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ARTICLE

Rare variants in β-Amyloid precursor protein (APP) and Parkinson's disease

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Many individuals with Parkinson's disease (PD) develop cognitive deficits, and a phenotypic and molecular overlap between neurodegenerative diseases exists. We investigated the contribution of rare variants in seven genes of known relevance to dementias (β-amyloid precursor protein (*APP*), *PSEN1/2*, *MAPT* (microtubule-associated protein tau), fused in sarcoma (*FUS*), granulin (*GRN*) and TAR DNA-binding protein 43 (*TDP-43*)) to PD and PD plus dementia (PD+D) in a discovery sample of 376 individuals with PD and followed by the genotyping of 25 out of the 27 identified variants with a minor allele frequency <5% in 975 individuals with PD, 93 cases with Lewy body disease on neuropathological examination, 613 individuals with Alzheimer's disease (AD), 182 cases with frontotemporal dementia and 1014 general population controls. Variants identified in APP were functionally followed up by Aβ mass spectrometry in transiently transfected HEK293 cells. PD+D cases harbored more rare variants across all the seven genes than PD individuals without dementia, and rare variants in *APP* were more common in PD cases overall than in either the AD cases or controls. When additional controls from publically available databases were added, one rare variant in *APP* (c.1795G>A(p.(E599K))) was significantly associated with the PD phenotype but was not found in either the PD cases or controls of an independent replication sample. One of the identified rare variants (c.2125G>A (p.(G709S))) shifted the Aβ spectrum from Aβ40 to Aβ39 and Aβ37. Although the precise mechanism remains to be elucidated, our data suggest a possible role for *APP* in modifying the PD phenotype as well as a general contribution of genetic factors to the development of dementia in individuals with PD.
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INTRODUCTION

Linkage analyses as well as genome-wide association and exome sequencing studies have uncovered at least 20 genes associated with idiopathic Parkinson's disease (PD). Still, to date, the identified genes only explain a small portion of the genetic burden in PD. It is likely that genetic factors involved in bringing about a PD phenotype comprise both genetic variants of strong effect, which alone are causative, as well as variants of weaker effect, which contribute to disease risk or phenotypic modification.

A significant overlap between different neurodegenerative diseases has been described on the neuropathologic, the genetic and the phenotypic level.[1–4] Neuropathologically, the overlap is exemplified by the coexistence of hallmark features of both Alzheimer's disease (AD) and PD in individuals with Lewy body disease.[1] On the genetic level, common genetic variants in microtubule-associated protein tau (*MAPT*) represent risk factors for PD[3,4] whereas, at the same time, rare variants of strong effect in *MAPT* have long been recognized as a cause of frontotemporal dementia (FTD).[2] Phenotypically, it is known that at least 30% of individuals with PD develop dementia[5,6] and that age has been described as a major predisposing factor for the development of cognitive impairment.[7] Accordingly, we sought to assess the contribution of genetic factors known to be involved in dementias such as AD[8–11] or FTD[2,12–14] to the PD phenotype.

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METHODS

Standard protocol approvals, registrations and patient consents

Ethics review board approval was obtained at all participating institutions, with the primary review board located at the Technische Universität München, Munich, Germany. All the participants provided written informed consent for participation in the study.

Participants, variant screening and genotyping

We used Idaho LightScanner (BioFire Defense, Salt Lake City, UT, USA) melting curve analysis to screen the coding regions and exon–intron boundaries of β-amyloid precursor protein (*APP*), presenilin 1 and 2 (*PSEN1* and *PSEN2*), tau (*MAPT*), TAR DNA-binding protein 43 (*TDP-43*), granulin (*GRN*) and fused in sarcoma (*FUS*) in 376 individuals with PD (188 with PD without dementia, 188 with PD plus dementia as diagnosed according to the guidelines set forth by the task force of the Movement Disorder Society[15]) and 376 KORA-AGE controls (*APP* and *MAPT* only; Supplementary Figure 1). In the case of altered melting patterns suggestive of variants, Sanger sequencing ensued.

Variants identified during the screening phase were genotyped in 975 PD cases, 93 independent neuropathologically confirmed cases of Lewy body disease, 613 AD, 182 FTD cases and 1014 controls using Sequenom MALDI-TOF mass spectrometry. For technical reasons, *MAPT* c.1637G>A (p.(R546H)) and *PSEN2* c.211C>T (p.(R71W)) were not included. Two 3 base pair (bp) deletions in *APP* were assessed by fragment analysis as described previously.[16] One variant (*APP* c.1795G>A (p.(E599K))) that showed significant association in the first sample was also assessed in a second independent sample of 715 PD cases and 948 healthy controls from Spain. Significance was judged using the χ^2-test. For the genotyping experiments, *P*-values were corrected using the Bonferroni method. *P*-values given for burden tests represent nominal *P*-values. For a detailed description, see Supplementary Figure 1.

The following transcripts and genomic sequences were used in primer design and variant annotation: *APP*—NM_000484.3, NG_007376.1; *PSEN1*—NM_000021.3, NG_007386.2; *PSEN2*—NM_000447.2, NG_007381.1; *FUS*—NM_004960.3, NG_012889.2; *GRN*—NM_002087.2, NG_007886.1; *MAPT*—NM_001123066.3, NG_007398.1; *TDP-43*—NM_007375.3, NG_008734.1. Primer sequences are available upon request.

Immunohistochemistry

Cortical and midbrain sections of the individual harboring the *APP* c.1795G>A (p.(E599K)) variant were stained for Aβ and alpha-synuclein. Staining procedure and antibodies can be found in the supplement.

Cloning, transfections and analysis of Aß-spectrum

cDNA of the pCDNA3.1+APP695sw vector containing all identified *APP* variants were transiently transfected into HEK293 cells and Aβ was analyzed by mass spectrometry as depicted in the supplement in the culture medium.

RESULTS

Variant screening of 'dementia genes' in individuals with PD

Within the coding regions and exon–intron boundaries (±10 bp) of *APP*, *PSEN1*, *PSEN2*, *MAPT*, *FUS*, *TDP-43* and *GRN*, we identified a total of 27 rare variants with minor allele frequency (MAF) <5% in 376 individuals with PD ($n=188$; 70.4±11.73 years, 28.4% female) or PD+D ($n=188$; 72.0±6.1 years, 33.0% female). Interestingly, more individuals with PD+D (10.11%) than solely PD (4.26%) harbored a rare variant with MAF <5% in any of the seven 'dementia genes' (19 PD+D individuals with a variant *vs* 8 PD individuals with a variant; $P=0.0027$, χ^2-test). Four individuals harbored the *GRN* c.1297C>T (p.(R433W)) (rs63750412) variant and one *GRN* c.103G>A (p.(G35R)). One novel variant in *PSEN1* (c.442A>G (p.(I148W))) within two amino acids of variants known to affect the function as well as three previously reported variants in *PSEN2* (c.185G>A (p.(R62H)) (rs58973334), c.211C>T (p.(R71W)) (rs140501902), c.389C>T (p.(S130L)) (rs63750197)) were found. No variants were identified in either *TDP-43* or *FUS*. Nine were also found by the NHLBI-GO exome sequencing project.[17] (Table 1) For a detailed discussion of the phenotype of variant carriers, please refer to the supplement. (Supplementary Table 1)

For *APP* and *MAPT*, the screening was performed in the above 376 PD cases and 376 KORA-AGE controls. In *APP*, 11 rare variants with MAF <5% (seven missense, two 3-bp deletions, two nearsplice variants) were seen. In total, 10 cases but only 4 general population controls carried a rare *APP* variant. None of these variants have previously been reported in individuals with a neurodegenerative condition. In *MAPT*, we identified a total of 10 rare variants (9 missense, 1 stop). Overall, seven cases and five controls harbored a rare *MAPT* variant. (Table 1,Supplementary Table 1). Analysis by common prediction algorithms yielded contradicting results for most variants (Table 1), thus warranting additional frequency assessment and functional study.

Frequency assessment in individuals with PD, AD and FTD

Frequency assessment for 25 of the 27 variants identified in the screening phase was carried out in a sample consisting of 975 PD patients (including the 376 used above), 613 AD patients, 182 FTD patients, 93 neuropathologically confirmed cases of Lewy body disease and 1014 controls (also including the 376 used above). 68.0% of the variants were very rare with MAF <0.1% in the control sample. When compared with controls, the *APP* c.1795G>A (p.(E599K)) variant was significantly more frequent in the PD phenotype than in controls ($P=0.009$, χ^2-test; Supplementary Table 2) prior to correction for multiple testing. When publically available data from the NHLBI-ESP exomes[17] (*APP* c.1795G>A (p.(E599K)) MAF=0.15% in KORA and 0.11% in NHLBI-ESP exomes *vs* 0.66% in PD cases) were added to the controls, the finding remained significant even after Bonferroni correction for multiple testing (14 out of 1068 cases *vs* 12 out of 5310 controls; $P_{\text{nominal}}=3.8\times10^{-7}$, $P_{\text{corrected}}=9.5\times10^{-6}$, χ^2-test). Exclusion of the 376 PD cases and 376 controls used in the discovery phase of the study did not alter this finding (11 out of 692 cases *vs* 11 out of 4934 controls; $P_{\text{nominal}}=4.0\times10^{-7}$, $P_{\text{corrected}}=1.0\times10^{-5}$, χ^2-test). However, when trying to replicate this finding in a Spanish PD case/control sample, we did not find any *APP* c.1795G>A (p.(E599K)) carriers in either cases or controls, possibly suggesting a population-specific effect of *APP* c.1795G>A (p.(E599K)) in Central Europeans. *APP* c.1795G>A (p.(E599K)) was the only variant identified in the 93 Lewy body disease cases. Neuropathologically, this case was indistinguishable from other LBD cases and showed no obvious special pathology. Clinically, this individual had suffered from classical, levodopa-responsive PD with an age of onset at 59 years. Her mother had also had PD. Histology revealed both Lewy bodies in the substantia nigra (SN) and some amyloid plaques in the frontal and parietal cortex and the hippocampus, in line with a diagnosis of idiopathic PD (Figure 1).

Burden tests analyzing the load of rare variants were performed for both *APP* and *MAPT*. This revealed an excess of rare variants with MAF <5% in *APP* in PD (27 individuals with a variant out of 975) when compared with either controls alone (13 out of 1014, $P=0.018$, χ^2-test), AD cases (4 out of 613, $P=0.002$, χ^2-test) or the combined sample of controls, AD and FTD cases ($P=2.22\times10^{-4}$, χ^2-test). This excess of variants with MAF <5% in *APP* in PD (17 individuals with a variant out of 599) was also seen after exclusion of the 'discovery' samples when compared with the joined sample of controls, AD and FTD cases (14 out of 1433, $P_{\text{corrected}}=0.014$, χ^2-test) and to AD cases alone (4 out of 613, $P_{\text{corrected}}=0.014$, χ^2-test). When compared with the controls only (9 out of 638), variants were nearly twice as frequent

Table 1 Rare variants in 'dementia genes' identified in variant screening

				Cases					
Gene	*Genomic position (hg19)*	*dbSNP138*	*Variant*	*PD (n = 188)*	*PD+D (n = 188)*	*Controls (n = 376)*	*Frequency assessment (975 PD:93LBD:613 AD:182 FTD:1014KORA)*	*NHLBI-ESP[a] (EA only)*	*CADD prediction[b] C-score*
APP	chr21:27,423,376, C>T	rs149995579	p.(A201V)		1		2:0:0:0:1	A = 3/G = 8597	1.04
APP	chr21:27,394,297_27,394,299delCTT	N/A	c.722_724delAAG		1		4:0:1:0:4	not found	13.04
APP	chr21:27,354,793, A>G	N/A	c.1091-3T>C	1			1:0:0:0:0	not found	7.06
APP	chr21:27,347,438, C>T	N/A	p.(R468H)			1	0:0:0:0:1	not found	31.00
APP	chr21:27,328,030, C>T	rs201547994	p.(A500T)			1	0:0:0:0:2	not found	25.70
APP	chr21:27,284,167, C>T	rs140304729	p.(E599K)	1	2	1	13:1:3:0:3	T = 9/C = 8591	25.40
APP	chr21:27,284,163, G>A	rs200088099	p.(T600M)			1	0:0:0:0:1	not found	22.10
APP	chr21:27,269,955_27,269,957delTCC	N/A	c.1992_1994delGGA		1		4:0:0:1:0	not found	16.18
APP	chr21:27,269,961, G>A	rs200260102	p.(T663M)		1		1:0:0:0:0	not found	13.01
APP	chr21:27,264,120, C>T	rs201269325	p.(G709S)		1		1:0:0:0:0	not found	35.00
APP	chr21:27,254,092_27,254,093 delAA	rs112965435	c.2212-10_2212-11delTT		1		1:0:0:0:1	not found	1.40
GRN	chr17:42,426,635, G>A	N/A	p.(G35R)		1		1:0:0:0:0	not found	21.70
GRN	chr17:42,429,500, C>T	rs63750412	p.(R433W)	1	3		2:0:0:0:0 homo &.6:0:5:2:6 hetero	T = 24/C = 8576	16.16
MAPT	chr17:44,039,716, C>T	N/A	p.(R5C)		1		1:0:0:0:0	not found	21.00
MAPT	chr17:44,039,717, G>A	rs63750959	p.(R5H)			1	0:0:0:0:1	not found	17.89
MAPT	chr17:44,039,824, G>A	rs115239819	p.(A41T)	1			1:0:0:0:0	not found	2.41
MAPT	chr17:44,067,341, C>T	rs143956882	p.(S427F)		1	1	2:0:4:0:3	T = 18/C = 8582	20.20
MAPT	chr17:44,067,403, C>T	rs200099007	p.(R448*)	1			2:0:4:0:3	T = 2/C = 8598	41.00
MAPT	chr17:44,068,850, G>A	rs143624519	p.(A469T)	1	1	2	3:0:4:0:5	A = 23/G = 8577	15.33
MAPT	chr17:44,071,314, C>G	N/A	p.(P511R)			1	1:0:0:0:2	not found	11.87
MAPT	chr17:44,073,840, G>A	N/A	p.(R546H)			1	n/a	not found	21.80
MAPT	chr17:44,096,064, A>G	N/A	p.(I695V)	1			1:0:0:0:0	not found	9.17
MAPT	chr17:44,101,491, C>T	rs63750991	p.(T762M)	1			1:0:0:0:0	not found	24.10
PSEN1	chr14:73,640,377, A>G	N/A	p.(I148W)		1		1:0:0:0:0	not found	9.29
PSEN2	chr1:227,071,449, G>A	rs58973334	p.(R62H)		1		4:0:3:0:5	A = 22/G = 8578	15.31
PSEN2	chr1:227,071,475, C>T	rs140501902	p.(R71W)		1		n/a	T = 32/C = 8568	18.06
PSEN2	chr1:227,073,271, C>T	rs63750197	p.(S130L)		1		2:0:2:0:4	T = 9/C = 8591	28.10

Abbreviations: AD, Alzheimer's disease; APP, β-amyloid precursor protein; chr, chromosome; D, dementia; FTD, frontotemporal dementia; GRN, granulin; MAPT, microtubule-associated protein tau; N/A = not annotated, n/a = not available because this class of variants cannot be tested using the given prediction algorithm; N/S = not scored; PD, Parkinson's disease; SNP, single-nucleotide polymorphism.
Gray boxes in the controls column symbolize that the given gene was not screened in controls. The following transcripts and genomic sequences were used in variant annotation: *APP*—NM_000484.3, NG_007376.1; *PSEN1*—NM_000021.3, NG_007386.2; *PSEN2*—NM_000447.2, NG_007381.1; *FUS*—NM_004960.3, NG_012889.2; *GRN*—NM_002087.2, NG_007886.1; *MAPT*—NM_001123066.3, NG_007398.1; *TDP-43*—NM_007375.3, NG_008734.1.
[a]Exome Variant Server, NHLBI-GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/; accessed 1 December, 2012).
[b]Combined Annotation Dependent Depletion (CADD), Seattle, WA (URL: http://cadd.gs.washington.edu; accessed 10 November, 2014).

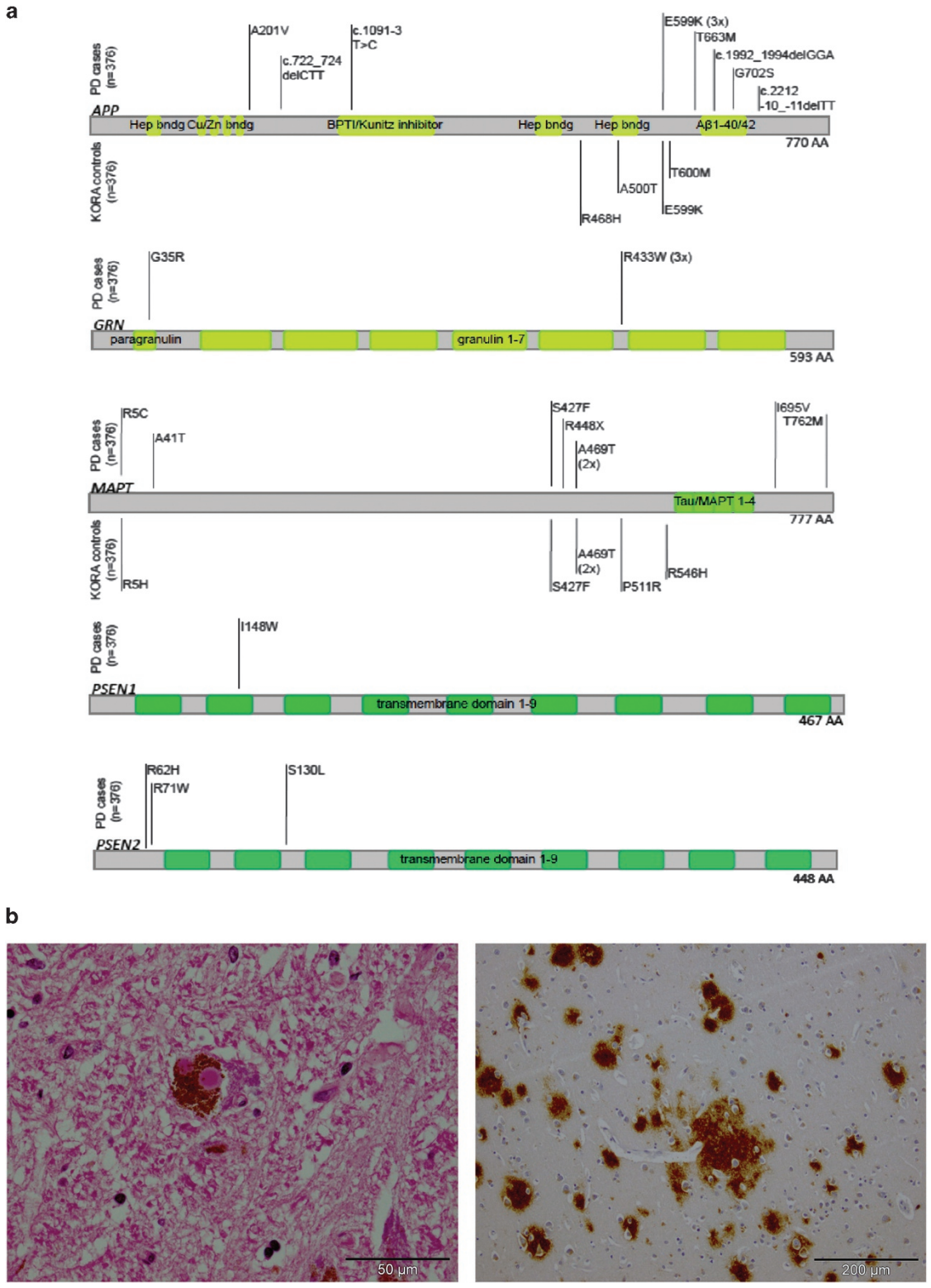

Figure 1 Location of rare variants in *APP*, *GRN*, *MAPT*, *PSEN1*, *PSEN2* and histological features of an individual harboring the c.1795G>A (p.(E599K)) variant of *APP*. (**a**) Variants with MAF <5% found in PD cases are depicted above the schematic illustration of each gene, those found in controls – if the gene was analyzed in controls – below the gene. If variants were present more than once in the discovery sample, the number of occurrences is given in parentheses. Domain annotations were taken from Uniprot (accessed 12 December, 2012). He*P*, heparin; AA, amino acids. (**b**) Depiction of a classical nigral Lewy body (left, antibody: anti-alpha-synuclein KM51, 1:1000, Novocastra/NCL-ASYN, counter stain: hematoxylin–eosine) and cortical Aβ plaques (right, antibody: 4G8, 1:2000, Signet) found in an individual with classical idiopathic PD and the *APP* c.1795G>A (p.(E599K)) variant. The neuropathology was in line with a cases of Lewy body disease (Braak stage 6) with additional Alzheimer-associated alterations (Braak and Braak Stage II), cerebral amyloid angiopathy (Thal stage 1) and beginning argyrophillic grain disease.

($MAF_{PD} = 1.41\%$ *vs* $MAF_{KORA} = 0.71\%$) but this result fell short of statistical significance ($P_{corrected} = 0.24$, χ^2-test). The frequency of rare variants in *MAPT* was similar in all the groups and remained unchanged after the omission of the initial 376 PD cases and 376 controls.

Impact of rare variants in *APP* on Aβ processing

Aβ spectral analysis was performed to further evaluate a potential functional effect of the identified coding variants in *APP*. In all but one, the Aβ spectrum reflected the wild-type situation. However, *APP* c.2125G>A (p.(G709S)), located within the Aβ domain, shifted the spectrum from Aβ40 as the main species to Aβ39 and – to a lesser extent – Aβ37 (Figure 2, Supplementary Figure 2).

DISCUSSION

Screening of seven genes known to be strong genetic factors in AD or FTD in a sample comprising both individuals with PD and PD+D revealed a number of rare variants not previously described. Interestingly, identified variants in *APP* were more common in PD with and without dementia than in either controls or AD. Next, to a mere chance occurrence, there are several possible explanations for this finding. For one, rare variants in known dementia genes could represent phenotype modifiers in PD. This is supported by the fact that in the screening sample, rare variants were more frequent in the PD+D group than in the PD group when all seven genes were analyzed together. Also, the 'dementia gene' variants could contribute to the overall 'neurodegenerative burden', which reflects an increased susceptibility for neurodegenerative conditions in general. In this scenario, an excess of genetic alterations in a specific pathway plus additional non-genetic factors could then tip the balance toward one neurodegenerative phenotype or the other or create phenotypes in which features of multiple neurodegenerative diseases and symptoms coexist. Alternatively, this could also mean that the phenotypic spectrum of AD or FTD is broader than previously recognized and could include PD-like aspects.

The boldest proposal would be that rare variants of strong effect in *APP* or *MAPT* alone could cause PD. *Mapt*$^{-/-}$ mice have recently been shown to develop not only memory deficits but also PD-specific features such as a loss of neurons in the SN and reduced locomotion.[18] Common variants in *MAPT* are an established risk factor for PD[3,4] and the relevance of allelic series – that is, both common variants of weak effect and rare variants of strong effect in one gene – to PD has already be shown.[4,19] Yet, in our sample, rare variants in *APP*, not *MAPT*, were enriched in PD. However, since a physical interaction between MAPT and APP and a role of MAPT in trafficking APP to the cell membrane has been reported,[18,20] rare variants in APP could have a similar effect with regard to PD as MAPT variants.

One of the identified APP variants (c.2125G>A (p.(G709S))) shifts the Aβ proteome spectrum from Aβ40 to Aβ39 and Aβ37 indicating that it likely interferes with γ-secretase cleavage. This could possibly be due to an alteration in the site at which APP interacts with γ-secretases, a mechanism recently postulated for increased Aβ37 production in response to an artificial APP variant (c.2095A>G (p.(K699E))) 10 amino acids N-terminal of our variant.[21] None of the other *APP* variants showed an altered Aβ spectrum. However, further studies are necessary to exclude that these variants could affect the structure and, accordingly, the aggregation potential of generated Aβ as has been demonstrated for some AD-linked variants (reviewed in Haass *et al*[22]).

Yet, from our data we cannot conclude that an Aβ-related function is truly relevant to a potential (modifying) role in PD. Next to the well-recognized role in amyloid production, recently several other functions have been identified.[23,24] APP has been described to serve as a neuronal ferroxidase, which oxidizes Fe^{2+} and loads Fe^{3+} on to the iron transport protein transferrin.[24] Moreover, iron accumulates in mice lacking *App*.[24] As iron accumulation in the SN is a known feature of PD,[25] it would be imaginable that APP dysfunction could also predispose to increased iron accumulation in the SN. *App*$^{-/-}$ mice also show increased cerebral levels of dopamine and catecholamines owing to a lack of amine catabolism via the amine oxidase function of App.[23] Increased APP expression due to *APP* variants potentially related to PD could lead to cerebral dopamine deficits and a PD phenotype. Accordingly, APP's ferroxidase[24] and amine oxidase[23] activities could even more plausibly fit a potential role in PD pathogenesis or phenotype modification and should be explored further.

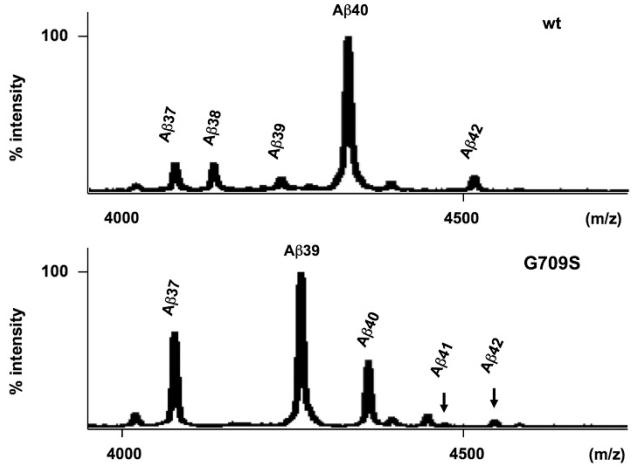

Figure 2 Functional effect of rare *APP* variants. When overexpressed in HEK293 cells, the *APP* c.2125 (p.(G709S)) variant shifts the Aβ spectrum from Aβ40 to Aβ39 and Aβ37, whereas none of the other rare APP variants examined showed alterations in the Aβ spectrum.

CONFLICT OF INTEREST

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