Neurobiology of Aging 62 (2018) 245.e1–245.e7

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Common and rare *TBK1* variants in early-onset Alzheimer disease in a European cohort

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https://doi.org/10.1016/j.neurobiolaging.2017.10.012

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ARTICLE INFO

Article history:
Received 13 April 2017
Received in revised form 31 August 2017
Accepted 15 October 2017
Available online 25 October 2017

Keywords:
Early onset Alzheimer's disease
TBK1
Loss-of-function
Frontotemporal dementia
RNA sequencing

ABSTRACT

TANK-binding kinase 1 (TBK1) loss-of-function (LoF) mutations are known to cause frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), often combined with memory deficits early in the disease course. We performed targeted resequencing of *TBK1* in 1253 early onset Alzheimer's disease (EOAD) patients from 8 European countries to investigate whether pathogenic *TBK1* mutations are enriched among patients with clinical diagnosis of EOAD. Variant frequencies were compared against 2117 origin-matched controls. We identified only 1 LoF mutation (p.Thr79del) in a patient clinically diagnosed with Alzheimer's disease and a positive family history of ALS. We did not observe enrichment of rare variants in EOAD patients compared to controls, nor of rare variants affecting NFκB induction. Of 3 common coding variants, rs7486100 showed evidence of association (OR 1.46 [95% CI 1.13–1.9]; *p*-value 0.01). Homozygous carriers of the risk allele showed reduced expression of TBK1 (*p*-value 0.03). Our findings are not indicative of a significant role for *TBK1* mutations in EOAD. The association between common variants in *TBK1*, disease risk and reduced TBK1 expression warrants follow-up in FTD/ALS cohorts.

1. Introduction

TANK-binding kinase 1 (TBK1) is a serine/threonine-protein kinase involved in autophagy and inflammatory response (Weidberg and Elazar, 2011). TBK1 interacts with the optineurin (OPTN) protein and with multiple interferon regulatory factors, mediating NF-kappa-B (NFκB) activity (Cirulli et al., 2015; Clement et al., 2008; Larabi et al., 2013). Recently, TBK1 has been demonstrated to regulate mitosis and microtubule stability via the TBK1-CEP170 complex (Pillai et al., 2015).

Loss-of-function (LoF) mutations in the *TBK1* gene, including frameshift mutations and inframe amino acid deletions have been identified as a cause of disease in the frontotemporal dementia (FTD)–amyotrophic lateral sclerosis (ALS) spectrum of neurodegeneration (Cirulli et al., 2015; Freischmidt et al., 2015; Gijselinck et al., 2015). In addition, several missense variants have been reported to lead to loss of function, for example, by inhibiting TBK1 interaction with OPTN (Freischmidt et al., 2015; Pottier et al., 2015). Recently, missense mutations compromising NFκB activation in the IFN pathway were found to be enriched among FTD patients compared with neurologically healthy control individuals, suggestive of intermediate penetrant risk variants (van der Zee et al., 2017). However, given the range of functions and substrates of TBK1 and the current absence of insight in the pathomechanism linking *TBK1* LoF to FTD/ALS, causal inferences should be made with caution.

Episodic memory loss and disorientation in time and/or space appear to be frequent early symptoms in carriers of a pathogenic *TBK1* LoF mutation (Van Mossevelde et al., 2015), even resulting in a clinical diagnosis of Alzheimer's disease (AD) in some carriers (Pottier et al., 2015; Van Mossevelde et al., 2015). This has led to the recommendation to consider genetic diagnostic testing for *TBK1* LoF mutations in case of clinical ambiguity between FTD and AD (Van Mossevelde et al., 2015).

Here, we report a massive parallel resequencing of *TBK1* in a large European cohort consisting of 1253 early-onset AD (EOAD) patients, and comparison with 2117 origin-matched unaffected control individuals, to investigate to what extent genetic variability in *TBK1* contributes to the occurrence of AD.

2. Materials and methods

2.1. Study population

The cohort under study consisted of 1253 EOAD patients originating from Flanders-Belgium (n = 273), Spain ($n = 375$), Portugal ($n = 104$), Italy ($n = 182$), Sweden ($n = 155$), Greece (n = 62), Germany ($n = 91$), and Czech Republic ($n = 11$) and 2117 age-matched European control individuals originating from Flanders-Belgium (n = 1042), Spain ($n = 334$), Portugal ($n = 124$), Italy ($n = 340$), and Sweden ($n = 277$) (Table 1). A detailed description of cohort procedures and characteristics is provided in (Verheijen et al., 2016). In the patient cohort, average onset age was 58.9 ± 6.2 years. Information on familial history of AD was present for 756/1253 (60%) patients. Of these, 338/756 (45%) individuals had a positive familial history (defined as presence of at least 1 first-degree relative with AD). Patients with known pathogenic mutations in genes *APP*, *PSEN1*, *PSEN2*, *ABCA7*, *C9orf72*, *MAPT*, *PRNP*, and *GRN* were excluded from the cohort (Cruts et al., 2012; Cuyvers et al., 2015a) (Cuyvers et al., 2015). Average age at inclusion for the control cohort was 67.5 ± 10.0 years. The percentage of women was 59% for both the patient and the control cohort.

Table 1
Cohort characteristics

Country of origin	Patients (n = 1253)	Controls (n = 2117)
Belgium	n = 273 57% female AAO = 63.5 ± 6.4	n = 1042 60% female AAI = 71.5 ± 9.8
Spain	n = 375 58% female AAO = 57.8 ± 4.8	n = 334 57% female AAI = 57.9 ± 4.9
Italy	n = 182 66% female AAO = 56.7 ± 7.1	n = 340 61% female AAI = 64.6 ± 8.9
Portugal	n = 104 61% female AAO = 56.9 ± 6.3	n = 124 69% female AAI = 66.3 ± 6.1
Sweden	n = 155 63% female AAO = 57.9 ± 4.6	n = 277 59% female AAI = 64.2 ± 5.5
Greece	n = 62 61% female AAO = 58.1 ± 3.7	n = 0
Germany	n = 91 52% female AAO = 58.6 ± 4.7	n = 0
Czech republic	n = 11 45% female AAO = 59.1 ± 7.7	n = 0

Key: AAI, age at inclusion (years ±standard deviation); AAO, age at onset.

2.2. Standard protocol approvals, registrations, and patient consents

All participants and/or their legal guardian gave written informed consent for participation in clinical and genetic studies. Autopsied patients or their legal guardian gave written informed consent for inclusion in neuropathological studies. Clinical study protocol and the informed consent forms for patient ascertainment were approved by the ethic committee of the respective hospitals at the cohort sampling sites. The genetic study protocols and informed consent forms were approved by the Ethics Committees of the University of Antwerp and the University Hospital of Antwerp, Belgium.

2.3. Genotyping

Sequencing of the *TBK1* coding region in EOAD patients was performed by target enrichment using MASTR technology (Multiplicom, Niel, Belgium) followed by Illumina sequencing. Details are provided in Supplementary Information. Sequence data of *TBK1* coding region were already available for 2117 control individuals, generated using the same procedures and equipment, and reported in the study by van der Zee et al. (2017). Shared ancestry of mutation carriers was determined using short tandem repeat genotyping, as described in the Supplementary Information.

2.4. RNA sequencing

RNA sequencing data were available for 58 AD patients (28 [48%] women, mean age at blood sampling 72 ± 4.9 years, and 29 [50%] *APOE* ε4-positive) and age-matched and ethnicity matched control individuals (8 [50%] women, mean age at blood sampling 75 ± 6.2 years, and 8 [50%] *APOE* ε4-positive) (Supplementary Table 1). RNA sequencing was performed on poly-A selected total RNA derived from lymphoblast cells using an Illumina HiSeq2000 sequencer as previously described (Verheijen et al., 2016). A detailed description of sequencing procedures can be found in the Supplementary Information.

2.5. Statistical analysis

Low frequency (minor allele frequency [MAF] between 0.01 and 0.05) and common (MAF ≥0.05) variants located in the *TBK1* coding region were tested for deviations from Hardy-Weinberg Equilibrium using PLINK (Purcell et al., 2007). Allele frequencies of common and low frequency variants in patients and controls were compared by χ^2 statistics. Odds ratios and 95% confidence intervals were calculated by logistic regression modeling, corrected for gender and *APOE* ε4 allele carrier status for each country of origin separately using PLINK, including individuals originating from Spain, Italy, Portugal, Sweden, and Belgium (n = 1015 patients and n = 1977 controls). Individuals originating from Czech Republic (11 patients, 0 controls), Greece (62 patients, 0 controls), and Germany (91 patients, 0 controls) were excluded from the analysis based on cohort size. Fixed-effects meta-analysis was performed using the R package rmeta. Nominal *p*-values were corrected for the number of variants tested using Bonferroni correction.

The effect of rare *TBK1* variants (MAF <0.01) on AD risk was assessed using an aggregation test on the same cohorts. Rare variant association analysis was performed across the full *TBK1* coding sequence and separately for each functional protein domain using an optimized Sequence Kernel Association Test (SKAT-O test), adjusted for sample size <2000, using the R package *SeqMeta*. SKAT-O meta-analysis was performed using standard beta weights, and included correction for gender and *APOE* ε4 carrier status of included individuals. Correction for multiple testing was performed using Šidák correction. Functional protein domains were determined according to the study by Gijselinck et al. (2015) and correspond to National Center for Biotechnology Information (NCBI) reference sequence NP_037386.1. Differences in *TBK1* expression were calculated using an unpaired nonparametric (Mann-Whitney) test.

2.6. In vitro NFκB activity

The effect of mutant *TBK1* on NFκB activity in the IFN pathway was investigated by in vitro luciferase assay as previously described (van der Zee et al., 2017). A detailed description is provided in the Supplementary Information.

3. Results

3.1. TBK1 mutation screening

We analyzed the coding sequence of *TBK1* in 1253 European early onset AD patients and 2117 origin-matched control individuals and identified 32 rare variants (MAF <0.01) in a total of 47 individuals, of whom 18 (38%) were patients (Supplementary Tables 2 and 3). In addition, we identified 2 low-frequency variants (MAF 0.01–0.05; 1 missense, 1 synonymous) and 1 synonymous common variant (MAF ≥0.05) (Supplementary Table 4).

The 32 rare variants included 1 LoF mutation (p.Thr79del) and 31 missense variants (Fig. 1). The LoF mutation and 7 of the 31 missense variants were only observed in the patient cohort and were all singleton variants with the exception of p.Ile397Thr, which was observed in 2 patients. In addition, 6 rare missense variants (19%) were present in both patient and controls, and 18 variants (58%) were exclusive to controls. Of the 7 patient-specific missense variants, 4 (57%) attained combined annotation dependent depletion (CADD) Phred score >20, whereas 11 of 17 (65%) control-specific variants and 4 of 6 (67%) shared variants attained CADD Phred score >20 (Supplementary Tables 2 and 3).

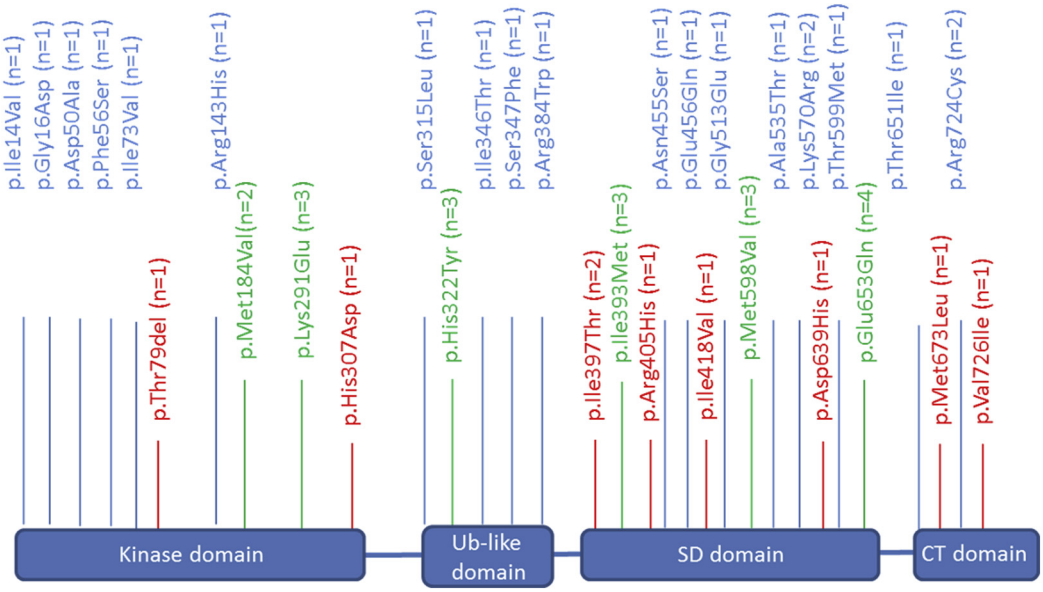

Fig. 1. Nonsynonymous rare *TBK1* variants identified in EOAD patients and control individuals. Variants in red denote variants present in patient cohort only. Variants in green denote variants present in both the patient and control population. Variants in blue denote variants present in the control population only. Functional domains are adapted from (Gijselinck et al., 2015), and based on uniprot information. Protein-level variant position is based on NP_037386.1. Here, (n =) depicts number of carriers in the patient/control cohort. Abbreviations: CT domain, C-terminal domain; SD domain, scaffold dimerization domain; Ub-like domain, ubiquitin-like domain. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Clinical characteristics of LoF mutation (TBK1 p.Thr79del) carrier

TBK1 p.Thr79del is a previously reported LoF mutation (van der Zee et al., 2017), which we observed in a patient of Spanish origin. The patient was clinically diagnosed with sporadic EOAD at age 62 years, with onset of first symptoms at 59 years. His clinical evaluation was considered consistent with AD-type dementia because of the following symptoms: (1) first symptoms were spatial disorientation and recent memory deficits; (2) cognitive evaluation (Supplementary Table 5) showed significant memory problems and deficits attributed to posterior cortex (visuospatial and visuoconstructive deficits); and (3) CT scan showed mild increase of the temporal horns, which could be an indirect sign of mesial temporal atrophy. At that time (2005), no CSF biomarker analysis or magnetic resonance imaging was performed. Repeated neuropsychological evaluation after 2 years showed a progressive cognitive decline still consistent with AD. Five years after onset of first symptoms, frontal features (hyperphagia, logopenia, and apraxia) became apparent, followed by bilateral parkinsonism at late stages (8 years after onset). The patient died at 69 years with no autopsy. Two siblings were later diagnosed with ALS with predominant bulbar signs. For one of the siblings diagnosed with ALS after the decease of this patient, mutation screening has been performed, confirming the presence of *TBK1* p.Thr79del. We previously identified this mutation in an unrelated Spanish FTD-ALS patient (van der Zee et al., 2017), who presented with a behavior disorder and was diagnosed with FTD fulfilling Rascovsky criteria. After 3 years, he developed rapidly progressive bulbar muscle weakness. Brain autopsy revealed frontolobar degeneration with TDP-43 positive inclusions and argyrophilic grain disease (stage III). Allele sharing analysis using a panel of flanking short tandem repeat markers indicated that the 2 mutation carriers have a common distant ancestor (Supplementary Table 6).

3.3. Effect of rare TBK1 variants on NFκB activation

The effect of the identified rare variants on NFκB induction was assessed in vitro by a luciferase reporter assay (Fig. 2). This included 14 *TBK1* variants, that is, the inframe deletion p.Thr79del, all 7 rare missense variants identified in the patient population, and all 6 missense variants identified in the patient/control population. The effects of the 18 rare missense variants identified in the control population only were previously reported, using the same procedures and equipment (van der Zee et al., 2017).We observed reduced NFκB induction for 5 variants, which included 2 variants observed only in the patient cohort, p.Thr79del and p.Ile418Val.

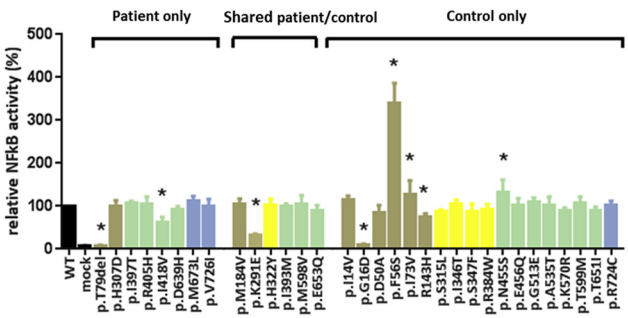

Fig. 2. Impact of mutant *TBK1* on NFκB activity in the IFN pathway. NFκB activity is shown for all patient only, shared patient/control and control only rare variants identified. Y-axis represents activity of NFκB relative to the reference wild type TBK1 vector transfection condition. Bars in brown reflect variants present in the kinase domain, bars in yellow reflect variants present in the ubiquitin-like domain, bars in green reflect variants present in the scaffold dimerization domain, and bars in blue represent variants present in the C-terminal domain. Asterisks above the bars indicate significant difference from the wild-type level after Bonferroni correction ($p < 0.001$). Mock refers to empty vector containing no TBK1. Error bars depict standard deviation. Abbreviation: WT, wild-type TBK1 vector. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Meta-analysis of *TBK1* rare variant burden

Country of origin	Rare alleles/total alleles patients	Rare alleles/total alleles controls	SKAT-O *p*-value
Belgium	5/534 (0.9%)	12/2064 (0.6%)	0.4
Spain	4/650 (0.6%)	6/592 (1.0%)	0.5
Italy	2/358 (0.6%)	1/592 (0.2%)	0.6
Portugal	2/196 (1.0%)	1/160 (0.6%)	0.6
Sweden	2/292 (0.7%)	4/546 (0.7%)	0.2
SKAT-O meta-analysis *p*-value	15/2030 (0.7%)	24/3954 (0.6%)	0.3

Rare variant burden analysis was performed using SKAT-O meta-analysis corrected for gender and *APOE* ε4 status, including individuals originating from Belgium, Spain, Italy, Portugal, and Sweden with nonmissing information on gender and *APOE* ε4 status. Individuals originating from Greece, Germany, and Czech Republic were excluded from analysis due to subthreshold cohort size. Percentages are based on alleles.

Confirming our earlier report, p.Thr79del showed near complete disruption of NFκB activity to 8.6 ± 1.1% of wild-type control activity level (p-value < 0.0001) (Fig. 2). Missense variant p.Ile418Val, located in the scaffold dimerization domain, showed reduced NFκB induction to 63.4 ± 11.6% of wild type control level (p-value 0.001). This mutation was detected in a Spanish sporadic patient with very early disease onset (48 years). A third variant, p.Lys291Glu, located in the kinase domain and showing reduced NFκB activation to 32.0 ± 4.0% (p-value < 0.0001), was detected in 1 patient (a Swedish female sporadic patient, onset age 52 years) and in 2 unaffected Belgian control individuals aged 67 and 70 years at inclusion. In addition, 2 missense variants specific to the control population showed reduction of NFκB activation (p.Gly16Asp [near complete reduction] and p.Arg143His [74.8 ± 3.0%]). Both variants are located in the kinase domain. Interestingly, 3 control-only missense variants showed an increase of NFκB activation. Two of these variants, p.Phe56Ser and p.Ile73Val are located within the kinase domain, whereas one p.Asn455Ser is located within the scaffold dimerization domain (Supplementary Table 3).

3.4. Rare variant association analysis

The frequency of rare variants in *TBK1* was 1.4% (18/1253) in the patient cohort and 0.6% (8/1253) for patient-only variants. Mutation frequency in the control cohort was 1.4% (29/2117) and 0.9% (20/2117) for control-only variants. Mutation frequency in patients with known familial history of AD was 1.5% (5/338) and 0.9% (3/338) for patient-only variants. SKAT-O meta-analysis performed across the entire *TBK1* coding region and for each of the 4 TBK1 protein domains separately showed no significant enrichment of rare variants in patients (SKAT-O p-value 0.3; Table 2, Supplementary Table 7). When limiting the analysis to the predicted most deleterious variants (CADD Phred score >20), we observed nominal significant enrichment of rare mutations in patients for the TBK1 scaffold dimerization domain (SKAT-O p-value 0.04), although we should note the small number of variant carriers included in this test (n = 6) (Supplementary Tables 8 and 9).

3.5. Single-variant association analysis of low-frequency and common variants

Two low-frequency variants were located in the *TBK1* coding region, rs35635889 (p.Val464Ala) and rs41292019 (p.Asn22Asn) (Hardy-Weinberg p-value 0.55 and 0.19, respectively), and 1 common synonymous variant rs7486100 (p.Ile326Ile) (MAF patients = 0.47; MAF controls = 0.42; Hardy-Weinberg p-value 0.49). Single-variant logistic regression analysis showed significant association between the rs7486100-T allele and EOAD in a recessive model (OR 1.46 95% CI [1.13–1.9], Bonferroni-corrected p value 0.012) (Supplementary Table 4A). All 3 variants were subsequently tested for disease association in a European FTD/FTD-ALS/ALS cohort previously described in (van der Zee et al., 2017). However, none of the variants showed association after correction for multiple testing in this cohort (Supplementary Table 4B). Homozygous rs7486100-TT carriers demonstrated decreased TBK1 expression levels in lymphoblast cell lines (2575 normalized read counts [standard error 188.9], n = 11) compared to AA and AT carriers (2932 [standard error 66.5], n = 66, Mann-Whitney p-value 0.028) (Supplementary Fig. 1).

4. Discussion

TBK1 LoF mutations have recently been identified as an important cause of FTD and ALS, with a notable tendency of carriers to display memory deficits early in the disease course. In addition, *TBK1* is regarded extremely intolerant of LoF mutations according to the Exac database (pLI = 1) (Lek et al., 2016). We performed a systematic screening of the coding sequence of *TBK1* in a large European cohort of 1253 EOAD patients to investigate the frequency of *TBK1* LoF mutations in AD patients, whether due to confounding of clinical presentation or due to a direct effect on AD risk. In contrast to studies on FTD and ALS reporting mutation frequencies from 1% to 4% (Cirulli et al., 2015; Freischmidt et al., 2015; Gijselinck et al., 2015; Pottier et al., 2015), we detected only 1 *TBK1* LoF mutation among EOAD patients, in a Spanish patient clinically diagnosed with EOAD (p.Thr79del; overall LoF carrier frequency 0.08%). *TBK1* p.Thr79del is an inframe deletion located in the kinase domain of TBK1, which was demonstrated to result in LoF due to a 50% reduction of TBK1 protein in postmortem human brain, reduction of TBK1 protein expression and absence of phospho-TBK1 in HEK293T cells overexpressing this mutation, and loss of NFκB activation (van der Zee et al., 2017). The same mutation was previously identified in an apparently unrelated FTD/ALS patient of the same nationality ascertained at a different research center (van der Zee et al., 2017). Of note, clinical follow-up of the AD patient carrying this mutation revealed symptoms compatible with FTD later in the course of the disease. Two siblings developed ALS, of which one could be tested genetically and was confirmed to carry the *TBK1* p.Thr79del mutation. Unfortunately, the diagnostic procedure of the index patient did not include biomarker analyses that could support either AD or FTLD diagnosis, and no autopsy was performed. The presence of the pathogenic *TBK1* mutation, however, suggests that this patient may represent an example of the atypical clinical presentation previously reported among FTD patients carrying a pathogenic *TBK1* LoF mutation (Pottier et al., 2015; Van Mossevelde et al., 2015). Although carriers of mutations in *APP*, *PSEN1*, *PSEN2*, *ABCA7*, *C9orf72*, *MAPT*, *PRNP*, and *GRN* were excluded from the study, we cannot exclude the possibility that the clinical phenotype of neurodegeneration in this patient was caused or modified by an as yet unknown mutation.

We did not observe an enrichment of rare variants in EOAD patients compared to controls (carrier frequency of 1.4% in both), in line with previous association studies performed on FTD and ALS patients (Freischmidt et al., 2015). Rare variants exclusively observed in patients were not more often novel and/or predicted to be pathogenic (based on CADD score) than rare variants only observed in controls. In addition, missense mutations with compromised NFκB activation capacity that have recently been proposed as rare risk variants for FTD (van der Zee et al., 2017) were not enriched among EOAD patients. Only 2 missense variants identified in patients resulted in reduced NFκB induction. One of those (p.Ile418Val) was observed in a patient with a clinical diagnosis of AD with very early onset age. The mutation affects the

scaffold dimerization domain and showed a modest effect on NFκB activation. Interaction between the scaffold dimerization domain and the kinase domain is required for TBK1 kinase activity and transphosphorylation activity in vitro (Shu et al., 2013). In absence of a positive family history, we were unable to further investigate the genetic evidence of pathogenicity of this mutation. The other mutation (p.Lys291Glu) showing decreased NFκB induction is located within the kinase domain. This mutation was detected in 1 patient and 2 control individuals. Both control-only missense variants disrupting NFκB activation, p.Gly16Asp, and p.Arg143His are also located within the kinase domain. Of note, it is presently unknown whether loss of NFκB activation is the molecular mechanism linking *TBK1* LoF mutations to neurodegeneration. Therefore, these results should be interpreted with caution. Nonetheless, lack of association between EOAD and rare *TBK1* missense variants, whether or not predicted or demonstrated to affect TBK1 functionality, argues against a significant role of rare *TBK1* variants in EOAD.

No associations of *TBK1* common and low frequency variants with dementia spectrum disorder diagnoses have yet been reported. In our meta-analysis covering EOAD cohorts originating from 5 European countries, a common synonymous variant (rs7486100) showed association in a recessive model. In light of the hypothesis that loss of TBK1 can contribute to neurodegeneration, we tested the effect of the risk allele on TBK1 expression. Interestingly, RNA sequence analysis on lymphoblast cell lines of homozygous carriers of the risk-increasing allele rs7486100-T showed decreased TBK1 expression. In line with this, carriers of the rs7486100-T allele showed decreased expression of TBK1 in multiple tissues according to the Gtex database, with lowest expression levels in homozygous rs7486100-T carriers. This variant tags a ~150 kb region of strong linkage disequilibrium extending to the flanking genes, and there is no evidence supporting a direct regulatory effect of rs7486100 (RegulomeDB score 6), warranting further investigation of common eQTLs in the region and their association with neurodegeneration spectrum disorders. Of note, however, rs7486100 did not show association with late-onset AD in a meta-analysis of genome-wide association studies (Lambert et al., 2013).

In conclusion, this investigation of common and rare variants in *TBK1* in a large European cohort of EOAD indicates that genetic variability in *TBK1* does not contribute significantly to the risk of EOAD. A common variant associated with decreased TBK1 expression may be enriched in EOAD patients compared to controls, but this requires further confirmation given the lack of association in late-onset AD. In diseases with stronger evidence of a genetic link between TBK1 LoF and neurodegeneration, such as FTD and ALS, further investigation of common variants affecting TBK1 expression is warranted. Our data revealed only 1 *TBK1* LoF variant among 1253 EOAD patients (LoF carrier frequency 0.08%), in a patient without a biomarker-supported or autopsy-confirmed diagnosis of AD. In light of the development of frontal features later in the course of the disease, this leaves open the possibility that this patient had FTLD with atypical clinical presentation due to early symptoms compatible with AD, in line with previous identification of *TBK1* LoF variants in FTD/ALS patients with initial clinical diagnosis of AD (Pottier et al., 2015; Van Mossevelde et al., 2015).

Missense mutations leading to significant reduction of NFκB activation were detected in AD patients, but despite the fact that these mutations may act as risk alleles in FTD (van der Zee et al., 2017), they were not significantly enriched in EOAD patients compared to controls. Although our findings do not support a role for TBK1 in the pathogenesis of EOAD, the recurring ambiguity of clinical diagnosis in carriers of pathogenic *TBK1* mutations necessitates further research on the clinical presentation of *TBK1* carriers.

Disclosure statement

SE received research funding from Janssen Pharmaceutica and from ADx Neurosciences and was/is consultant for Innogenetics/Fujirebio Europe, Lundbeck, Pfizer, Novartis, UCB, Roche diagnostics, Nutricia/Danone, Lilly, and Biogen. Other co-authors declare that they have no conflict of interest.

Acknowledgements

The authors thank the personnel of the Genomic Service Facility and of the Bioinformatics Unit of the VIB Department of Molecular Genetics for their support of the genetic analyses. The authors are indebted to brain donors and relatives for generous brain donation for research and to the Neurological Tissue Bank of the IDIBAPS Biobank, Barcelona, Spain, for data and sample procurement. The Stockholm site (Caroline Graff) wishes to express their acknowledgements to Associate Professor Inger Nennesmo for the neuropathological assessments, Huei-Hsin Chiang, Jenny Björkström, Lena Lilius, Charlotte Forsell, Marie Fallström, (Department of Neurobiology, Care Sciences and Society [NVS], Center for Alzheimer Research, Division of Neurogeriatrics, Karolinska Institutet and Department of Geriatric Medicine, Genetics Unit, Karolinska University Hospital, Stockholm, Sweden), and The Brain Bank at Karolinska Institutet.

At the Antwerp site, the data generation was in part funded by the Belgium Science Policy Office Interuniversity Attraction Poles program (http://www.belspo.be), the Alzheimer Research Foundation (S#13023) (http://alzh.org), the Flemish Government Initiated Methusalem Excellence Program to CVB, the Research Foundation Flanders (G043211N) (FWO, http://www.fwo.be), the University Research Fund, the Flemish Government initiated Flanders Impulse Program on Networks for Dementia Research, the MetLife Foundation Research Award to CVB, the EU FP7 project AgedBrainSYSBIO under grant agreement no. 305299 (http://ec.europa.eu/research/fp7). The Brescia IRCCS Fatebenefratelli site was funded by Ricerca Corrente, Italian Ministry of Health. EGT is supported by grants SAF2010-18277, PI14/00099, and FEDER funds (Madrid, Spain). The Florence site is funded by Fondazione Cassa di Risparmio di Pistoia e Pescia (grant 2014.0365 to BN), and a grant from Ministry of Health no. RF-2010-2319722 to SS. The Prague site was partly supported by research programs PRVOUK-P26/LF1/4 and P27/LF1/1 and OPPK CZ.2.16/3.1.00/24509 (Charles University, Prague, Czech Republic). AL receives a grant (PI14/00282) from the Spanish Ministry of Economy and Competitiveness ISCIII and co-funded by the European Regional Development Fund (ERDF). The Stockholm site (CG) was financially supported by Swedish Brain Power, Swedish Research Council (grant numbers 521-2010-3134; 2015-02926), Gun and Bertil Stohne, Gamla tjänarinnor, Demensfonden, Sweden Alzheimer Foundation, King Gustaf V, and Queen Victoria's Foundation of Freemasons and StratNeuro at Karolinska Institute (KI), Swedish Brain Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2017.10.012.

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