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CALHM1 P86L polymorphism does not alter amyloid-β or tau in cerebrospinal fluid

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ABSTRACT

Recently, the *P86L* alteration in *CALHM1* (calcium homeostasis modulator-1) was reported to be associated with Alzheimer's disease (AD). Moreover, the risk allele increased amyloid-β (Aβ) levels in conditioned media from cultured cells. Therefore, we hypothesized that *CALHM1 P86L* may modulate Aβ or tau levels in cerebrospinal fluid (CSF). Nearly 200 individuals with AD or other cognitive disorders were included for CSF analysis and *CALHM1* genotyping. No significant differences in CSF levels of Aβ42, tau or phospho-tau were found across the various *CALHM1* genotypes. In conclusion, we found no evidence that *CALHM1 P86L* is associated with altered CSF levels of the investigated AD biomarkers.

Alzheimer's disease (AD) is a genetically heterogeneous disorder believed to be initiated by the deposition of amyloid-β (Aβ) peptides in the brain. Apart from rare familial early-onset forms caused by mutations in the genes for amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) several risk genes have been suggested, of which only a fraction show consistent results upon meta-analysis [1]. Of these, only the apolipoprotein E (*APOE*) has been established to modulate the risk for AD. Carriers of one *APOE* ε4 allele have a 3–4 times increased disease risk whereas *APOE* ε4 homozygotes are 10–15 times more susceptible to AD [10].

The pathogenic mechanisms underlying the association between *APOE* ε4 and AD risk are only partly understood, but several lines of evidence suggest that the ε4 allele increases the deposition of Aβ, particularly of more aggregation-prone Aβ42 peptides. Consistent with this, AD patients with the *APOE* ε4 allele have increased levels of aggregated Aβ in brain (as shown by PET-PIB imaging) [6] along with decreased levels of Aβ42 in cerebrospinal fluid (CSF) [4,5,11,12]. The cause for decreased CSF Aβ42 in AD is not completely understood, although it has been assumed to reflect the increased deposition of Aβ plaques in the diseased brain.

Recently, a novel gene on chromosome 10 (10q24.33) was reported to modulate the risk for late-onset sporadic AD [3]. In that study, several independent case-control cohorts were genotyped for a Pro to Leu alteration at codon 86 (*P86L*; rs2986017) in the gene for calcium homeostasis modulator-1 (*CALHM1*), a transmembrane glycoprotein. Heterozygotes for the leucine-allele were found to have approximately 30% increased risk for AD, whereas homozygotes appeared to have an almost 80% risk increase [3] as compared to proline homozygotes. Much like most other proposed genetic associations in AD, the relevance of this potential risk effect remains to be investigated, as no association with disease risk was found in at least three independent follow-up studies [2,8,9]. According to

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Table 1
Cerebrospinal fluid (CSF) levels of Aβ42, total-tau (t-tau) and phospho-tau (p-tau) among Swedish and Finnish subjects with different *CALHM1 P86L* genotypes (rs2986017).

	rs2986017 genotype			Total
	CC	CT	TT	
Swedish				
n (%)	37 (55.2)	25 (37.3)	5 (7.5)	67
t-tau [ng/ml] (SD)	607.3 (440.8)	457.2 (219.3)	498.0 (182.0)	543.1 (361.6)
p-tau [ng/ml] (SD)	94.2 (61.1)	72.2 (33.5)	63.8 (17.5)	83.7 (51.0)
Aβ42 [ng/ml] (SD)	469.4 (242.3)	519.2 (240.2)	530.0 (218.2)	492.5 (237.8)
Finnish				
n (%)[a]	77 (64.7)	38 (31.9)	4 (3.4)	119
t-tau [ng/ml] (SD)	488.2 (253.5)	627.4 (395.5)	457.0 (0)	531.6 (310.7)
p-tau [ng/ml] (SD)	66.8 (21.7)	77.8 (45.2)	67.0 (0)	71.0 (31.5)
Aβ42 [ng/ml] (SD)	485.6 (191.7)	453.3 (152.3)	552.2 (124.5)	477.0 (177.5)

[a] Number of available samples for t-tau and p-tau measurements: CC = 28, CT = 16, and TT = 1.

the biochemical assessments, *CALHM1 P86L* risk allele was found to affect APP processing in a calcium-dependent fashion, and to increase Aβ levels in the conditioned media of cultured Chinese hamster ovary (CHO) cells [3]. This was attributed to an impairment of the normal function of CALHM1, which would result in an increase of extracellular levels of Aβ. Collectively, these data suggest that CALHM1 may act as an important regulator of Aβ metabolism also in the human brain.

In this study, we investigated whether or not the proposed *CALHM1 P86L* risk allele affects CSF levels of Aβ42, total tau and phospho-tau in patients with AD or other cognitive conditions. CSF from a total of 186 individuals was analyzed and all cases were

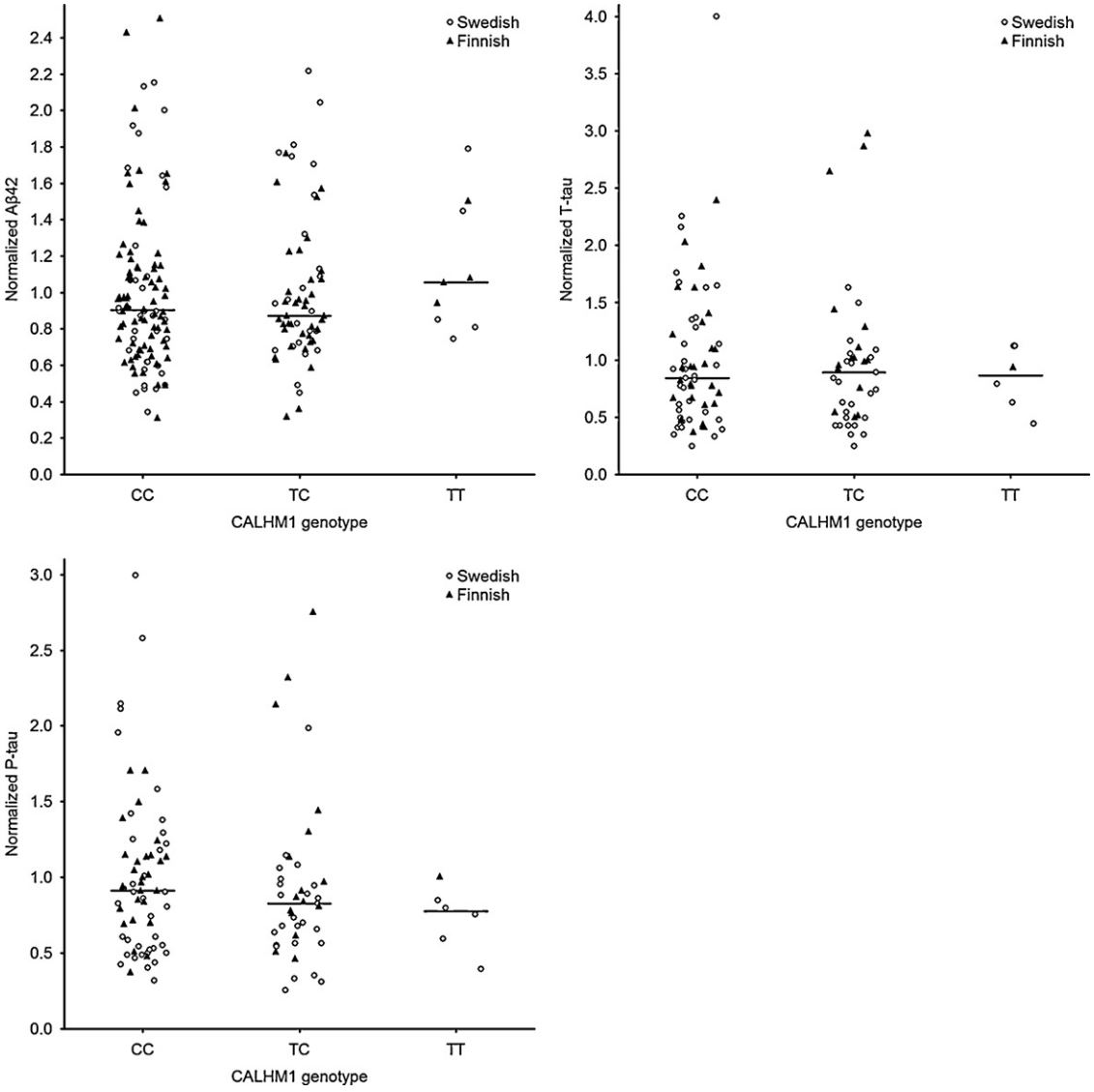

Fig. 1. Normalized CSF levels of Aβ42, total tau and p-tau with respect to the *CALHM1 P86L* genotypes in Swedish and Finnish sample sets. Horizontal line shows median level of the combined sample set.

genotyped for *CALHM1 P86L*, allowing us to investigate the influence of *CALHM1 P86L* on CSF biomarker levels.

A total of 70 (age ± SD 66.8 ± 8.2 years, 48% females) Swedish cases, recruited from the Memory Disorder Unit at Uppsala University Hospital, underwent lumbar puncture as part of clinical dementia investigation. The *CALHM1* genotype was successfully determined on 67 of the cases. Of these, 18 patients were diagnosed with probable AD based on NINCDS-ADRDA criteria [7]. The remaining 49 patients were diagnosed with other cognitive diagnoses, such as mild cognitive impairment ($n = 34$), non-specified dementia ($n = 1$), subjective memory disturbance ($n = 10$), and frontotemporal dementia ($n = 4$). Moreover, 119 AD cases (age-at-onset 70.5 ± 7.1 years, 72% females) from eastern Finland underwent lumbar puncture as part of a dementia investigation at the Department of Neurology of Kuopio University Hospital. All patients fulfilled the NINCDS-ADRDA criteria for probable AD [7]. Informed consent was obtained from all Swedish and Finnish subjects and the study was approved by the Regional Ethical Committee in Uppsala and The Ethics Committee of Kuopio University Hospital/Kuopio University, respectively.

Genotypes for the *CALHM1 P86L* (rs2986017) variant were generated as previously described [2], either by single-base extension followed by high-efficiency fluorescence polarization (HEFP) detection, or by direct sequencing. Genotyping efficiency was 96%, and the error rate below 0.2%. The Swedish and Finnish CSF samples were analyzed for Aβ42 (INNOTEST® ß-AMYLOID$_{(1-42)}$, Innogenetics, Ghent, Belgium). Moreover, the samples were also analyzed for total tau (t-tau, INNOTEST hTAU Ag, Innogenetics), and tau phosphorylated at Thr^{181} (p-tau, INNOTEST PHOSPHO-TAU$_{(181P)}$, Innogenetics).

Kruskal–Wallis ANOVA followed by a Mann–Whitney *U* test was used to investigate possible differences in CSF Aβ42, tau or p-tau between subjects with the different *CALHM1* genotypes. In order to combine data from Swedish and Finnish samples, marker levels were normalized based on the mean concentration of each biomarker in the CC genotype carriers. Power analysis indicated a power of 0.84 to detect a 1.2-fold increase in Aβ42 levels between the *CALHM1* CC and CT genotypes. However, due to the lower number of samples and higher variation power to detect 1.2-fold increase in tau and p-tau was only 0.36 and 0.47, respectively. All statistical analyses were performed by the Statistica software (StatSoft Inc. Tulsa, OK, USA).

When analyzing the Swedish and Finnish cohorts separately, CSF levels of Aβ42, total tau, and p-tau did not differ with respect to the *CALHM1* genotype (Table 1). Although absolute CSF Aβ42, tau, and p-tau concentrations were very similar between the Swedish and Finnish groups (Fig. 1), their respective levels were normalized within each cohort, allowing a combined analyses of samples. When analyzing the combined Swedish–Finnish sample set, no significant differences were detected between heterozygous and homozygous carriers of the "risk allele" (leucine or T) as compared to subjects homozygous for the "wild-type" (proline or C) allele. Finally, no differences were found when analyzing the AD and non-AD subgroups separately.

Based on recently described findings [3], we investigated whether CSF levels of Aβ42 are affected between carriers and non-carriers of the proposed AD risk allele (*P86L*) in *CALHM1*. In addition, we also analyzed total and phosphorylated tau levels, since both of these biomarkers are known to negatively correlate with CSF Aβ levels. However, the results should be interpreted cautiously, because of the low sample numbers and high inter-individual variations in biomarker levels. When analyzing CSF Aβ42, t-tau and p-tau from Swedish and Finnish patients, we did not detect any significant differences with respect to the *CALHM1* genotype. Furthermore, separate subgroup analyses on AD ($n = 18$) and non-AD ($n = 48$) cases in the Swedish cohort also failed to show any correlation between the *CALHM1* genotype status and the investigated CSF biomarkers. In conclusion, we did not find any evidence that the *CALHM1 P86L* variation affects levels of Aβ and tau in CSF from cases with AD or other cognitive disorders.

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