TMEM106B and *ApoE* polymorphisms in *CHMP2B* mediated frontotemporal dementia (FTD-3)

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Abstract

Single-nucleotide polymorphisms (SNPs) in the *TMEM106B* gene have been identified as a risk factor in frontotemporal dementia (FTD). The major allele of SNP rs3173615 is a risk factor in sporadic FTD, while the minor allele seems protective in *GRN*- and *C9orf72*-mediated FTD. The role of Apolipoprotein E (ApoE) in FTD is uncertain, though an established risk factor in Alzheimer's disease.

In a unique Danish family, inherited FTD is caused by a mutation in the *CHMP2B* gene located on chromosome 3 (FTD-3). In this family, both risk factors *TMEM106B* and *ApoE* were analyzed and correlated to Age At Onset (AAO) and progression in terms of institutionalization (AAI) and death (AAD). Although TMEM106B and CHMP2B share cellular function in that both localize to endolysosomes, *TMEM106B* genotypes appeared to have no influence on the clinical disease course. ApoE ε4 was found to be a protective factor with later AAO and AAI, while ε2 seemed to aggravate the disease with earlier AAO and AAD. These results indicate ApoE ε2 as a risk factor in FTD-3 and suggest a protective role of ε4.

Abbreviations

AAD: Age at Death, AAI: Age at Institutionalization, AAO: Age at Onset, AD: Alzheimers Disease, ApoE: Apolipoprotein E, bvFTD: Behavioral variant Frontotemporal Dementia, CHMP2B: charged multivesicular body protein 2B, CI: Confidence Interval, ESCRT: Endosomal Sorting Complex Required for Transport, FReJA: Frontotemporal dementia in Jutland Association, FTD: Frontotemporal Dementia, FTD-3: Chromosome 3-linked Frontotemporal Dementia, GWAS: Genome Wide Association Study, HR: Hazard Ratio, LD: Linkage Disequilibrium, m: Median Survival Time, SNP: single-nucleotide polymorphism, TMEM106B: Transmembrane Protein 106B (*TMEM106B)*

Keywords
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SNP rs3173615
ApoE

1 Introduction

Frontotemporal dementia (FTD) is the second most common cause of early onset dementia (< 65 years) surpassed only by Alzheimer's disease (AD). It is broadly characterized as either language variant FTD or behavioral variant FTD (bvFTD), the latter presenting with changes in personality, disinhibited behavior, lack of judgement, empathy and insight, while memory is often spared. Progression is diverse and most patients are institutionalized due to neuropsychiatric symptoms, while a later deterioration of motor function causes death.

While most other forms of dementias are mainly sporadic, FTD cases often present with a family history of neurodegenerative disease. The pattern of inheritance is often autosomal dominant and consequently a number of disease causing mutations have been identified in *MAPT*, *GRN*, *VCP*, *FUS*, *TARDBP* and *C9orf72* (Paulson and Igo, 2011; Rohrer et al., 2009).

In a large Danish family with an autosomal dominantly inherited FTD, the cause of disease has been identified as a single base mutation in the *CHMP2B* gene on chromosome 3 (FTD-3) resulting in early onset FTD (Brown et al., 1995; Gydesen et al., 2002; Skibinski et al., 2005; Urwin et al., 2009). The pedigree now encompasses more than 500 individuals distributed over twelve branches and six generations. Each branch is derived from one of the twelve children of the first case, a woman born in 1876 (Brown et al., 1995; Gydesen et al., 2002; Lindquist et al., 2008). The Danish FTD-3 family is unique not only in size, but also in carrying the disease causing *CHMP2B* truncating mutation (c.532-1G>C) (Skibinski et al., 2005). However, a distinct truncating mutation in CHMP2B has been identified in a Belgian familial FTD patient (van der Zee et al., 2008).

CHMP2B encodes the protein charged multivesicular body protein 2B which is a component of ESCRT-III (endosomal sorting complex required for transport) which participates in delivering protein cargoes to endosomes for lysosomal degradation (Henne et al., 2011). One of the pathological hallmarks of FTD-3 is enlarged endosomal structures in the frontal cortex of the brain as well as p62-positive cytoplasmic inclusions (Holm et al., 2007; Urwin et al., 2010). In addition we have recently reported that neurons in FTD-3 patient brains and in mutant CHPM2B mice have large autofluorescent inclusions reminiscent of lysosomal storage pathology (Clayton et al., 2015). Studies in patient fibroblasts and overexpression cell models have shown that the endosomal pathway is impaired in cells harboring the mutation and implicate

that the autophagocytic pathway is also affected (Clayton et al., 2015; Filimonenko et al., 2007; Lee and Gao, 2009, 2008; Urwin et al., 2010).

Recently, a genome wide association study (GWAS) has associated FTD with the transmembrane protein 106B (*TMEM106B)* gene, identifying single-nucleotide polymorphisms (SNPs) in this gene as a possible risk factor in sporadic FTD and amyotrophic lateral sclerosis (ALS) as well as a modifier of disease onset in *GRN* and *C9ORF72* mutation carriers (Cruchaga et al., 2011; Finch et al., 2011; Gallagher et al., 2014; Lattante et al., 2014; Van Blitterswijk et al., 2014; Van Deerlin et al., 2010; Van Der Zee et al., 2011; Vass et al., 2011). A C->G polymorphism located in exon 6 causes a threonine (T) to serine (S) substitution in the C-terminal of the transmembrane domain. The minor allele (G) appears to be protective in *C9orf72* expansion carriers, while the major allele (C) seems to be a risk factor in FTD (Van Der Zee et al., 2011).
TMEM106B, which at present is the most well replicated risk factor for FTD, is a glycosylated type 2 transmembrane protein found in the late endosomes and lysosomes and the levels of protein are modulated by lysosomal activity (Chen-Plotkin et al., 2012; Lang et al., 2012). The protein is highly expressed in neurons (Brady et al., 2013; Lang et al., 2012) and plays a role in the trafficking of neuronal lysosomes (Schwenk et al., 2013; Stagi et al., 2014).
Interestingly, recent findings show that TMEM106B associates with CHMP2B in cultured mouse cortical neurons and HEK293T cells (Jun et al., 2015).

The apolipoprotein E (*ApoE)* ε4 allele is a risk factor in sporadic AD while the *ApoE* ε2 allele seems to be protective (Boccardi et al., 2004; Giau et al., 2015; Gustafson et al., 1997; Raber, 2008; Raichlen and Alexander, 2014); however, the role of ApoE in FTD is unclear. Several studies have investigated the correlation of *ApoE* genotypes and disease but the results are somewhat contradictive (Bernardi et al., 2006; Boccardi et al., 2004; Chiò et al., 2016; Engelborghs et al., 2006; Giau et al., 2015; Gustafson et al., 1997; Mehta et al., 2007; Minthon et al., 1997; Pickering-Brown et al., 2000; Riemenschneider et al., 2002; van Blitterswijk et al., 2014a; Verpillat et al., 2002). Presence of *ApoE* ε2 allele significantly modulated risk of FTD in ALS patients independent of *C9orf72* expansion status, whereas *ApoE* ε4 was ineffectual (Chiò et al., 2016).
In this study we sought to clarify whether *TMEM106B* modifies disease in our FTD-3 cohort. As FTD-3 has complete penetrance in the mutation carriers, the influence of the *TMEM106B* genotype was evaluated in relation to age at symptom onset (AAO) and disease progression in terms of age at institutionalization (AAI) and age at death (AAD).
Further, we analyzed the role of *ApoE* in FTD-3, similarly correlating genotypes to AAO and disease progression.
As both CHMP2B and TMEM106 is involved in endolysosome function, we expected *TMEM106B* genotype to influence the clinical presentation of FTD-3, but differences in symptom onset and institutionalization did

not reach a significant level in a family based proportional hazards model. Consequently, the protective effect of the minor allele was not substantiated in this cohort.

We found carriers of the *ApoE* ε2 allele to have an earlier AAO and AAD, while the *ApoE* ε4 allele was protective in terms of AAO and AAI. These findings suggested an influence of *ApoE* genotypes in FTD-3 which is pathomechanisticly different from sporadic AD.

2 Materials and Methods

2.1 Study population

The FTD-3 family has been subject to extensive studies during more than 20 years within the Frontotemporal dementia in Jutland Association (FReJA) collaboration and biological material has been collected during this period for linkage analyses, gene identification and functional studies (Gydesen et al., 2002). Clinical characteristics have been recorded in 45 cases of disease, providing information about natural history, clinical characteristics and AAO.

Blood samples were collected from family members in eleven branches of the family. The eight branches in which FTD-3 has been identified are shown in Figure 1. As is clear from the pedigree, it would have been possible to deduce carrier status in parents of homozygotes; e.g. in a case of homozygosity of the *TMEM106B* minor allele, it could be deduced that also the parent would be carrier of the minor allele. This would however preselect for major carriers, and consequently only directly tested individuals were included in the analysis.

At the time of sampling, some had developed clinical FTD while most were at 50% risk of carrying the *CHMP2B* mutation. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-1-2012-041), and written informed consent was obtained from each participant before enrollment.

All blood samples were stored in the Danish Dementia Biobank. Samples from a total number of 80 individuals were included, and genotypes of *TMEM106B* and *ApoE* were established

Individuals not carrying the *CHMP2B* mutation were classified as controls, while mutation carriers who had not yet had onset of symptoms were classified as presymptomatic. In clinically affected individuals, the age at onset (AAO) was defined as the time for first symptoms reported by relatives.

While symptom onset is often difficult to assess, progression was estimated as age at institutionalization (AAI) and age at death (AAD). The Danish Consolidation Act on Social Services provides guidelines for the criteria for institutionalization, and although individual or regional differences may be of influence, AAI was considered a reliable marker of disease progression.

2.2 Procedures

DNA was purified using Maxwell 16 DNA purification kit (Promega) according to manufacturer's directions. *TMEM106B* genotypes for rs3173615 were Sanger sequenced according to manufacturer's instruction using BigDye Terminator v.1.1 cycle sequencing kit (Life Technologies) on an ABI 3130XL Genetic Analyser. CLC Main Workbench 7 software was used to analyze the sequences. *ApoE* genotyping for the ε2, ε3 and ε4 alleles was performed with a TaqMan qPCR assay as described earlier (Koch et al., 2002). All primer sequences are available on request.

2.3 Statistical analysis

Data were analyzed using SAS® software (Enterprise Guide 7.1, 2014, SAS Institute Inc., Cary, NC, USA). Overall frequencies of genotypes were evaluated using chi square calculations. In affected individuals AAO was normally distributed and independent of gender. Primary analysis of AAO using Cox proportional hazards models included 20 affected family members and 14 presymptomatic *CHMP2B* mutation carriers from eight branches (Figure 1). AAO was correlated to *TMEM* and *APOE* genotypes respectively in recessive, dominant and co-dominant models. All p-values are stated without correction for multiple models. Though all individuals were members of the same family, some individuals are closer related than others. This fact was taken into account by using a Cox analysis with robust standard errors allowing for non-independent observations (Gharibvand and Liu, 2009; Lin and Wei, 1989). With this approach we allowed for correlation between subjects in branch and siblingship. This approach is common in biostatistics and has been well applied in similar analyses of genetic susceptibilities in families (Couch et al., 2001). As samples were available from several generations in the family, the possibility of confounding was further reduced by including year of birth as a covariate in the Cox model. Analyses of AAI and AAD were carried out in similar Cox models. As AAI and AAD were closely correlated to AAO, disease progression was subsequently evaluated in Cox models of duration from AAO to AAI and to AAD (calculated as AAI-AAO and AAD-AAO respectively). In the co-dominant analysis of *ApoE* the ε3/ε3 was chosen as the neutral genotype.

3 Results

3.1 Genotypes of the cohort

In the cohort 46 individuals did not carry the *CHMP2B* mutation and were classified as controls. Twenty mutation carriers were clinically affected, while 14 mutation carriers were classified as presymptomatic. Characteristics of the cohort are provided in Table 1.

Initially, the frequencies of the *TMEM106B* and *ApoE* alleles in the *CHMP2B* family were assessed (Table 1). As expected, frequencies did not differ significantly between mutation carriers and controls for neither *TMEM106B* ($p=0.613$, Fisher's Exact Test) nor *ApoE* ($p=0.818$, Fisher's Exact Test). We found, however, that frequencies of both *TMEM106B* and *ApoE* alleles differed from the European background population as

summarized in www.ensebl.org (X^2=9.97, p=0.002), which was not unexpected either, considering that all samples were from within the first four generations of the same family.

While all combinations of the *TMEM* genotypes were represented in the cohort, (heterozygotes and homozygotes for the minor and the major allele, respectively), not all *ApoE* combinations were represented. The cohort lacked ε2/ε2 carriers entirely, and none of the affected individuals carried an ε2/ε3 genotype.

			TMEM106B			*ApoE*					
	No.	M/F	C/C	C/G	G/G	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
Affected	20	12/8	12 (0.60)	5 (0.25)	3 (0.15)	0	0	3 (0.16)	7 (0.37)	7 (0.37)	2 (0.10)
Presymptomatic	14	8/6	9 (0.64)	4 (0.29)	1 (0.07)	0	1 (0.07)	2 (0.14)	4 (0.29)	5 (0.36)	2 (0.14)
Controls	46	18/28	25 (0.54)	16 (0.35)	5 (0.11)	0	6 (0.07)	7 (0.15)	12 (0.26)	14 (0.30)	10 (0.22)

Table 1: Characteristics of the Danish FTD-3 cohort.
M/F: Male/Female
C: Major allele of the *TMEM106B* rs3173615 gene, G: Minor allele of the *TMEM106B* rs3173615gene. Frequencies in parentheses.
E2, E3 and E4: Apolipoprotein E polymorphisms. Frequencies in parentheses.
In one affected individual ApoE genotypes was not available.

3.2 Initial analysis of Age at Onset, Age at Institutionalization and Age at Death

Initial analysis found AAO correlated to year of birth (p=0.010) and to family branch (p=0.037). This latter correlation is illustrated in Figure 2. Though AAO seemed to cluster in certain siblingships, this correlation was not significant (p=0.073). Consequently, the following Cox analyses were nested only by branch and adjusted for year of birth.

As would be expected, AAI and AAD were closely correlated to AAO (p=0.002 and p=0.018 respectively).

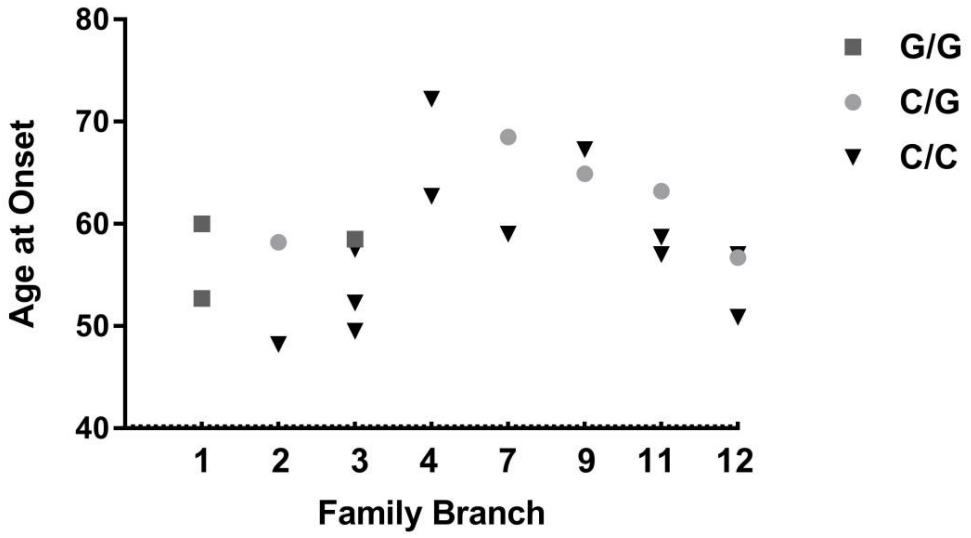

Figure 2:
AAO in family branches.
Symptomatic FTD-3 was identified in eight of the original twelve branches of the FTD-3 family. As illustrated, AAO was correlated to family branch, necessitating family based statistics nesting for branch.

3.3 Influence of *TMEM106B* on Age at Onset and disease progression

To assess whether *TMEM106B* influenced the disease course of FTD-3, AAO was compared to genotype in co-dominant, minor carrier dominant and minor carrier recessive models (the two latter being equivalent to major carrier recessive and major carrier dominant models respectively). Similar analyses were carried out for AAI and AAD, and the results are visualized in Figure 3. Although carriers of the *TMEM106B* rs3173615 minor allele (as either CG or GG) seemed protected in terms of later disease onset (median survival time (m) 59.3 (95% CI 52.7, 64.9)) when compared to individuals homozygous for the major allele (m 58.1 (95% CI 50.9, 62.7)), these finding were not significant in the Cox analysis (HR 0.988 (95% CI 0.419, 2.336), p=0.979). Similarly, minor carriers seemed protected from rapid progression in terms of a higher AAI (m 73.0 (95% CI 60.4, 76.7) than non-minor carriers (m 67.2 (95% CI 61.1, 72.0)). However, when correlating for AAO and family branch in a Cox analysis, this finding failed to reach significance (HR 0.608 (95% CI 0.220, 1.681), p=0.337). The influence of genotype on AAD ($p<0.0001$) was caused by the fact that only one minor carrier in the cohort had died, a woman living with the disease for 27 years reaching the age of 87 (as described in Gydesen et al., 2002, supplementary material, Case 3-III).

Neither minor recessive nor co-dominant modeling showed any significant influence on disease onset and progression.

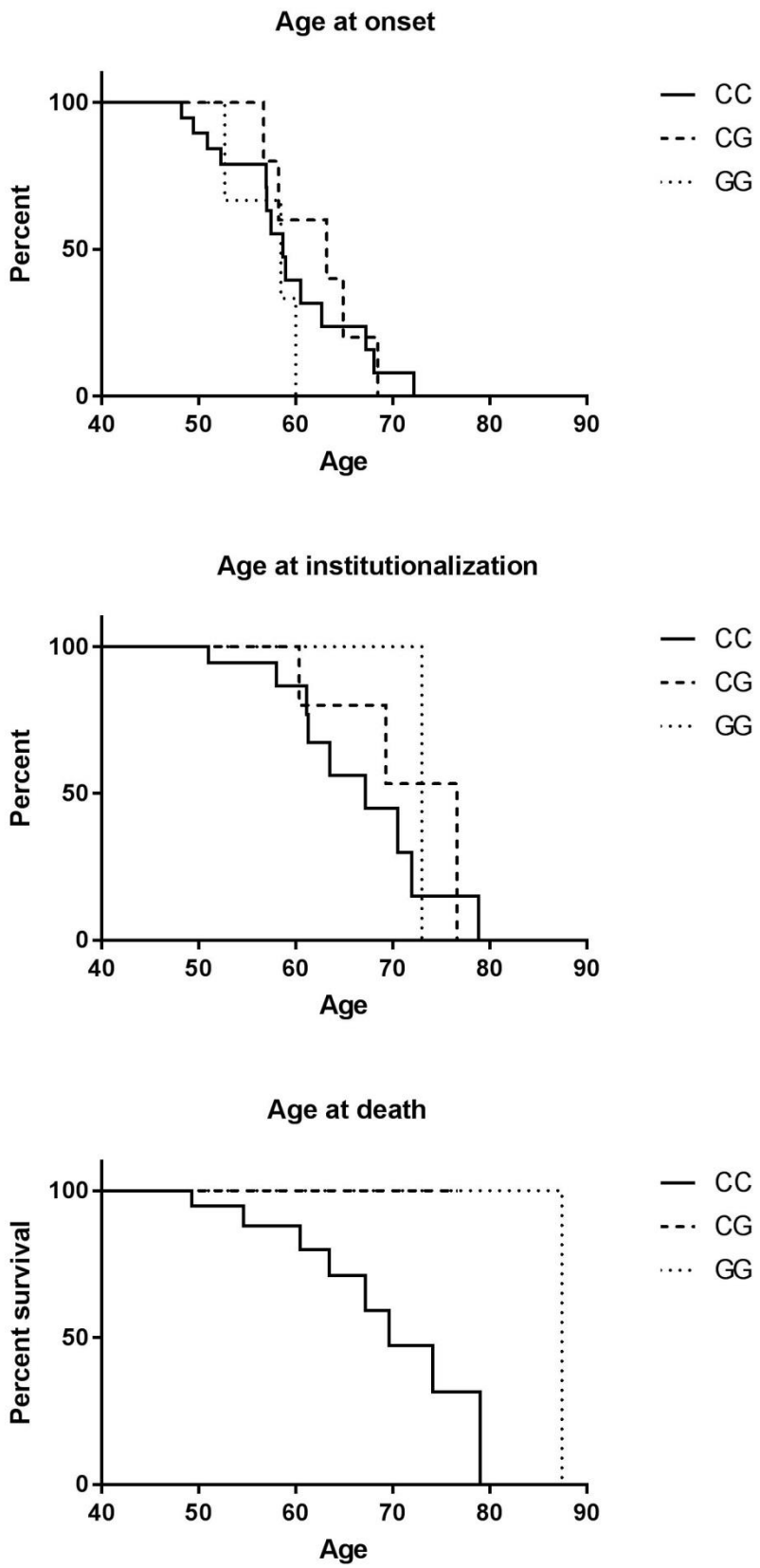

Figure 3

AAO, AAI and AAD in *CHMP2B* mutation carriers, stratified by *TMEM106B* genotype.

The subsequent analyses of proportional hazards ratios (Cox) were adjusted for year of birth and nested by family branch.

3.4 Influence of *ApoE* on Age at Onset and disease progression

Similar analyses of the influence of *ApoE* genotypes on FTD-3 disease were performed in co-dominant and ε4-, ε3- and ε2 -carrier dominant models. The overall impression from the results visualized in Figure 4 was that ε4 had a protective influence in terms of onset, institutionalization and death, while ε2 seemed a risk factor for early onset and rapid progression. In fact, ε3/ε4 and ε4/ε4 carriers had AAO late (m 60.3 (95% CI 58.2, 64.9) and 63.7 (95% CI 59.0, 68.5) respectively) when compared to ε3/ε3 and ε2/ε4 carriers (56.8 (95% CI 48.2, 62.7) and 52.3 (95% CI 49.5, 57.5) respectively). Similarly differences were seen on progression with ε3/ ε4 and ε4/ ε4 carriers being institutionalized later (m 70.5 (95% CI 61.1, 73.0) and 74.3 (95% CI 72.0, 76.7) respectively) than ε3/ε3 and ε2/ε4 carrier (63.5 (95% CI 60.4, NA) and 58.0 (95% CI 51.0, NA) respectively), and ε3/ε4 and ε4/ε4 carriers reaching a higher age before death (m 87.5 (95% CI 63.5, 87.5) and 74.1 (single observation) respectively) than ε3/ε3 and ε2/ε4 carrier (m 69.6 (95% CI 49.3, NA) and 67.2 (95% CI 54.6, 67.2) respectively).

In the co-dominant Cox analysis these differences reached significance for ε4 homozygotes in terms of AAO (HR 0.229 (95% CI 0.077, 0.680), p=0.0080) and AAI (HR 0.047 (95% CI 0.008, 0.274), p=0.0007).When compared to ε2 carriers the ε4 genotype was protective in AAO for both ε3/ε4 carriers (HR 0.201 (95% CI 0.072, 0.557), p=0.0021) and ε4 homozygotes (HR 0.078 (95% CI 0.030, 0.202), p<0.0001).

The prolonged survival from AAO to AAD were near significant in ε4 homozygotes (HR 0.134 (95% CI 0.017, 1.036) p=0.0541). The ε4 dominant model did not reach significance, probably due to the influence from ε2/ε4 carriers. When reproduced in the ε2 dominant model, the apparent risk of carrying ε2 (Figure 4) was near-significant in terms of AAO (HR 3.268 (95% CI 0.976, 10.989), p=0.0549) and significant in terms of AAD (HR 5.556 (95% CI 1.748, 17.857), p=0.0037).

There were no ε2 homozygous carriers in the cohort, nor any ε2/ε3 genotypes amongst affected family members. Consequently, as ε4 seemed protective in terms of AAO and there were no ε2 carriers not also carrying the ε4 allele, it was difficult to clarify the exact risks in ε2 carriers.

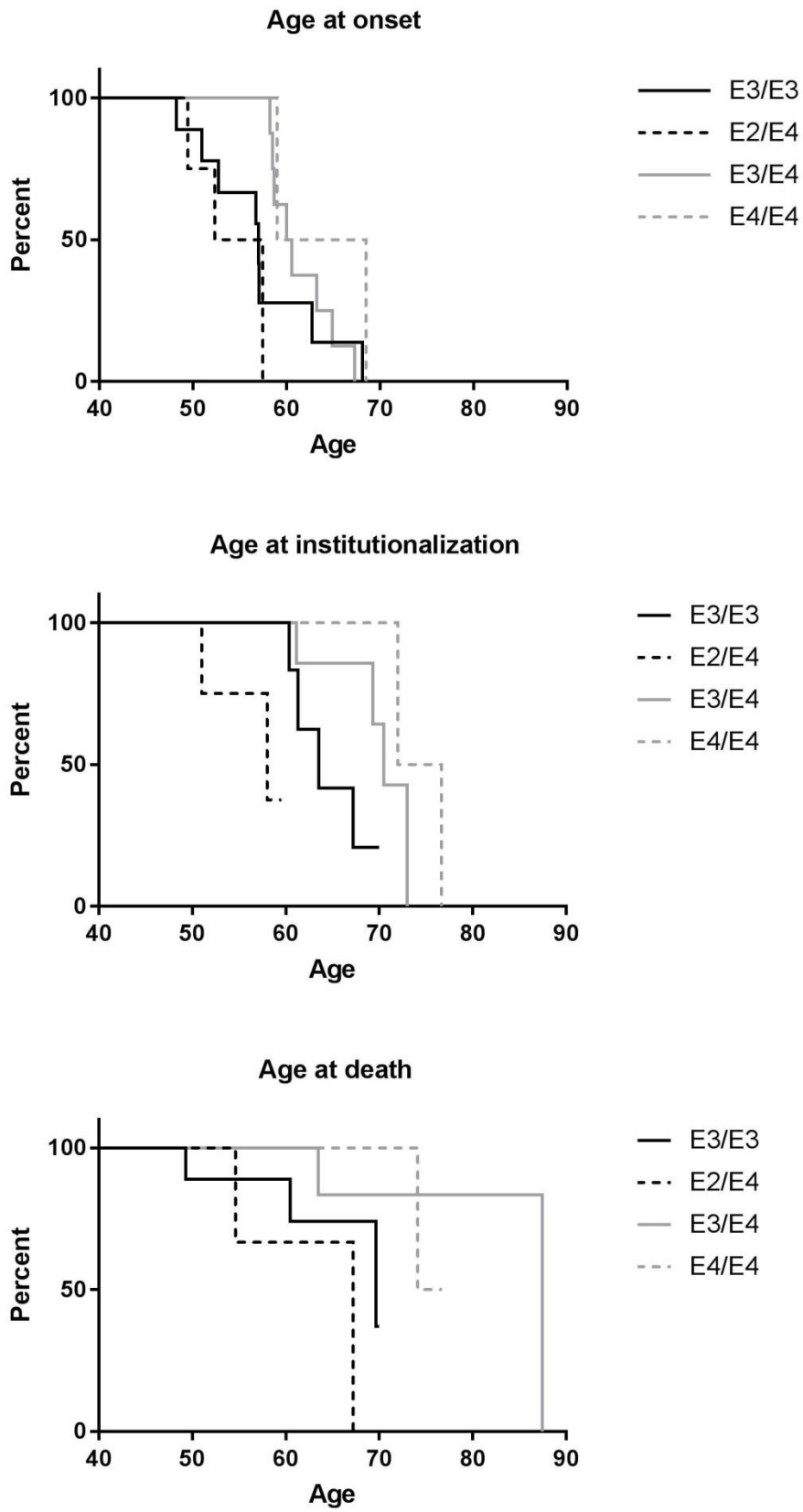

Figure 4

AAO, AAI and AAD in *CHMP2B* mutation carriers, stratified by *ApoE* genotype.

The subsequent analyses of proportional hazards ratios (Cox) were adjusted for year of birth and nested by family branch.

Discussion

In the Danish FTD-3 family we included 34 *CHMP2B* mutation carriers and correlated *TMEM106B* rs3173615 and *ApoE* genotypes to disease presentation. Although *TMEM106B* minor carriers had symptom onset and were institutionalized at a later age than major homozygous carriers, these apparently protective effects could not be reproduced in a family-based Cox model. This was somewhat surprising not only because previous studies have demonstrated that *TMEM106B* is a risk factor in the presentation of FTD, but also because TMEM106B is related to CHMP2B on a cellular level.

The minor allele of the rs3173615 has earlier been shown to be protective against FTD in *C9orf72* expansion carriers, and several studies on *GRN* mutation carriers and *C9orf72* expansion carriers have demonstrated that the minor allele of *TMEM106B* rs3173615 is not only protective of the development of FTD, but also beneficially modifies the disease in terms of penetrance, AAO, disease duration and age at death (Cruchaga et al., 2011; Finch et al., 2011; Gallagher et al., 2014; Lattante et al., 2014; Van Blitterswijk et al., 2014).

TMEM106B is localized in late endosomal and lysosomal compartments and is involved in lysosomal transport (Brady et al., 2013; Lang et al., 2012; Schwenk et al., 2013; Stagi et al., 2014). Likewise, CHMP2B is involved in endolysosomal trafficking by the ESCRT-mediated pathway and mutations in *CHMP2B* disrupts endosomal trafficking causing abnormal endosomes and lysosomal storage pathology in patient and mice brain (Clayton et al., 2015; Ghazi-Noori et al., 2012; Holm et al., 2007; Urwin et al., 2010).

Another linkage between *TMEM106B* and *CHMP2B* was suggested recently when a study found *TMEM106B* sequestered to *CHMP2B*-positive structures in a cellular model (Jun et al., 2015).

A similar pathophysiologic mechanism has been proposed in *GRN* mediated FTD after the finding that not only does TMEM106B colocalize to late endosomes, but the overexpression of TMEM106B elevates levels of intracellular progranulin and causes enlarged and less acidic endolysosomes (Brady et al., 2013; Chen-Plotkin et al., 2012). Taken together, these findings suggest a common molecular pathology in the endolysosomal pathway in FTD caused by mutated CHMP2B and progranulin. As TMEM106B seems to modulate the GRN pathology, we expected to see a similar effect in the CHMP2B pathology. In *C9orf72* mediated FTD, *TMEM106B* has been associated with disease risk (Lattante et al., 2014; Van Blitterswijk et al., 2014), and one study correlated *TMEM106B* genotype with AAO (Gallagher et al., 2014). Although loss of the normal function of C9orf72 is not thought to be the primary cause of *C9orf72* mediated FTD (Mizielinska and Isaacs, 2014), loss of function could play a modulary role. In this context it is intriguing that accumulating evidence points to a role for C9orf72 in endolysosomal trafficking and autophagy (Busch et al., 2016; Farg et al., 2014; Levine et al., 2013; Sellier et al., 2016; Zhang et al., 2012). Therefore, *TMEM106B*, *GRN*, *C9orf72* and *CHMP2B* FTD appear linked via their roles in endolysosomal function.

Although we failed to demonstrate a protection by the *TMEM106B* minor allele amongst our *CHMP2B* carriers, this lack of a significant effect could be due to our small sample size.

In our cohort, carrying the *ApoE* ε4 allele was found to delay symptom onset, institutionalization and death, suggesting a protective effect of this allele. This effect was apparent in heterozygous carriers, and was further strengthened in ε4 homozygous carriers with significantly lower hazard ratios in AAO and AAI. Our analysis of *ApoE* was weakened by the few carriers of the ε2 isoform in the cohort. Nevertheless, our analysis found an increased risk of early onset in ε2 carriers to be near-significant. Although evaluation of progression was further weakened by the small number of individuals who had been institutionalized (N=13) and had died (N=8) we did find a significantly earlier AAD in ε2 carriers. The protein ApoE has been extensively studied in sporadic AD, where the isoform ε4 is established as a risk factor. The role of ApoE in FTD is however poorly understood. Associating *ApoE* genotypes to FTD have produced conflicting results. Carrying the ε4 allele has been suggested as a risk factor in sporadic FTD (Bernardi et al., 2006; Gustafson et al., 1997), while a larger meta-analysis found no significant association between ε4 and FTD (Verpillat et al., 2002). In a heterogeneous cohort of sporadic FTD, ε4 was associated with early AAO (Minthon et al., 1997), while this was found not to be the case in a pathologically more homogenous group (Pickering-Brown et al., 2000). In genetically homogeneous cohorts, ε4 was found to be a risk factor for penetrance of FTD in VCP-mutation carriers (Mehta et al., 2007), and was found to be a risk factor for decreased survival in *C9orf72* FTD (van Blitterswijk et al., 2014b).

To the best of our knowledge, the results presented here are the first to find *ApoE* ε4 associated with AAO in a genetically homogeneous FTD. Suggesting ε4 to be protective of FTD is somewhat contradictory to earlier findings, but given the diversities of former studies, a possible protective effect cannot be ruled out.

While the role of the *ApoE* allele ε4 in FTD is debatable, we add to the increasing evidence of ε2 as a risk factor. The results of our study suggested the *ApoE* isoform ε2 as an aggravating factor in FTD-3, since the allele was found to correlate with earlier AAO and AAD. This is not inconsistent with earlier findings of ε2 as a risk factor for sporadic FTD (Gustafson et al., 1997; Lehmann et al., 2000; Verpillat et al., 2002), and the presence of the ε2 allele has been associated with the occurrence of FTD in ALS (Chiò et al., 2016). Our findings support this correlation, strengthening it with the prospect of an earlier AAO in ε2 carriers in a genetically homogenous cohort. Previous studies on *ApoE* and genetically verified FTDs did not include the ε2 allele in analysis (Mehta et al., 2007; van Blitterswijk et al., 2014b). Thus, we are yet to see the possible risk of ε2 reproduced in other genetically homogenous FTD subtypes.

The molecular mechanisms of ApoE is widely described in cholesterol metabolism (Giau et al., 2015; Raichlen and Alexander, 2014), and although an interaction with amyloid has been demonstrated (Deroo et al., 2015), the exact role of ApoE in AD is still poorly understood. The role of ApoE in FTD is less well established, and we propose further investigations in this modifier of familial FTD.

Conclusion

With the finding of a possible protective role of *ApoE* ε4 in FTD and the increasing evidence of ε2 as a risk factor, we suggest further investigations in the role of *ApoE* in FTD as a modifier of sporadic as well as familial FTD.

Although both CHMP2B and TMEM106B are involved in the endolysosomal pathway, we failed to demonstrate *TMEM106B* as a modifier of clinical FTD-3.

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FReJA consortium: Gade A, Englund E, Fisher E, Stockholm J, Thusgaard T.

Conflicts of interest

None.

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