Data on the family are very sparse but the mutation was also found in the daughter with “subjective memory impairment”. However, chorein levels were not analysed and neither “tau-negative Alzheimer's disease” nor a causal association between the two were convincingly demonstrated.

As to the label of “probable tau-negative”, also known as “plaque-only”, Alzheimer's disease, we are unaware of established or clinically applicable criteria to diagnose this entity in living subjects. Truly, the results of PiB-PET imaging argue for abnormal cortical amyloid deposition in the patient and the CSF markers appear compatible with low formation of tau positive tangles in her brain<sup>5</sup>, although there are reports on the contrary<sup>6</sup>. To the best of our

knowledge no consensus, however, exists with respect to diagnosing the neuropathological condition of “dementia of the Alzheimer type without neocortical neurofibrillary tangles”<sup>7</sup> in the clinic, let alone differentiating it into “probable” or into less or more distinct types of “tau-negative Alzheimer’s disease”.

The sparse data presented on the patient’s children are insufficient to diagnose a familial condition and, in particular, segregation of the mutation with clinical traits was not proven.

We have previously reported a ChAc pathogenic mutation including deletion of exons 70-73 of *VPS13A* plus exons 6-7 of *GNA14* (c.9189+8647\_oGNA14:c.723+897del), a founder mutation in French-Canadian population<sup>8</sup>. Whether exactly this “French-Canadian” mutation is present in the Brazilian proband of Lazarczyk et al. could be solved with a simple PCR. Should the mutation be different, and especially if ChAc was disproven (e.g. through normal chorein and CK levels, perhaps also acanthocyte quantification), a fine characterization of the breakpoint would be needed since the patient’s deletion may also involve further genes downstream of *VPS13A*. Furthermore, removal of *VPS13A* terminal exon will result in an mRNA including new spliced sequence at its 3’ end that could lead to a chimeric protein.

Thus, the patient’s deletion might be clinically relevant either because of effects on genes other than *VPS13A* or because of the formation of an abnormal protein that contains some chorein moieties.

Further, despite the claim as to functional effects that reduced levels of chorein may have, no evaluation of the proband’s chorein levels was done. Although the patient’s findings are rather atypical for ChAc, neither age of onset nor clinical presentation completely exclude it<sup>1</sup>. Absence of chorein would prove a diagnosis of ChAc, also arguing for a second mutation not detected by exon sequencing. Such scenarios would occur, for example, with activation of intronic cryptic splicing sites. Some clarification could also be provided by measurement of blood CK levels, typically elevated in ChAc.

The authors claim that reduction in chorein expression selectively decreases A $\beta$ 40 secretion. Their *in vitro* experiments may support this hypothesis, even if the variation shown in Fig.2F suggests a rather small difference from control cells. Taking into account that reduction of chorein levels was achieved by siRNA transfection and only checked by measuring mRNA levels but not actual protein levels, interpretation of those results should be done very carefully. More detailed analyses, using homogenous cultures of cells with known *VPS13A* mutations, would probably be required to properly evaluate the suggested role of chorein in amyloid processing.

To clinically test for an association of reduced A $\beta$ 40 CSF levels with *VPS13A* mutations one would need to screen patients with low A $\beta$ 40 CSF (typically Alzheimer’s cases) for *VPS13A* mutations and, *vice versa* measure CSF markers in ChAc patients. Circumstantial evidence against the association is provided by the high prevalence of AD and the absolute rarity of ChAc and by the lack of neuropathological Alzheimer changes in ChAc brains. In this light, the co-occurrence of AD and a *VPS13A* mutation in the patient of Lazarczyk et al. does not appear causally related.

Finally, we want to comment on the issue of “chorein hemizygosity”. The authors’ statement “chorein hemizygosity is not clinically silent, and reduction in chorein expression has a detrimental impact on different tissues” is extrapolated from one single family<sup>9</sup> but ignores the much higher number of reports to the contrary. The “siblings with heterozygous 495+1G>A mutation” and “cognitive impairment” of Connolly et al.<sup>10</sup> that are referred to for further support do actually weaken the argument. These patients cannot just have a single *VPS13A* mutation since a heavily reduced level of chorein in brain tissue was shown. While only a 495+1G>A mutation was found, it can be assumed that a second mutation would be detected using different screening approaches.

In summary, we argue that the association of AD and heterozygous *VPS13A* mutation that Lazarczyk et al. report may be purely fortuitous. Further analyses are needed but currently a speculation of causal association clearly appears premature.

## References

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