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### **Low Florbetapir PET Uptake and Normal A $\beta$ 1-42 Cerebrospinal Fluid in an APP Ala713Thr Mutation Carrier**

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**Abstract.** According to the literature, the *APP* Ala713Thr mutation is associated with Alzheimer’s amyloid angiopathy. We describe a case of dementia clinically compatible with frontotemporal dementia in a *APP* Ala713Thr mutation carrier in which both [<sup>18</sup>F]Florbetapir PET uptake and A $\beta$ <sub>1-42</sub> cerebrospinal fluid levels were low. Further evidences are required to establish if this association is only incidental.

**Keywords:** *APP* Ala713Thr, familial Alzheimer’s disease, florbetapir PET, frontotemporal dementia

## INTRODUCTION

The revised National Institute on Aging-Alzheimer’s Association (NIA-AA) diagnostic criteria for dementia due to Alzheimer’s disease (AD) encourage the use of biomarkers in order to improve AD cases identification [1], and according

to International Working Group-2 diagnostic criteria, the diagnosis of AD in the presence of a pathophysiological biomarker. The accuracy of amyloid positron emission tomography (PET) imaging in detecting amyloid plaques has been demonstrated [3], and Sorbi et al. found a correlation between amyloid PET and cerebrospinal fluid (CSF) A $\beta$ <sub>1-42</sub> levels and amyloid plaques in the brain [4] as well as between amyloid PET uptake [5]. In the presence of an AD causative mutation, the certainty that the dementia is caused by AD pathology is in-

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A 51-year-old Italian man was admitted to our clinic because of gradual change in behavior and personality, cognitive decline, and urinary incontinence; symptoms started one year before the first visit. Family history included a maternal aunt affected by unspecified early onset dementia (55 years) who died at the age of 75. No other cases of neurodegenerative/vascular dementia were known in the family. The mother died at 76 years because of renal failure in diabetes mellitus, while the father was affected by syphilis and died at the age of 78 years.

Information about health status of his cousins were not available. The patient underwent clinical-neuropsychological assessment and conventional brain magnetic resonance imaging (MRI); subsequently, since the diagnosis was uncertain, supplemental investigations on biomarkers were performed: brain [ $^{18}\text{F}$ ]fluorodeoxyglucose ([ $^{18}\text{F}$ ]FDG) PET, CSF analysis, genetic analysis for frontotemporal dementia (FTD), genetic analysis for FAD and lastly amyloid PET. All exams, except the amyloid PET, were carried out one year after the symptoms onset (baseline); neuropsychological evaluation was performed at baseline and at 6-month follow-up (Table 1), while amyloid PET was made 3 years after the symptoms onset.

The extensive neuropsychological evaluation was conducted with the Mini-Mental State Examination (MMSE) [10] and by tests assessing memory (Rey Auditory Verbal Learning Test [11], Babcock Story Recall Test [12], Digit Span Forward and Backward [13]), attention (Trail Making Test A and B [12]), language (Verbal Fluency [11, 14]), executive functions (Frontal Assessment Battery [15], Stroop Test [16]), visuospatial abilities (Rey-Osterrieth Complex Figure Test [17]), praxia (qualitative evaluation), mood

assessment.

With regards to the genetic analysis, DNA was extracted from peripheral blood leukocytes by standard procedures, by utilizing the QIAamp DNA kit (Qiagen, Hilden, Germany). Genetic analysis for FTD causative mutations (*MAPT*, *MAP2K*, *MAP3K*) and then for FAD causative mutations (*MAPT*, *MAP2K*, *MAP3K* and *APP*) were performed following standard procedures [23]. The Apolipoprotein E genotype was determined using PCR and HinfI digestion. The combination of the two single nucleotide polymorphisms (rs429358 and rs7412) on the *APOE* gene was determined by Lumbar puncture and CSF collection. The patient was found out according to good clinical practice. The CSF sample was analyzed for total-Tau, phosphorylated-Tau,  $\text{A}\beta_{1-42}$  by commercial enzyme-linked immunosorbent assay (innogenetics ELISA). The CSF values were: for total-Tau <300 pg/ml, for phosphorylated-Tau <60 pg/ml, and for  $\text{A}\beta_{1-42}$  >6 pg/ml.

## RESULTS

At neurological examination, signs of parkinsonism and left hand resting tremor were observed, without other extrapyramidal signs. Neuropsychological assessment showed significant apathy, memory impairment, reduced verbal initiative and verbal fluency, short-term memory impairment, slight executive functions, and functional decline (instrumental self-care deficit). The MMSE score was below reference norms (21), the Neuropsychiatric Inventory score was relatively high (28) because of delusions, severe apathy, motor rigidity, stereotyped behaviors, dietary changes, and daytime sleepiness. The clinical picture was suggestive of early onset dementia in the prodromal spectrum.

Trail Making Test	(55")	(50")
A		
B	73" (110")/1 Err	not evaluable
Stroop Test		
Time Interference Effect	17.5" (18)	not evaluable
Errors Interference Effect	(0)	not evaluable
Phonemic Word Fluency	6.5 (5)	11.5 (10)
Semantic Word Fluency	20.5 (19)	14.5 (13)
Neuropsychiatric Inventory	(28/144)	(40/144)
Activities of Daily Living Scale	(4/6 preserved)	(3/6 preserved)

Brain MRI showed diffuse fronto-temporo-parietal atrophy (Pasquier score was 14 and the most affected regions were the fronto-temporals) with bilateral involvement of hippocampus (Scheltens score was 3 on the right side, 2 on the left side), without evident vascular burden (Fazekas score was 1) (Fig. 1a). The MRI pattern was not suggestive for any specific dementia subtype.

Brain [<sup>18</sup>F]FDG PET scan (Fig. 1b) revealed severe bilateral hypometabolism in the anterior prefrontal, anterior cingulate, medial frontal, orbitofrontal cortices, in the hippocampi, amygdala, temporal pole, and caudate nuclei (mainly on the right side). Moderate bilateral hypometabolism in the insula and posterior cingulate cortex was also evident, whereas the metabolism in the posterolateral parietal cortex was preserved. This pattern of predominantly anterior hypometabolism has been interpreted as suggestive of neurodegenerative disease not in the AD spectrum but more likely in the FTD spectrum.

With regards to CSF biomarkers, A $\beta$ <sub>1-42</sub> and phospho-Tau were within the normal range (respectively, 771 pg/ml and 35 pg/ml), whereas total-Tau was slightly increased (354 pg/ml); according to current evidence, these values and their ratios were not suggestive of AD (total Tau/A $\beta$ <sub>1-42</sub> value (0.46) was not suggestive of AD profile according to Duits

et al. [25], phospho-Tau/A $\beta$ <sub>1-42</sub> not suggestive of AD profile according to et al. [26]).

Based on these results and on the diagnosis of behavioral FTD was

Genetic analysis for FTD causative mutations (*MAPT*, *GRN*, *C9orf72*) was negative. In the presence of early onset dementia, genetic causative mutations was also performed. The occurrence of an *APP* Ala713T heterozygous mutation in the presence of the genotype.

Lastly, amyloid imaging with PET was performed and no significant retention was found (Fig. 1c). The disease onset, the clinical picture (characterized by mutism and compulsive behavior, impairment of attention and executive function, gait, and dysautonomic dysfunction with hypersalivation).

According to all the data, an alternative diagnosis of "frontal variant AD", in the absence of a causative mutation and in the absence of CSF biomarkers, has been assumed. According to the non-genetic biomarkers profile, according to Rascovsky's criteria [27], a clinical picture of possible FTD, behavioral variant FTD, was postulated.

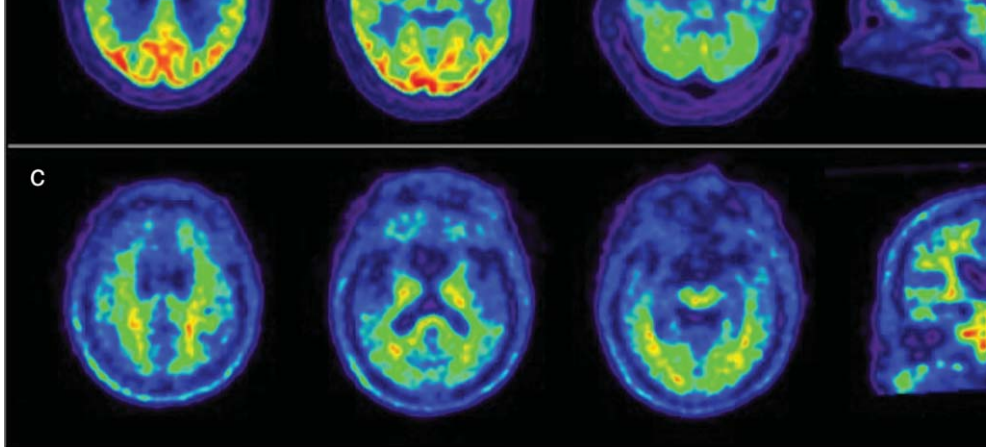


Fig. 1. Neuroimaging data a) MRI, axial T2-weighted fluid-attenuated inversion recovery images and coronal T2-weighted images, evidence of cortical and hippocampal atrophy; b) [ $^{18}\text{F}$ ]FDG-PET: severe hypometabolism in frontal and caudate nuclei; c) [ $^{18}\text{F}$ ]Florbetapir PET: no tracer retention in grey matter.

## DISCUSSION

The potential AD pathogenicity of *APP* Ala713Thr (g.275329G>A) variant is confirmed by segregation of the mutation with the disease [8, 9, 28, 29], absence of the mutation in control subjects [8, 9, 28], and results of bioinformatics analysis [30].

This variant is reported in mutation databases as pathogenic (<http://www.molgen.ua.ac.be/ADmutations> [31], <http://www.alzforum.org/mutations> [32], <http://www.hgmd.cf.ac.uk/> [33]); it has been described associated with autosomal dominant FAD, with both early and late onset, and cerebrovascular lesions. Despite these considerations, asymptomatic subjects of heterozygous carriers have been reported [8, 9]; since these cases were mainly younger than 65 years [8] or than the average age at onset of respective affected family members [9], they cannot be considered spared from the disease. Moreover, an

incomplete penetrance of the mutation was proposed in order to explain this phenotype. Genetic or environmental factors could be responsible for the expression/unexpression of the mutation [7].

In the present case, the genetic diagnosis contrasts with the lack of evidence of amyloid pathology assessed by both CSF analysis and amyloid imaging. Some cases of negative amyloid imaging have been reported in FAD in both Arctic APP carriers [34] and *APP* Glu693del mutation carriers, also in rare sporadic cases of disease. The absence of compound B PET ([ $^{11}\text{C}$ ]PiB PET) signal is unexpected cerebral amyloid burden.

PiB binding was found to be dependent on certain forms of A $\beta$ , such as A $\beta_{42}$  and A $\beta_{40}$ , or high-affinity binding sites which are not present in each amyloid plaque. The absence of [ $^{18}\text{F}$ ]Florbetapir uptake in the

variant (oligomeric toxicity without alteration in A $\beta$  ratio [37]), it is conceivable that CSF A $\beta$ <sub>1-42</sub> value remains in the normal range and that CSF A $\beta$ <sub>1-40</sub> (not evaluated in this case) decreases. This hypothesis is based on the evidence that the reduced A $\beta$ -peptides in CSF match the peptides accumulated in the amyloid plaques [38].

Instead of AD diagnosis, the co-occurrence of an altered A $\beta$  sequence with a FTD phenotype could suggest an association between *APP* Ala713Thr mutation and the FTD pathology. A causative role of *APP* Ala713Thr in pathology different from AD has not been previously supposed, whereas mutation located at the same codon (the *APP* Ala713Val) was found associated to schizophrenia with cognitive deficit [39], suggesting that mutations involving codon 713 can be pleiotropic.

In conclusion, we describe the first case of *APP* Ala713Thr mutation carrier associated to both low [<sup>18</sup>F]Florbetapir PET uptake and normal A $\beta$ <sub>1-42</sub> CSF. It remains to be clarified whether *APP* Ala713Thr mutation is linked to AD without *in vivo* evidence of A $\beta$  biomarkers or more likely to other pathology as FTD, and the debate is unsolvable without histopathological examination. Based on our findings, we suggest that in the occurrence of an AD causative mutation, more than one criterion is necessary for the *in vivo* evidence of AD pathology and that pathophysiological biomarkers should be studied even in carriers of a pathogenic mutation, in order to confirm the genetic diagnosis or, alternatively, to suppose other diagnosis and the relative pathogenic mechanism.

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