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Cerebrospinal Fluid $A\beta_{42}$ Levels and *APP* Processing Pathway Genes in Parkinson's Disease

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Abstract

Background—Of recent interest is the finding that certain CSF biomarkers traditionally linked to Alzheimer's disease (AD), specifically amyloid beta protein (Aβ), are abnormal in PD CSF. The aim of this exploratory investigation was to determine if genetic variation within the amyloid precursor protein (APP) processing pathway genes, correlate with cerebrospinal fluid (CSF) $A\beta_{42}$ levels in Parkinson's disease (PD).

Method—PD (n=86) and control (n=161) DNA were genotyped for 19 regulatory region tagging single nucleotide polymorphisms (SNPs) within nine genes (*APP, ADAM10, BACE1, BACE2, PSEN1, PSEN2, PEN2, NCSTN* and *APH1B*) involved in the cleavage of APP. SNP genotypes were tested for their association with CSF biomarkers and PD risk while adjusting for age, gender, and *APOE* ε4 status.

Results—Significant correlation with CSF $A\beta_{42}$ levels in PD was observed for two SNPs, (*APP* rs466448 and *APH1B* rs2068143). Conversely, significant correlation with CSF $A\beta_{42}$ levels in controls was observed for three SNPs (*APP* rs214484 and rs2040273 and *PSEN1* rs362344).

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Conclusion—The results of this exploratory investigation suggest that an *APP* SNP and an *APH1B* SNP are marginally associated with PD CSF $A\beta_{42}$ levels in *APOE* ε4 non-carriers. Further hypotheses generated include that decreased CSF $A\beta_{42}$ levels are in part driven by genetic variation in APP processing genes. Additional investigation into the relationship between these findings and clinical characteristics of PD, including cognitive impairment, compared to other neurodegenerative diseases, such as AD, are warranted.

Keywords

APP; ADAM10; BACE1; BACE2; PSEN1; PSEN2; PEN2; NCSTN; APH1B; Parkinson's disease; Cerebrospinal fluid

Introduction

Amyloid precursor protein (APP) is an integral membrane protein that is best known as the protein whose processing generates amyloid beta (Aβ), a peptide that is the primary component of the amyloid plaques characteristic of Alzheimer's disease (AD). Aβ peptides of varying sizes are found in cerebrospinal fluid (CSF). Low CSF $A\beta_{42}$ levels are a promising biomarker for AD[1] and have been described in Parkinson's disease (PD)[2].

Many proteins are involved in the post-translational cleavage of APP into Aβ. An α-secretase, ADAM10, cleaves APP in one processing pathway that does not form an amyloidgenic Aβ peptide[3–7]. In another processing pathway, APP cleavage by β-secretases BACE1 or BACE2 produces a peptide, which subsequently can undergo γ-secretase cleavage to produce an amyloidgenic Aβ peptide[8–12]. The γ-secretase protein complex is composed of several subunits including presenilin (either PSEN1 or PSEN2), nicastrin (NCSTN), APH1B and PEN2[13–15], and is uniquely involved in APP cleavage according to tissue specificity, or the stability of the protein complex[16–20].

Rare autosomal-dominant forms of early-onset familial AD, are caused by mutations in the *PSEN1*, *PSEN2* or APP[21]. The majority of the rare coding mutations in APP alter processing of APP so that the relative levels of $A\beta_{42}$ are increased[22–24]. Triplication of the APP gene, due to chromosome 21 trisomy in Down's Syndrome, is associated with increased APP expression and early amyloid plaque formation[25–28]. In addition, APP promoter polymorphisms have been associated with AD[29,30].

Aβ peptides of varying sizes are normally present in both the brain and CSF. Low CSF $A\beta_{42}$ levels are associated with increased Aβ deposition in the brain[31–33], age and *APOE* ε4 genotype in cognitively normal adults[34], and in AD and mild cognitive impairment[35–37].

Some studies, but not all, report reduced CSF $A\beta_{42}$ associated with PD or cognitive decline in PD[38–40]. In addition, the *APOE* ε4 allele appears to be a risk factor across the Lewy body disease (LBD) spectrum, including PD[41].

Our group has reported an association between CSF biomarker levels and APP processing genes in AD[42]. However, to the best of our knowledge, the genetic influences of APP processing related genes on AD-associated CSF biomarkers have not been studied in PD.

Therefore, we hypothesized that genetic variation within regulatory regions of APP processing genes, would correlate with CSF $A\beta_{42}$ levels in PD according to *APOE* ε4 status. Specifically, the aim of this investigation was to determine if genetic variation in common transcriptional regulatory regions of *APP, ADAM10, BACE1, BACE2, PSEN1, PSEN2, PEN2, NCSTN* and *APH1B* correlate with PD CSF $A\beta_{42}$ levels. A total of 19 single nucleotide polymorphisms (SNPs) were analyzed while taking into account age, gender, and *APOE ε4* status.

Methods

Subjects

All procedures were approved by the institutional review boards of the participating institutions. Following informed consent, all PD subjects (n=86) underwent evaluation that consisted of medical history, family history, physical and neurologic examinations, and laboratory tests. All PD subjects fulfilled criteria for a diagnosis of PD[43].

All control subjects (n=161) underwent thorough clinical and neuropsychological assessment as prescribed by the Alzheimer Centers' uniform data set[44]. All control subjects had a Clinical Dementia Rating (CDR) scale score of 0, and underwent consensus conference review.

Cerebrospinal Fluid

All CSF samples were collected in the morning after participants fasted overnight. CSF samples were collected as previously described[34,45]. Results reported here are from assays run from comparable lumbar puncture fractions to limit variability from rostrocaudal concentration gradients. Concentrations of $A\beta_{42}$ in the 5th to 10^{th} ml of collected CSF Aβ42 were measured using the INNO-BIA AlzBio3 kit obtained from Innogenetics (Gent, Belgium) following the manufacturer's instructions except that the CSF samples were diluted 1:4 before performing the assay. CSF $A\beta_{42}$ was measured using multiplexed Luminex reagents from InnoGenetics according to manufacturer's instructions and as previously described[46]. All CSF samples were analyzed using a LiquiChip Luminex 200TM Workstation (Qiagen, Valencia, CA). Intra-assay coefficient of variation was <10 % for all assays. Assays were performed blind to clinical diagnosis.

Genes and SNP selection

The nine studied genes were chosen for their biologically characterized role in encoding proteins that are involved in the processing of APP. SNPs were chosen within these genes according to the following criteria; (1) the SNP was located within a known or putative regulatory region of the gene. Tagging SNPs were chosen to capture regulatory regions when the actual regulatory region SNP was not available; (2) the SNP had a minor allele frequency (MAF) of ≥ 0.1 in HapMap Northern European population (CEU) and a minor genotype frequency in our study sample of ≥ 0.01; and (3) the SNP genotyping assay was commercially available. Based on these criteria, a total of 19 SNPs were selected (Table 2). Additionally, SNPs rs429358 and rs7412 were genotyped to determine *APOE ε4* status.

SNP Genotyping

Genomic DNA (10 ng) was genotyped using TaqMan allelic discrimination detection on 384 well plates as previously described[47]. Briefly, for each reaction, SNP TaqMan Assay (Applied Biosystems), TaqMan Universal PCR Master Mix (Applied Biosystems) and DNA were pipetted into each well. PCR was carried out using a 9700 Gene Amp PCR System (Applied Biosystems). Plates were then subjected to an end-point read on a 7900 Real-Time PCR System (Applied Biosystems). The results were first evaluated by cluster variations; the allele calls were then assigned automatically before being integrated into the genotype database.

Seven PD patients with an age-at-onset of younger than 40 years were screened for Parkin (*PARK2*) mutations using a method previously described[48]. One patient was found to be a compound heterozygote and was excluded from the analysis leaving 85 PD subjects for analysis (Table 1).

Statistical Analysis

Logistic regression was used to compare SNP genotype (FF major homozygotes, EF heterozygotes, EE minor homozygotes) or collapsed genotype (EF, EE compared to FF; minor allele positive compared to minor allele negative) between PD and controls both with and without adjusting for age, gender and *APOE* ε4 status. SNP genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE) using the chi-squared test with one degree of freedom[49] (Table 2). All subsequent analyses involving SNPs were based on the collapsed genotype group.

Linear regression models were used to compare PD and control CSF $A\beta_{42}$ protein levels with and without adjusting for gender, age, and *APOE* ε4 (Figure 1, Panel A) and to evaluate the relationship between *APOE* ε4 status and CSF $A\beta_{42}$ levels while taking into account gender and age (Figure 1; Panels B).

Linear regression main effect models both with a single SNP or all SNPs were used to examine the relationship between SNP (for collapsed genotype) and CSF $A\beta_{42}$ levels with and without adjusting for gender, age, and *APOE* ε4 status (Figure 2). Additional regression models included a SNP-by-*APOE* ε4 status interaction term or a term for group (1) ε4+ and SNP allele +, (2) ε4+ and SNP allele −, (3) ε4− SNP and allele +, and (4) ε4− and SNP allele −, both with and without adjusting for age and gender (Figure 3).

Diagnostic plots of model residuals were inspected to assess any major departures from normality or homoscedasticity. Statistical analyses were performed in R (version 3.0.2; R Core Team, 2013; http://www.R-project.org.) or SPSS (Version 22). When correcting for multiple comparisons, the Holm (1979) was used.

Results

Sample Population

Subjects were 161 healthy cognitively normal control subjects with a mean age of 68 years, and 85 PD patients with a mean age of 67 years and a mean age-of-motor-onset symptom of

57 years (Table 1). All normal control subjects were reviewed by a consensus panel and designated as neurologically and cognitively normal based on cognitive testing and examination. All PD subjects fulfilled criteria for PD, based on history and examination[43]. All but two PD cases had a mini-mental status examination (MMSE), with a mean MMSE score of 27.6 (+/− 3.5) for these cases. Only thirteen cases had a MMSE below 26.[50] The percentage of female subjects was significantly higher in control subjects compared to PD subjects (Table 1). Out of 86 PD patients, there were 7 PD patients with an age-at-onset of younger than 40 years (Table 1). These 7 patients were screened for Parkin (*PARK2*) mutations. One patient was found to be a compound heterozygote. This patient was excluded from the analysis leaving 85 PD patients for the analyses.

SNP Genotype Frequency

All 19 APP processing gene SNPs passed the HWE test after correcting for multiple comparisons. *PSEN2* rs2802268 and *APH1B* rs2068143 frequency was significantly different between PD and controls. Significance did not remain after Holm multiple comparison correction (Table 2).

CSF $A\beta_{42}$ Levels by Disease Group and *APOE* ε4 Status

CSF $A\beta_{42}$ levels were not significantly different between PD and controls (Figure 1; Panel A). In the control sample, but not in PD, *APOE* ε4 carriers had significantly lower CSF $A\beta_{42}$ levels compared to non-carriers both with and without adjusting for gender and age (p = 0.006; p=0.012, respectively) (Figure 1; Panel B).

SNP Effect on CSF $A\beta_{42}$ Levels

The effect of SNP (collapsed genotypes) on CSF $A\beta_{42}$ levels was analyzed for each group (controls or PD) both with and without adjusting for the covariates; gender, age and *APOE* ε4-status. The results are presented graphically as beta coefficients and 95% confidence intervals without adjusting for covariates.

Within the PD group, $A\beta_{42}$ levels were significantly lower for minor allele carriers of the *APP* rs466468 (p = 0.014 adjusted, p = 0.015 unadjusted), the rs214484 (p = 0.032 adjusted, p = 0.036 unadjusted),and significantly higher for minor allele carriers of the *APH1B* SNP rs2068143 (p = 0.002 adjusted, p = 0.003 unadjusted). The rs2068143 adjusted value remained significant after Holm correction for multiple comparisons (p = 0.040). The rs466468 and rs2068143, but not rs214484, remained significant in main effect models containing all SNPs and covariates (p = 0.049, p = 0.011, p = 0.106, respectively).

Within the control group, $A\beta_{42}$ levels were significantly lower for minor allele carriers of the *APP* rs214484 (p = 0.002 adjusted, p = 0.007 unadjusted) and rs2040273 (p = 0.017 adjusted, p = 0.039 unadjusted), and for the *PSEN1* rs362344 (p = 0.023 adjusted, p = 0.040 unadjusted). The *APP* SNP rs214484 adjusted value within controls remained significant after Holm correction (p = 0.040) and after including all the SNPs and covariates in the model (p = 0.044) (Figure 2). When a SNP-by-*APOE* ε4 status interaction term was included in the model it was not significant within controls and not significant within PD patients, while *APOE* ε4 and SNP remained significant, suggesting an additive effect.

CSF $A\beta_{42}$ Levels by *APOE* ε4 Status and SNP status

The nature of this additive effect for 3 SNPs positive in the control group was evaluated further using linear regression models described in the methods section. For the one SNP that remained significant after correcting for multiple comparisons there was a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 carriers with the rs214484 G allele and *APOE* ε4 non-carriers without the rs214484 G allele with ($p = 0.0002$) and without adjusting for gender and age ($p = 0.0001$). There was a significant difference between *APOE* ε4 carriers with the rs2040273 G allele and *APOE* ε4 non-carriers without the rs2040273 G allele with ($p = 0.002$) and without adjusting for gender and age ($p = 0.001$). There was a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 carriers with the rs362344 T allele and *APOE* ε4 non-carriers without the rs362344 T allele with ($p = 0.001$) and without adjusting for gender and age ($p = 0.001$). There was also a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 carriers with the rs362344 T allele and *APOE* ε4 non-carriers with the rs362344 T allele with ($p = 0.027$) and without adjusting for gender and age ($p = 0.020$) (data not shown).

The nature of this additive effect for 2 SNPs positive in the PD group was evaluated further using the linear regression models described in the methods section. There was a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 carriers with the rs466448 G allele and *APOE* ε4 non-carriers without the rs466448 G allele with ($p = 0.030$) and without adjusting for gender and age ($p = 0.031$) (Figure 3; Panel A). There was also a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 non-carriers with the rs466448 G allele and *APOE* ε4 non-carriers without the rs466448 G allele with ($p = 0.035$) and without adjusting for gender and age ($p = 0.036$) (Figure 3; Panel A). There was a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 carriers with the rs2068143 A allele carriers and *APOE* ε4 non-carriers without the rs2068143 A allele with ($p = 0.027$) and without adjusting for gender and age ($p = 0.025$) (Figure 3; Panel A). There was also a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4 non-carriers with the rs2068143 A allele and *APOE* ε4 non-carriers without the rs2068143 A allele with ($p = 0.021$) and without adjusting for gender and age ($p = 0.016$) (Figure 3; Panel A).

Discussion

This exploratory investigation demonstrates that genetic variation within regulatory regions of APP processing pathway genes correlate with CSF $A\beta_{42}$ levels in PD and normal controls.

There was not a significant difference in CSF $A\beta_{42}$ levels between PD and controls (Figure 1, Panel A). Some, but not all, previous studies describe PD CSF $A\beta_{42}$ levels as lower than in controls[51–57]. A more consistent finding is an association between lower CSF $A\beta_{42}$ levels and cognitive impairment in PD[38,56,57]. Upon stratification by *APOE* ε4, control, but not PD, *APOE* ε4 carriers had significantly lower CSF $A\beta_{42}$ levels, both with and without age adjustment, compared to *APOE* ε4 non-carriers (Figure 1, Panel B). These results are supported by previous reports that show cognitively normal subject CSF $A\beta_{42}$ levels decrease with increasing age in *APOE* ε4 carriers[34]. In addition, a lack of association between PD CSF $A\beta_{42}$ levels and *APOE* ε4 has been described[58,59].

One *APP* rs214484 was significantly associated with CSF $A\beta_{42}$ levels in cognitively normal controls after correcting for multiple comparisons and after adjusting for gender, age and *APOE* ε4 (Figure 2). *APP* rs2040273 and *PSEN1* rs362344 nominally correlated with CSF $A\beta_{42}$ levels in controls and *APP* rs466448 and *APH1B* rs2068143, nominally correlated with CSF $A\beta_{42}$ levels in PD (Figure 2). To our knowledge this is the first investigation to find an association between SNPs in the APP processing pathway and CSF $A\beta_{42}$ levels in PD. However, in support of these findings, a recent report suggests that disturbed APP processing has an effect on the variable rate of motor and functional decline in PD[60]. Coding mutations in the APP gene that influence the processing of APP and increase the production of Aβ cause a rare early onset form of AD and emphasize the importance of the APP processing pathway in AD cognitive decline[29,6121]. Our group previously reported an association between soluble CSF biomarkers and APP processing genes in AD[42]. Specifically, a SNP allele located at the α-secretase *ADAM10* locus was associated with CSF APPα levels in AD and was significantly different between AD and controls. However, there was no association between APP processing genes and CSF $A\beta_{42}$ levels in AD[42]. Taken together, a lack of association between APP processing genes and CSF $A\beta_{42}$ levels in AD in the previous study, but an association in PD in the present study, suggests that a different biological factor may play a role in the pathobiology of amyloid pathology in PD. Furthermore, the association between an *APP* 3' region SNP in controls (rs214484) and an *APP* 5' region SNP in PD (rs466448), generates further hypotheses regarding CSF $A\beta_{42}$ levels and *APP* genetic variants as therapeutic targets or markers of disease specific neurodegeneration.

To determine if these 5 APP pathway SNPs in combination with *APOE* ε4 have an additive effect on CSF $A\beta_{42}$ levels, all 5 SNPs were grouped by *APOE* ε4 in regression models. All 5 SNPs exhibited an additive effect where the carriers of both *APOE* ε4 and one of the APP processing SNP alleles had significantly lower CSF $A\beta_{42}$ levels compared to *APOE* ε4 non-carriers and APP processing SNP allele non-carriers. Interestingly, rs466448 and rs2068143 were the only SNPs that showed significantly lower PD CSF $A\beta_{42}$ levels within the *APOE* ε4 non-carriers, suggesting that these SNPs may influence PD CSF $A\beta_{42}$ levels, but not control levels, regardless of *APOE* ε4 status (Figure 3). Rs466448 is located in the 5' region at position -1023 upstream of TSS of the *APP* promoter[62] and is within an ENCODE described promoter associated histone mark (H3K4Me3)[63] suggesting that allele specific variation may play a mechanistic role in *APP* expression. In addition, rs214484 is located in the 3' region of *APP* within an ENCODE described H3K27Ac mark often found near active regulatory elements[63] further suggesting that *APP* expression level may be influenced by genetic variation at these locations.

The rs2068143 is located in the 3' region of *APH1B* in intron 4 in an ENCODE DNase I hypersensitivity cluster as well as an enhancer associated histone mark (H3K4Me1)[63] suggesting that allele specific variation may play a mechanistic role in *APH1B* expression. Inactivation of the Aph1B subunit of γ-secretase in a mouse model of AD has been described. In this model inactivation of Aph1B led to improvements in AD relevant phenotypic features[64]. Given that the genetic variation of this gene has not been extensively investigated, and given that only 2 SNPs were analyzed in the present investigation, further

analysis is needed to determine if rs2068143 is a surrogate marker for another genetic variant at the *APH1B* locus. Interestingly, there is a modest effect by all these described SNPs after multiple comparison adjustment. However, the information provided by this exploratory investigation may be important as it suggests that *APOE* ε4 non-carriers have a different APP pathway related association with CSF $A\beta_{42}$ compared to *APOE* ε4 carriers. This information suggests that it may be important, in future PD therapeutic strategies related to amyloid treatment of cognitive decline, to take into account *APOE* ε4 status.

An advantage of this investigation, in contrast to genome wide association studies, is that a small number of SNPs (n=19), within the context of a specific biological pathway were analyzed, and therefore increased the power to test the hypothesis that the APP processing pathway genes influence PD CSF $A\beta_{42}$ levels. A limitation of this investigation's approach was that only a few APP processing genes were tested. Thus, many other contributors to APP processing may have been missed, such as genes related to the clearance or deposition of Aβ in the brain. In addition, a limited number of SNPs were used to capture putative regulatory genetic variation within and surrounding the genes of interest. Therefore, these results must be approached with caution since many important SNPs may have been missed. Another limitation of this investigation is that sAPPα and sAPPβ were not measured. Previously, our group described an association between CSF sAPPα and *ADAM10* in AD[42]. Others have described CSF sAPPα, sAPPβ and various CSF $A\beta_{42}$ fragments in PD, Lewy body disease, and AD[60,65,66]. Unfortunately, we did not have sAPPα and sAPPβ data available for this PD cohort.

Others have reported correlation between cognitive decline in PD and CSF $A\beta_{42}$[66]. In the present investigation the relationship between cognition (MMSE) and SNP was evaluated (data not shown) and there was not a significant association for any of the SNPs or CSF $A\beta_{42}$ levels. However, we did not have cognitive data available for this cohort beyond MMSE, which has been shown to have low sensitivity to cognitive impairment in PD. Thus, additional cognitive testing would be necessary to adequately assess this issue. Given the results described here, future investigation of the relationship between CSF $A\beta_{42}$, APP pathway genes and cognition in PD may be warranted.

In conclusion, the main novel findings are that an *APP* SNP and an *APH1B* SNP are marginally associated with PD CSF $A\beta_{42}$ levels in *APOE* ε4 non-carriers. Further hypotheses generated by this exploratory investigation, include that decreased CSF $A\beta_{42}$ levels are in part driven by genetic variation in APP processing genes. This information may have important implications for therapeutic strategies related to amyloid treatment of cognitive decline that take into account *APOE* ε4 status. Investigation into the relationship between these genes and clinical characteristics of PD, such as cognitive impairment, compared to other neurodegenerative diseases, are warranted. Characterization of the functional influence of *APP* and *APH1B* genetic variation on expression may lead to a better understanding of PD, help identify novel PD biomarkers and help identify PD therapeutic targets.

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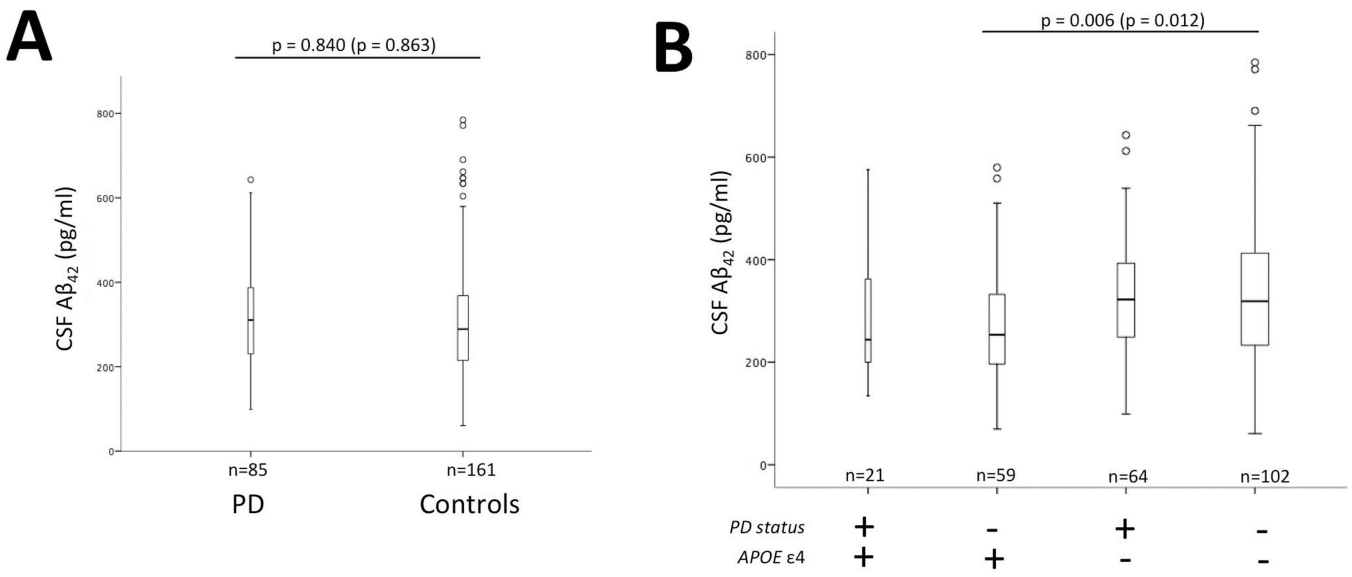

Figure 1.

Cerebrospinal fluid (CSF) levels stratified by disease status and *APOE* status. There is not a significant difference in CSF $A\beta_{42}$ levels between Parkinson's disease (PD) and cognitively normal control subjects (Controls) (Panel A). There is a significant difference in CSF $A\beta_{42}$ levels in controls between *APOE* ε4+ and *APOE* ε4− but not PD (P-values are Bonferroni corrected for multiple comparisons: Panel B). Bars represent interquartile range for CSF $A\beta_{42}$ levels. P-values are adjusted for covariates gender and age. P-values in parentheses are not adjusted for covariates and bar graphs are not adjusted for covariates. The horizontal line within the quartiles represents the median. The vertical lines represent minimum and maximum CSF $A\beta_{42}$ levels. Circles represent outliers.

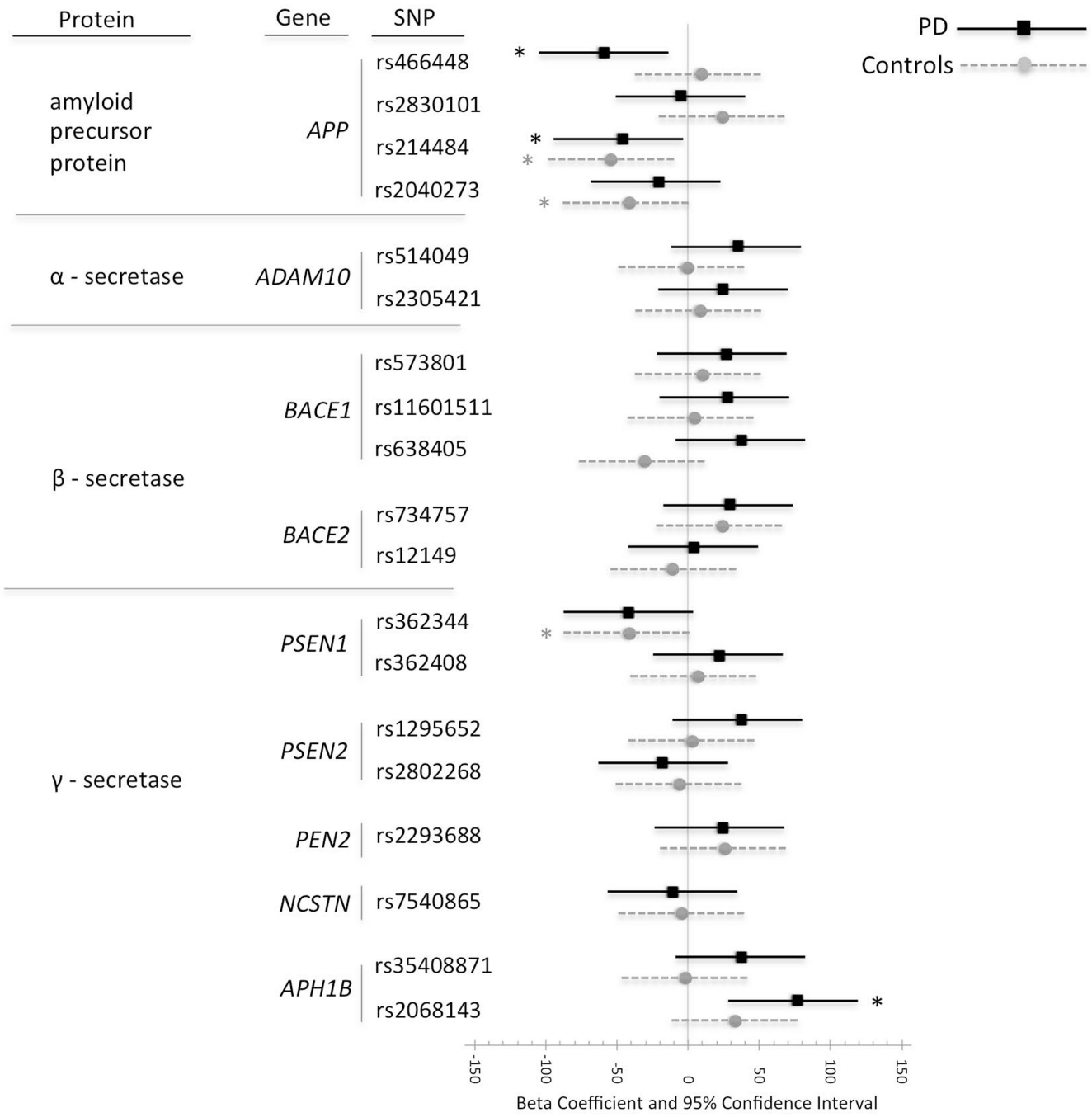

Figure 2.

Linear regression beta-coefficients for cerebrospinal fluid (CSF) $A\beta_{42}$ levels in cognitively normal control subjects (Controls: grey circle) or Parkinson's disease patients (PD: black square) for each collapsed genotype. A confidence interval that does not cross the vertical line at zero indicates that the difference in genotype is significant ($p < 0.05$) before adjusting for multiple comparisons as indicated by asterisk (*). A beta coefficient to the left of the vertical line indicates lower CSF $A\beta_{42}$ levels for the collapsed genotype group (EE, EF) than

for the major homozygote genotype (FF). Beta coefficients, 95% confidence intervals and p-values in parentheses are not adjusted for covariates.

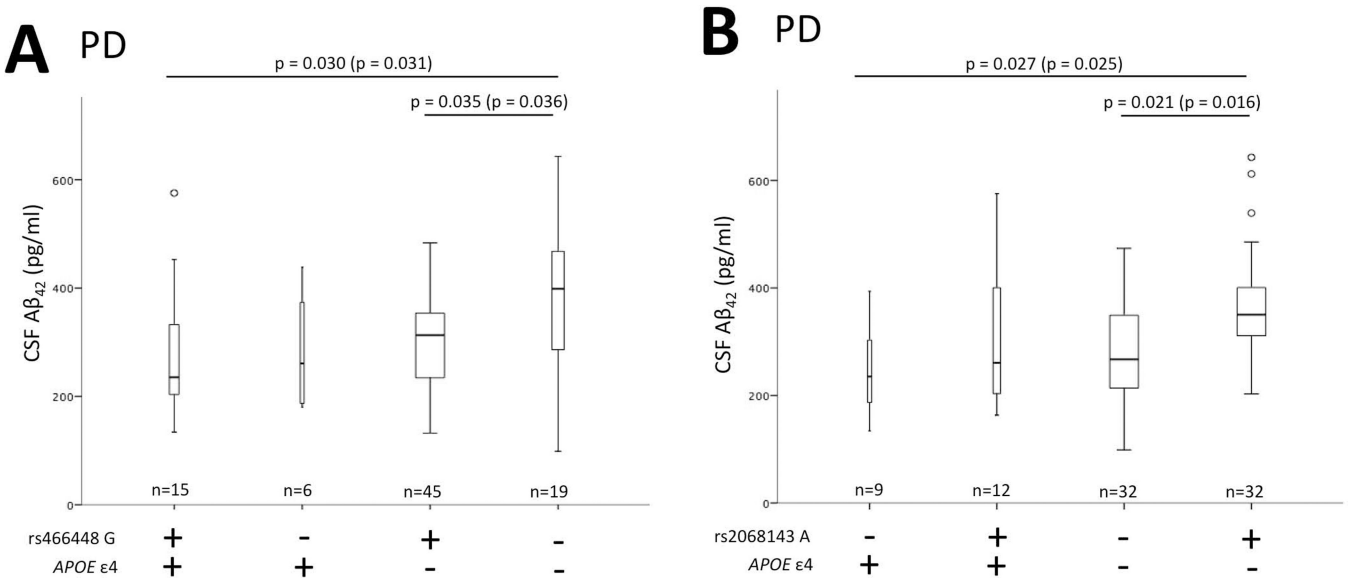

Figure 3.

PD CSF levels stratified by *APOE* and SNP. There is a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4+ rs466448 G+ and *APOE* ε4− rs466448 G− (p=0.030) as well as between *APOE* ε4− rs466448 G+ and *APOE* ε4− rs466448 G− (p=0.035) (Panel A). There is a significant difference in CSF $A\beta_{42}$ levels between *APOE* ε4+ rs2068143 A− and *APOE* ε4− rs2068143 A+ (p=0.027) as well as between *APOE* ε4− rs2068143 A− and *APOE* ε4− rs2068143 A+ (p=0.021) (Panel B). Bars represent interquartile range for CSF $A\beta_{42}$ levels and width of bars a adjusted for number of subjects. P-values are adjusted for covariates gender and age. P-values in parentheses are not adjusted for covariates and bar graphs are not adjusted for covariates. The horizontal line within the quartiles represents the median. The vertical lines represent minimum and maximum CSF $A\beta_{42}$ levels. Circles represent outliers. All p-values are Bonferroni corrected for multiple comparisons.

Table 1

Population description.

	PD	Controls	χ^2 p-value
	n=85	n=161	
% Female	24	58	< 0.0001
% White	98	92	0.170
% *APOE ε4+*	25	37	0.060
Mean Age (Minimum-Maximum)	67 (48–83)	68 (52–88)	0.454
Mean Age-at-motor symptom onset (Minimum-Maximum)	57 (28–76)		
MMSE (Minimum-Maximum)	27.6 (10–30)		

Table 2

Genotype description and frequency distribution.

Gene ID	Chromosome Location	SNP	NCBI Gene Location	Alleles	PD Genotype Frequency			Control Genotype Frequency			PD vs Controls			
					EE	EF	FF	EE	EF	FF	Unadjusted Genotype p-value	Adjusted Genotype p-value	Unadjusted Collapsed Genotype p-value	Adjusted Collapsed Genotype p-value
Amyloid Precusor Protein														
APP	21q21.3	rs466448	5' region	G/a	0.19	0.52	0.29	0.25	0.49	0.26	0.324	0.122	0.578	0.136
		rs2830101	intron 1	G/a	0.08	0.46	0.46	0.13	0.41	0.46	0.601	0.394	0.990	0.728
		rs214484	intron 17	C/g	0.07	0.47	0.46	0.11	0.42	0.47	0.697	0.909	0.917	0.987
		rs2040273	3' region	A/g	0.13	0.51	0.36	0.16	0.43	0.41	0.888	0.661	0.491	0.545
α-Secretase														
ADAM10	15q22.1	rs514049	5' region	C/a	0.13	0.46	0.41	0.22	0.45	0.34	0.089	0.191	0.237	0.298
		rs2305421	Intron 13	A/g	0.04	0.21	0.75	0.04	0.27	0.70	0.414	0.778	0.345	0.933
β-Secretase														
BACE1	11q23-24	rs573801	5' region	G/a	0.12	0.28	0.60	0.03	0.42	0.55	0.631	0.466	0.478	0.695
		rs11601511	intron 0	G/c	0.01	0.22	0.76	0.02	0.24	0.74	0.556	0.463	0.661	0.697
		rs638405	exon 5	C/g	0.14	0.53	0.33	0.16	0.50	0.34	0.929	0.449	0.848	0.948
BACE2	21q22.3	rs734757	intron 1	C/t	0.08	0.49	0.42	0.19	0.44	0.37	0.097	0.102	0.438	0.434
		rs12149	exon 9	C/t	0.22	0.47	0.31	0.25	0.47	0.28	0.557	0.252	0.665	0.417
γ-Secretase														
PSEN1	14q24.3	rs362344	3' region	C/t	0.05	0.35	0.60	0.05	0.35	0.60	0.999	0.900	0.970	0.956
		rs362408	3' region	G/a	0.04	0.18	0.79	0.02	0.23	0.75	0.762	0.736	0.520	0.608
PSEN2	1q42.2	rs1295652	5' region	A/g	0.05	0.33	0.62	0.07	0.37	0.56	0.295	0.194	0.330	0.224
		rs2802268	3' region	T/g	0.04	0.28	0.68	0.06	0.40	0.55	**0.048**	0.083	**0.040**	0.078
PEN2 (PSENEN)	19q13.1	rs2293688	intron 2	C/g	0.11	0.47	0.42	0.12	0.48	0.39	0.570	0.964	0.625	0.925
NCSTN	1q22-q23	rs7540865	intron 5	G/t	0.07	0.48	0.45	0.11	0.41	0.48	0.978	0.414	0.577	0.223
APH1B	15q22.2	rs35408871	Intron 4	G/a	0.01	0.25	0.74	0.02	0.24	0.73	0.748	0.984	0.889	0.820

Gene ID	Chromosome Location	SNP	NCBI Gene Location	Alleles	PD Genotype Frequency			Control Genotype Frequency			PD vs Controls			
					EE	EF	FF	EE	EF	FF	Unadjusted Genotype p-value	Adjusted Genotype p-value	Unadjusted Collapsed Genotype p-value	Adjusted Collapsed Genotype p-value
		rs2068143	intron 4	G/a	0.16	0.35	0.48	0.07	0.34	0.60	**0.020**	0.081	0.088	0.152

Adjusted p-values are based on a logistic regression model with diagnosis as the dependent variable and SNP as a predictor variable along with the covariates gender, age and *APOE* ε4 status.

P-values < 0.05 are in bold but do not remain significant after adjustment for multiple comparisons.