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Increased amyloidogenic APP processing in *APOE* ϵ 4-negative individuals with cerebral β -amyloidosis

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Increased APP (amyloid precursor protein) processing causes β -amyloid ($A\beta$) accumulation in autosomal dominant Alzheimer's disease (AD), but it is unclear if it also affects sporadic $A\beta$ accumulation. We tested healthy controls and patients with mild cognitive symptoms ($N=331$) in the BioFINDER study, using cerebrospinal fluid (CSF) $A\beta$ 40 as a surrogate for amyloidogenic APP processing. We find that levels of brain $A\beta$ fibrils (measured by 18F-flutemetamol PET) are independently associated with high CSF $A\beta$ 40 ($P<0.001$) and *APOE* ϵ 4 ($P<0.001$). The association between CSF $A\beta$ 40 and brain $A\beta$ is stronger in *APOE* ϵ 4-negative than in positive people ($P=0.0080$). The results are similar for CSF $A\beta$ 38 and for a combination of CSF $A\beta$ 38 and CSF $A\beta$ 40. In conclusion, sporadic $A\beta$ accumulation may be partly associated with increased amyloidogenic APP production, especially in *APOE* ϵ 4-negative subjects. The risk for sporadic AD may consequently depend on increased $A\beta$ production, in addition to decreased $A\beta$ clearance.

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Brain accumulation of amyloid β ($A\beta$) is a hallmark of Alzheimer's disease (AD) which may precede dementia by up to two decades^{1–3} and be quantified by cerebrospinal fluid (CSF) biomarkers or positron emission tomography (PET) imaging^{4,5}. $A\beta$ accumulation is thought to be caused by an imbalance of $A\beta$ production and clearance from the brain⁶. The *APOE* $\epsilon 4$ allele is the main genetic susceptibility factor for late-onset AD and sporadic $A\beta$ pathology⁷. This is likely because the *APOE* $\epsilon 4$ gene product apoE4 has reduced capacity to clear $A\beta$ peptides from the brain⁸. However, $A\beta$ accumulation also occurs in the absence of *APOE* $\epsilon 4$ (ref. 7) and ~40–50% of AD patients lack the *APOE* $\epsilon 4$ allele⁹. In autosomal dominant forms of AD, $A\beta$ pathology is believed to be caused by increased amyloidogenic processing of APP (amyloid precursor protein), that is, increased $A\beta$ production¹⁰ but variations in APP processing have not been thoroughly explored as risk factors in 'sporadic' AD. Using a large cohort of non-demented subjects, the aim of this study was to test if *APOE* $\epsilon 4$ and biomarker surrogates of amyloidogenic APP processing were independently associated with brain $A\beta$ accumulation. We used CSF levels of $A\beta 40$ to estimate amyloidogenic APP processing. The rationale for this was that $A\beta 40$ is a major $A\beta$ isoforms produced by neurons by concerted β - and γ -secretase cleavages of APP (the same processing pathway that results in $A\beta 42$)¹¹ but is generally not related to $A\beta$ plaque pathology (in contrast to CSF $A\beta 42$, which is reduced in the presence of $A\beta$ plaques¹²). Note that previous studies testing the correlation between CSF $A\beta 40$ and PET $A\beta$ have not co-varied for the presence of *APOE* $\epsilon 4$. We hypothesized that there would be independent correlations between $A\beta$ accumulation and the predictors *APOE* $\epsilon 4$ and CSF $A\beta 40$, and that increased amyloidogenic APP processing would be related to $A\beta$ accumulation mainly in *APOE* $\epsilon 4$ -negative subjects. We also hypothesized that CSF $A\beta 40$ would not be associated with *APOE* $\epsilon 4$ (that is, CSF $A\beta 40$ would not be affected by apoE4-mediated impaired $A\beta$ clearance). Finally, we hypothesized to see similar results when using CSF $A\beta 38$ instead of CSF $A\beta 40$ to estimate amyloidogenic APP processing.

Our results confirmed our hypothesis. We show that 18F-flutemetamol PET levels are independently associated with high CSF $A\beta 40$ ($P < 0.001$) and *APOE* $\epsilon 4$ ($P < 0.001$) and that the association between CSF $A\beta 40$ and brain $A\beta$ is stronger in *APOE* $\epsilon 4$ -negative than in positive people ($P = 0.0080$). The results are similar when using CSF $A\beta 38$ or a combination of CSF $A\beta 38$ and CSF $A\beta 40$ to estimate amyloidogenic APP production. We conclude that sporadic $A\beta$ accumulation may be

partly associated with increased amyloidogenic APP production, especially in *APOE* $\epsilon 4$ -negative subjects. Thus, the risk for sporadic AD may partly depend on increased $A\beta$ production, in addition to decreased $A\beta$ clearance.

Results

Cohort characteristics. The cohort consisted of 331 participants (cognitively normal controls (CN) 121, subjective cognitive decline (SCD) 102 and mild cognitive impairment (MCI) 108). Demographics and data on cognition and biomarkers are summarized in Table 1 (see Table 2 for demographics stratified by *APOE* status). In sum, *APOE* $\epsilon 4$ positivity was more common in SCD and MCI than in CN, CSF $A\beta 42$ levels were lower in MCI compared with the other groups, and the frequency of PET $A\beta$ positivity was lowest in CN and highest in MCI. CSF $A\beta 38$ and CSF $A\beta 40$ did not differ between the diagnostic groups. *APOE* $\epsilon 4$ was not associated with CSF $A\beta 40$ or with CSF $A\beta 38$ (Fig. 1). The lack of association between *APOE* $\epsilon 4$ and CSF $A\beta 40$ and CSF $A\beta 38$ supports our assumption that these CSF $A\beta$ peptides are unaffected by apoE4-mediated clearance of $A\beta$.

***APOE* $\epsilon 4$ and high CSF $A\beta 40$ independently predict PET $A\beta$.**

Figure 2 shows the observed PET $A\beta$ and CSF $A\beta 40$ data, with estimated slopes in the *APOE* $\epsilon 4$ -positive and -negative groups. In a linear regression model with PET $A\beta$ as the dependent variable, high levels of CSF $A\beta 40$ ($\beta = 1.05 \times 10^{-4}$, $P < 0.001$), *APOE* $\epsilon 4$ -positivity ($\beta = 0.406$, $P < 0.001$) and the interaction between *APOE* $\epsilon 4$ and CSF $A\beta 40$ ($\beta = -5.61 \times 10^{-5}$, $P = 0.0080$) were all significant predictors of continuous PET $A\beta$. Note that since *APOE* $\epsilon 4$ and CSF $A\beta 40$ were both included as predictors the main effect of CSF $A\beta 40$ indicates the effect within *APOE* $\epsilon 4$ -negative subjects. The significant interaction between CSF $A\beta 40$ and *APOE* $\epsilon 4$ indicates that the correlation between CSF $A\beta 40$ and brain $A\beta$ was stronger in *APOE* $\epsilon 4$ -negative than in positive people (as seen in Fig. 2). The correlation between CSF $A\beta 40$ and PET $A\beta$ in the *APOE* $\epsilon 4$ -positive group was weaker than in the *APOE* $\epsilon 4$ -negative group, but remained significant ($\beta = 0.485 \times 10^{-4}$, $P = 0.010$). The results support the hypotheses that high CSF $A\beta 40$ and *APOE* $\epsilon 4$ are independent predictors of PET $A\beta$, and that the relationship between CSF $A\beta 40$ and PET $A\beta$ varies with *APOE* $\epsilon 4$ carrier status. As expected, CSF $A\beta 42$ was a significant covariate (low CSF $A\beta 42$ was correlated with PET $A\beta$, $\beta = -0.00120$, $P < 0.001$), but CSF $A\beta 40$, *APOE* $\epsilon 4$ and the interaction between CSF $A\beta 40$ and

Table 1 Demographics.					
	CN	SCD	MCI	All	P value
N	121	102	108	331	
Age (y)	73.7 (4.5)	70.2 (5.6)	71.2 (5.6)	71.8 (5.4)	<0.001
Sex (F)	63%	51%	40%	52%	0.0023
Education (y)	11.5 (3.28)	12.7 (3.28)	11.3 (3.38)	11.8 (3.39)	0.0038
MMSE (points)	29.0 (0.92)	28.5 (1.47)	27.2 (1.69)	28.3 (1.57)	<0.001
ADAS-cog, delayed recall (points)	2.1 (2.0)	3.3 (2.2)	6.4 (2.2)	3.9 (2.8)	<0.001
<i>APOE</i> $\epsilon 4$ (%) +	28%	42%	43%	37%	0.028
PET $A\beta$ (%) +	19%	37%	61%	38%	<0.001
PET $A\beta$ (SUVR)	1.30 (0.28)	1.41 (0.38)	1.70 (0.53)	1.46 (0.44)	<0.001
CSF $A\beta 38$ (ng l ⁻¹)	1,742 (404)	1,722 (421)	1,686 (421)	1,718 (414)	0.51
CSF $A\beta 40$ (ng l ⁻¹)	4,510 (1526)	4,893 (1852)	4,812 (1809)	4,727 (1728)	0.35
CSF $A\beta 42$ (ng l ⁻¹)	538 (187)	584 (251)	475 (216)	532 (221)	0.0031

ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PET, positron emission tomography; SCD, subjective cognitive decline; SUVR, standardized uptake value ratio. Continuous data are mean (s.d.). *APOE* $\epsilon 4$ + is defined as at least one $\epsilon 4$ allele. PET $A\beta$ positivity is defined as >1.42 SUVR (ref. 13). MMSE ranges from 0 to 30. ADAS-cog delayed recall ranges from 0 to 10 (word list learning test from the ADAS-cog battery, points indicate number of missed items). P values are for comparisons between diagnostic groups (using Kruskal-Wallis test for continuous variables and χ^2 -test for categorical variables).

Table 2 | Demographics by diagnostic group and APOE ε4.

N	CN		SCD		MCI		All	
	121		102		108		331	
	APOE ε4 –	APOE ε4 +	APOE ε4 –	APOE ε4 +	APOE ε4 –	APOE ε4 +	APOE ε4 –	APOE ε4 +
	87	34	59	43	61	47	207	124
Age (y)	73.5 (4.4)	74.1 (4.7)	70.1 (6.0)	70.3 (5.0)	71.0 (6.1)	71.5 (5.0)	71.8 (5.6)	71.8 (5.1)
Sex (F)	63%	62%	53%	49%	41%	38%	54%	48%
Education (y)	11.6 (3.3)	11.5 (3.3)	12.9 (3.5)	12.4 (2.9)	11.3 (3.4)	11.3 (3.3)	11.9 (3.5)	11.7 (3.2)
MMSE (points)	29.0 (0.97)	29.2 (0.78)	28.4 (1.55)	28.6 (1.35)	27.2 (1.75)	27.2 (1.62)	28.3 (1.58)	28.2 (1.57)
ADAS-cog, delayed recall (points)	2.2 (2.1)	1.7 (1.9)	3.2 (2.3)	3.5 (2.0)	6.0 (2.3)	6.9 (2.0)	3.6 (2.7)	4.3 (2.9)
PET Aβ (% +)	10%	41%	20%	61%	38%	91%	21%	67%
PET Aβ (SUVR)	1.22 (0.16)	1.48 (0.41)	1.27 (0.29)	1.59 (0.42)	1.51 (0.51)	1.95 (0.45)	1.32 (0.35)	1.70 (0.47)
CSF Aβ38 (ng l ⁻¹)	1,741 (420)	1,742 (365)	1,735 (457)	1,705 (367)	1,670 (436)	1,707 (405)	1,719 (435)	1,716 (380)
CSF Aβ40 (ng l ⁻¹)	4,573 (1,655)	4,348 (1,135)	5,076 (2,051)	4,643 (1,527)	4,792 (1,969)	4,838 (1,599)	4,781 (1,871)	4,636 (1,461)
CSF Aβ42 (ng l ⁻¹)	571 (179)	454 (181)	673 (240)	462 (213)	559 (211)	365 (168)	596 (212)	423 (192)

ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PET, or positron emission tomography; SCD, subjective cognitive decline; SUVR, standardized uptake value ratio.
Continuous data are mean (s.d.). APOE ε4 + is defined as at least one ε4 allele. PET Aβ positivity is defined as >1.42 SUVR (ref. 13).

APOE ε4 remained significant also when not adjusting for CSF Aβ42 (CSF Aβ40: $P=0.0089$; APOE ε4: $P<0.001$; interaction: $P=0.026$). Age ($\beta=0.0082$, $P=0.0094$) and diagnosis (SCD, $\beta=0.137$, $P=0.0011$; MCI, $\beta=0.299$, $P<0.001$) were also significant predictors of PET Aβ, but sex was not ($P=0.23$). White matter lesions (WML) were evaluated as a covariate, but were not significant ($P=0.68$) and were therefore excluded from the final model. We also evaluated plasma levels of Aβ40 as a covariate to exclude the possibility that the results depended on peripheral APP processing. Plasma Aβ40 was not a significant covariate ($P=0.99$) and including it in the model did not change the other estimates.

To further examine if clinically significant Aβ accumulation (defined as a composite standardized uptake value ratio (SUVR) >1.42 (ref. 13) was associated with CSF Aβ40, we evaluated a logistic regression model with PET Aβ positivity as the dependent variable. CSF Aβ40 (log odds = 9.18×10^{-4} , $P<0.001$) was a significant predictor in this model but APOE ε4 ($P=0.13$), and the interaction between CSF Aβ40 and APOE ε4 were not ($P=0.73$). Age and sex ($P=0.13$ – 0.14) were not significant but CSF Aβ42 ($P<0.001$) and diagnosis (SCD, $P=0.0016$; MCI, $P<0.001$) remained significant covariates. When CSF Aβ42 was excluded from the model, CSF Aβ40 ($P=0.014$) and APOE ε4 ($P<0.001$) were both significant predictors of PET Aβ positivity.

CSF Aβ38 as an independent predictor of PET Aβ. To corroborate our findings, we repeated the analyses using CSF Aβ38 instead of CSF Aβ40, with very similar results. When predicting continuous PET Aβ, the effects of CSF Aβ38 ($\beta=4.04 \times 10^{-4}$, $P<0.001$), APOE ε4 ($\beta=0.72$, $P<0.001$) and the interaction between APOE ε4 and CSF Aβ38 ($\beta=-3.40 \times 10^{-4}$, $P<0.001$) were all significant, and CSF Aβ42 was a significant covariate ($\beta=-0.00112$, $P<0.001$). CSF Aβ38 ($P=0.017$), APOE ε4 ($P<0.001$) and the interaction between APOE ε4 and CSF Aβ38 ($P=0.015$) remained significant when removing CSF Aβ42 from the model. When predicting PET Aβ positivity using logistic regression, CSF Aβ38 (log odds = 0.00332, $P<0.001$) and APOE ε4 (log odds = 4.47, $P=0.0075$) were independent predictors and there was a tendency for significant interaction between APOE ε4 and CSF Aβ38 ($P=0.061$). Again, CSF Aβ42 was a significant covariate (log odds = -0.0119 , $P<0.001$).

We also used a combination of CSF Aβ40 and CSF Aβ38 based on their molar amounts (CSF Aβ, mol l⁻¹). Again, the results were very similar. When predicting continuous PET Aβ, the effects of CSF Aβ ($\beta=3.70 \times 10^8$, $P<0.001$), APOE ε4 ($\beta=0.470$, $P<0.001$) and the interaction between APOE ε4 and CSF Aβ ($\beta=-2.20 \times 10^8$, $P=0.0030$) were all significant predictors. CSF Aβ42 remained a significant covariate ($\beta=-0.00120$, $P<0.001$). CSF Aβ ($P=0.0090$), APOE ε4 ($P<0.001$) and the interaction between APOE ε4 and CSF Aβ ($P=0.021$) remained significant when removing CSF Aβ42 from the model. When predicting PET Aβ positivity using logistic regression, CSF Aβ (log odds = 3.15×10^9 , $P<0.001$) was a significant predictor and CSF Aβ42 remained a significant covariate (log odds = -0.0129 , $P<0.001$).

CSF Aβ40 is highest in APOE ε4 – PET Aβ + subjects. In a linear regression model with CSF Aβ40 as the dependent variable and a four level combination of PET Aβ and APOE as the independent variable, the overall highest CSF Aβ40 levels were seen in the PET Aβ + & APOE ε4 – group ($\beta=732$, $P=0.015$, compared with the reference category PET Aβ – & APOE ε4 –, Fig. 3). PET Aβ + & APOE ε4 – subjects had 19% higher mean level of CSF Aβ40 (and 26% higher median level) compared with PET Aβ – & APOE ε4 – subjects. The model was adjusted for WML ($\beta=-18.8$, $P=0.00013$), age ($\beta=46.1$, $P=0.015$), sex (female sex, $\beta=-322$, $P=0.094$) and diagnostic group (SCD, $\beta=570$, $P=0.019$; MCI, $\beta=421$, $P=0.11$). When also adjusting for CSF Aβ42 as a covariate the effect of PET Aβ & APOE ε4 was even stronger, with higher CSF Aβ40 in the PET Aβ + & APOE ε4 – group compared with PET Aβ – & APOE ε4 – ($P<0.001$) and PET Aβ – & APOE ε4 + ($P<0.001$) but no significant difference compared with the PET Aβ + & APOE ε4 + group ($P=0.45$).

Discussion

We tested the hypothesis that biomarker surrogates of amyloidogenic APP processing (CSF Aβ40 and Aβ38) and APOE ε4 were independent predictors of brain Aβ fibril accumulation. In accordance with our hypotheses, CSF Aβ40 (and Aβ38 in a secondary analysis) and APOE ε4 were independent predictors of PET Aβ, and the effect of CSF Aβ40 was strongest in the APOE ε4-negative individuals. To our knowledge, this is the first study showing that increased Aβ production are associated with

Figure 1 | CSF Aβ38 and Aβ40 in APOE ε4- and APOE ε4+ subjects.

Observed data for CSF Aβ38 and CSF Aβ40 by APOE ε4 status. The individual observations are overlaid on boxplots (thick lines are medians, box limits are 25th and 75th percentiles). APOE ε4 did not affect levels of CSF Aβ38 (Mann-Whitney U-test, $P=0.75$; t-test, $P=0.95$; linear regression adjusted for age, sex and diagnosis, $P=0.99$) or CSF Aβ40 (Mann-Whitney U-test, $P=0.84$; t-test, $P=0.43$; linear regression adjusted for age, sex and diagnosis, $P=0.26$). This supports the notion that CSF Aβ38 and Aβ40 are unaffected by APOE ε4-mediated changes in Aβ clearance.

increased risk for sporadic brain Aβ accumulation. These novel results provide indirect evidence that brain Aβ pathology in humans may arise from two pathways, where one involves the APOE ε4 allele (likely causing reduced apoE4-mediated Aβ42 clearance), and the other involves increased amyloidogenic processing of APP. This may correspond to two pathways to sporadic AD, namely reduced clearance and increased production of Aβ peptides.

The amyloid cascade hypothesis postulates that Aβ pathology arises due to an imbalance between Aβ production and clearance⁶. It has been suggested that sporadic AD is mainly caused by poor clearance of peptides from the brain, whereas autosomal dominant AD is mainly caused by increased Aβ production, especially the Aβ42 variant. This is supported by a metabolic labelling study showing reduced Aβ clearance in sporadic AD dementia¹⁴, and studies showing increased amyloidogenic APP processing in early stages of autosomal dominant AD¹⁰. The main cause of reduced Aβ clearance in sporadic AD is likely APOE ε4, since the apoE4 protein isoform has reduced capacity to clear Aβ peptides compared with other apoE isoforms⁸, although it is possible that APOE ε4 may also contribute to increased AD risk by other mechanisms, for

Figure 2 | PET Aβ as a function of CSF Aβ40 and APOE ε4. Observed PET and CSF Aβ40 data. Slopes are modelled from a linear regression adjusted for CSF Aβ42, sex, age and diagnostic group. The shaded areas indicate 95% confidence intervals for the slopes. The dotted line indicates a cutoff for clinically significant PET Aβ load (1.42 SUVR). β-coefficients (divided by 10^{-4}) and P values for the slopes within APOE ε4-positive and separately for APOE ε4-negative subjects are shown in the legend. The interaction between CSF Aβ40 and APOE ε4 is significant, indicating that the correlation between CSF Aβ40 and PET Aβ differs by APOE ε4 status ($P=0.0080$). The results did not change significantly when removing outliers ($\text{CSF A}\beta 40 > 10,000 \text{ ng l}^{-1}$).

example, by affecting inflammation and neuronal repair^{15,16}. However, one APP gene polymorphism which reduces Aβ production is associated with reduced risk of AD in the general population¹⁷, which provides genetic evidence that variations in APP processing may also affect the risk for sporadic AD.

The main limitation of this paper was that we used an indirect measure of APP processing, which was estimated by CSF Aβ40. The rationale for this approach was that Aβ40 is a major Aβ isoform produced by neurons¹¹, which is not directly influenced by the presence of Aβ plaque pathology¹², and is not influenced by APOE ε4-mediated impaired Aβ clearance. The later was demonstrated by our finding that there was no overall difference in CSF Aβ40 depending on APOE ε4 status (Fig. 1). Alterations in CSF Aβ40 are therefore more likely to reflect differences in amyloidogenic APP processing rather than differences in Aβ clearance. However, we acknowledge that there may be variations in APP processing that are not captured by CSF Aβ40. We also performed analyses using CSF Aβ38 (another highly expressed Aβ isoform) and a combination of CSF Aβ38 and CSF Aβ40 (based on their molar amounts), with very similar results as when using CSF Aβ40 alone, which support our findings. A more direct estimate of Aβ production may be done by metabolic labelling¹⁴, but such methods are liable to bias due to the longitudinal drift of CSF biomarkers during continuous CSF sampling that depends on sampling frequency and volume¹⁸. Another limitation is that there may be other factors affecting CSF Aβ40 besides variations in APP processing. For example, reduced CSF Aβ40 is associated with chronic WML¹⁹, and WML may also be associated with Aβ pathology (although this is more common in MCI²⁰ and AD dementia²⁰ than in non-demented people²¹). It is not clear if the association between CSF Aβ40 and WML is due to a direct link between Aβ production and WML or if lower CSF Aβ40 levels reflect reduced neuronal Aβ secretion due to decreased brain activity in the presence of WML. Importantly, our results remained significant when adjusting for WML. Theoretically, CSF Aβ40 could also be influenced by peripheral APP processing,

Figure 3 | CSF A β 40 in different combinations of PET A β and APOE ϵ 4.

Observed CSF A β 40 for different combinations of APOE ϵ 4 and PET A β positivity and negativity. The individual observations are overlaid on boxplots (thick lines are medians, box limits are 25th and 75th percentiles). CSF A β 40 was significantly increased in the PET A β + & APOE ϵ 4 – group compared with PET A β – & APOE ϵ 4 –, which was the reference category ($P = 0.015$, using linear regression adjusted for age, sex, diagnosis and WML). No other group had significant different CSF A β 40 compared with the PET A β – & APOE ϵ 4 – group ($P = 0.62$ – 0.90). The groups were PET A β – & APOE ϵ 4 –, $N = 158$; PET A β – & APOE ϵ 4 +, $N = 39$; PET A β + & APOE ϵ 4 –, $N = 41$; and PET A β + & APOE ϵ 4 +, $N = 75$. The total $N = 313$ for this analysis was smaller than the total study population ($N = 331$) due to missing data for WML in 18 subjects (but the main results did not differ when WML was not included and the analysis was done on the whole study population). One data point is excluded from the graph for visual clarity (CSF A β 40 14110 ng l $^{-1}$, PET A β + & APOE ϵ 4 –).

but our results were stable when adjusting for plasma A β 40, suggesting that the effects did not depend on peripheral APP processing. We did not measure all other possible factors besides increased amyloidogenic APP that may contribute to A β deposition in APOE ϵ 4-negative subjects. For example, other AD risk genes (including *CLU* and *CRI*) may impact A β clearance in APOE ϵ 4-negative subjects²². We included several different diagnostic groups, including a SCD group. We noted that the frequency of A β positivity in our SCD subjects (37%) was higher than in a recent large meta-analysis by Jansen *et al.*³ where ~22% of SCD subjects were A β -positive, compared with ~25% of CN subjects. The reason for this difference is not clear, but we noted that our SCD subjects were on average 6 years older than the Jansen subjects, which may contribute to higher frequency of A β pathology. Furthermore, all our SCD subjects were referred to specialized memory clinics because of cognitive symptoms, while some of the Jansen SCD subjects may have been seen at other health care facilities, opening for the possibility that they had less severe complaints than the SCD subjects in our study. Another recent study on PET A β positivity in memory clinic SCD subjects found that 57% of SCD subjects were A β -positive compared with 31% of CN, which more resembles the findings in our cohort²³. Finally, we did not include an AD dementia group, since we know from a previous study that patients with severe AD dementia have lower CSF A β 40 than patients with mild dementia (this may reflect reduced capacity to

produce A β peptides as the disease progresses)²⁴. Including an AD dementia group in this study would therefore risk confounding the relationship between CSF A β 40 and PET A β .

Until now, there have been few attempts to examine the independent roles of APOE ϵ 4 and APP processing in the development of brain A β pathology in non-demented subjects. Previous studies did not find correlations between CSF A β 40 (or A β 38) and PET A β (ref. 12). This is likely because they did not covary for APOE ϵ 4 (and/or CSF A β 42). Adjusting for APOE ϵ 4 is important since the relationship between CSF A β 40 and PET A β differs between APOE ϵ 4-positive and -negative individuals. Furthermore, adjusting for APOE ϵ 4 and CSF A β 42 reduces the residual errors of the models, and some of this error may contribute to the variance of CSF A β 40. Once this error is removed the correlation between CSF A β 40 and PET A β can be better estimated. Our results add novel information and point to different possible pathways to A β pathology in humans. In sum, our results support the idea that sporadic A β accumulation may be partly associated with increased amyloidogenic APP production, especially in APOE ϵ 4-negative subjects. The risk for sporadic AD may consequently depend on increased A β production, in addition to decreased A β clearance. This provides novel insight into disease mechanisms in AD and may be important for development of drugs targeting A β metabolism in early stages of AD.

Methods

Study population. The study population came from the Swedish BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably). All available CN and non-demented patients with mild cognitive symptoms characterized as having SCD or MCI were included.

CN subject were originally enrolled from the population-based EPIC cohort. The inclusion criteria were: age ≥ 60 years old, MMSE 28–30, and fluent in Swedish. Exclusion criteria were: presence of subjective cognitive impairment, significant neurologic disease (for example, stroke, Parkinson's disease, multiple sclerosis), severe psychiatric disease (for example, severe depression or psychotic syndromes), dementia or MCI. All CN subjects underwent a thorough clinical assessment, including neurological, psychiatric and cognitive testing all performed by a medical doctor, in addition to MRI of the brain and relevant blood tests. The cognitive battery included MMSE, ADAS-cog (items 1–3), Trail Making A & B, Symbol Digit modalities, A quick test of cognitive speed, clock drawing, cube coping, letter S fluency and animals fluency. The medical doctor made a global assessment of whether the individual was cognitively healthy based on the test results in relation to education and age. All CN subjects had a Clinical Dementia Rating scale score of 0.

The SCD and MCI cases were recruited consecutively and were thoroughly assessed by physicians with special competence in dementia disorders. The inclusion criteria were: referred to a memory clinic due to possible cognitive impairment, not fulfilling the criteria for dementia, MMSE 24–30, age 60–80 years and, fluent in Swedish. The exclusion criteria were: cognitive impairment that without doubt could be explained by another condition (other than prodromal dementia); severe somatic disease; and refusing lumbar puncture or neuropsychological investigation. The classification in SCD or MCI was based on a neuropsychological battery and the clinical assessment of a senior neuropsychologist. The battery included tests for verbal ability (including A multiple-choice vocabulary test (SRB:1 (ref. 25) and semantic verbal fluency (Condition 2, D-KEFS (ref. 26)), episodic memory (including Rey Auditory Verbal Learning Test (RAVLT (ref. 27)), and Rey Complex Figure Test (RCFT (ref. 28))), visuospatial construction ability (including Block design (WAIS (ref. 29) and The copy trial of Rey Complex Figure Test), attention and executive functions (including Trail Making Test (D-KEFS (ref. 26) and Letter Verbal Fluency, Condition 1 (D-KEFS (ref. 26))). A senior neuropsychologist stratified all patients into those with SCD (no measurable cognitive deficits) or MCI according to the consensus criteria for MCI suggested by Petersen³⁰.

The Regional Ethics Committee in Lund, Sweden, approved the study. All subjects gave written informed consent. For more details, see ref. 13 and www.biofinder.se.

PET analysis. Brain A β was measured using ^{18}F -flutemetamol PET (refs 31,32). PET/CT scanning was conducted at two sites using the same type of scanner, a Philips Gemini TF 16. PET sum images from 90 to 110 min post injection were generated for the average uptake. MRI results were not used since this does not improve the quantification of ^{18}F -flutemetamol data³³. The images were analysed using the NeuroMarQ software provided by GE Healthcare. A volume of interest template was applied for nine bilateral regions (prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate and posterior cingulate/precuneus), combined in a global neocortical composite

signal³³. The SUVR was the global composite tracer uptake, normalized for the mean uptake in the cerebellar cortex (note that Thurfjell *et al.*³⁵ found that ¹⁸F-flutemetamol PET SUVR had >98% concordance with visual reads independent of which reference region that was used). Most analyses in this study used continuous PET A β but when indicated a previously defined cutoff for A β positivity was used (>1.42 SUVR, based on mixture modelling analysis¹³).

Cerebrospinal fluid analysis. All subjects underwent lumbar CSF sampling at baseline, following the Alzheimer's Association Flow Chart³⁵. Samples were stored in 1 ml polypropylene tubes at -80°C until analysis. CSF A β 38, A β 40 and A β 42 were analysed by ELISA assays (EUROIMMUN AG, Lübeck, Germany). All analyses were performed by board-certified laboratory technicians who were blinded for clinical data and diagnoses. The CSF samples were randomized to avoid group bias. The analyses were performed during two different runs in batch, plates 1–20 using lot no. E140224AB for A β 38, E130611AA for A β 40 and E130607AA for A β 42, and plates 21–24 using lot no. E150522BK for A β 38, E150302A1 for A β 40 and E150522AZ for A β 42. Aliquots of two different pools of CSF were used as internal control samples, with CVs of 13.8% for A β 38 for the first control with a mean of 695 pg ml $^{-1}$ and 7.9% for A β 38 for the second control with a mean of 1596 pg ml $^{-1}$; 17.9% for A β 40 for the first control with a mean of 1951 pg ml $^{-1}$ and 11.1% for A β 40 for the second control with a mean of 3992 pg ml $^{-1}$; and 16.3% for A β 42 for the first control with a mean of 227 pg ml $^{-1}$ and 15.1% for A β 42 for the second control with a mean of 216 pg ml $^{-1}$. To assure consistency in levels between the two runs, 40 CSF samples from the first run were re-analysed in the second run.

Plasma analysis. For plasma collection, blood was drawn into tubes containing EDTA as anticoagulant. After centrifugation (2000g, $+4^{\circ}\text{C}$, 10 min), plasma samples were aliquoted into polypropylene tubes and stored at -80°C pending biochemical analyses. Plasma A β 40 was analysed using Simoa immunoassay (Quanterix, Lexington, MA, USA).

White matter lesions. All patients were examined using a single 3T MR scanner (Trio, Siemens). Automated segmentation of WML was performed using the Lesion Segmentation Tool implemented in SPM8 (<http://www.applied-statistics.de/ist.html>), generating a total WML volume. Before this, manual segmentation for reference of WML was performed on FLAIR images co-registered to the native MPRAGE in four MCI patients, with the segmented volume ranging from 0.5 to 106.3 ml; the resulting optimal κ based on the Dice coefficient was 0.4 (ref. 36) and was used in the subsequent automated segmentation for all participants.

Statistical analysis. We tested correlations between CSF A β 40 and PET A β in different regression models. The main model was a linear regression model where the dependent variable was PET A β and the independent variables were CSF A β 40, APOE ϵ 4 (dichotomous), and the interaction between CSF A β 40 and APOE ϵ 4. Second, we tested the correlation between clinically significant PET A β accumulation and CSF A β 40 and APOE ϵ 4 in a logistic regression model with PET A β positivity as the dependent variable. Third, we tested a linear regression model with CSF A β 40 as the dependent variable and a four level combination of PET A β and APOE ϵ 4 as the independent variable (PET A β - & APOE ϵ 4-, PET A β - & APOE ϵ 4+, PET A β + & APOE ϵ 4- and PET A β + & APOE ϵ 4+). All models were adjusted for age (years), sex and diagnostic group. We also adjusted for CSF A β 42 to test if CSF A β 40 was associated with PET A β beyond CSF A β 42, and to reduce the residual error of the model, allowing a better estimate of the correlation between CSF A β 40 and PET A β . We adjusted for WML (ml) except for when WML was clearly nonsignificant, as detailed in the results section. The primary analyses were done using CSF A β 40, but we also performed analyses using CSF A β 38 and a combination of CSF A β 38 and A β 40 (based on their molar weights, A β 38: 4129.012 g mol $^{-1}$; and A β 40: 4327.148 g mol $^{-1}$ (ref. 37)). Statistical significance was determined at $P < 0.05$. All analyses were done using R (v. 3.0.1, The R Foundation for Statistical Computing).

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Author contributions

N.M. and O.H. had the full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design was formulated by N.M. and O.H. Acquisition, analysis or interpretation of data were done by N.M., P.S.I., S.P., H.Z., K.B., E.S., L.M. and O.H. Drafting of the manuscript was done by N.M. Critical revision of the manuscript for important intellectual content was done by P.S.I., S.P., E.S., L.M., H.Z., K.B. and O.H. Statistical analysis was done by N.M. and P.S.I. N.M. and O.H. obtained funding

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Additional information

Competing financial interests: N.M., P.S.I., S.P., E.S., L.H. and O.H. report no conflicts of interest. K.B. has served as a consultant for Eli Lilly, Novartis, Roche Diagnostics and Sanofi-Aventis, and at Advisory Boards for IBL International and lecturing for Fujirebio Europe and Lundbeck. K.B. and H.Z. are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Holding-based platform company at the University of Gothenburg.

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