Brain, Behavior and Immunity

Inflammatory markers of *CHMP2B* mediated frontotemporal dementia

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Abstract

Mutations in the *CHMP2B* gene cause a behavioral variant frontotemporal dementia (bvFTD) of autosomal dominant inheritance. The disease is most extensively described in the large Danish cohort known as the FTD-3 family. Although recognized as a disease of endolysosomal dysfunction, animal models have suggested an inflammatory component in the pathomechanism of disease. Early histopathology studies have found activated microglia in the brains of deceased FTD-3 patients, further suggesting a neuroinflammatory process.

In this cross-sectional study of the Danish FTD-3 family, serum and cerebrospinal fluid were analyzed for inflammatory and microglial markers in *CHMP2* mutation carriers and in non-carriers as controls. Apart from the *CHMP2B* gene, the cohort was considered genetically homogenous.

Kruskal-Wallis analyses found no differences in 30 biomarkers in the CSF, while serum levels of CCL4 were increased early and throughout life in *CHMP2B* mutation carriers.

In a novel approach of correlating biomarkers to disease burden, serum levels of IL-15, CXCL10, CCL22 and TNF-α were found significantly associated with cognitive decline, suggesting a circulating inflammatory response to the *CHMP2B* mediated neurodegeneration. CSF levels of sTREM2 appeared to increase more

rapidly with age in *CHMP2B* mutation carriers than in controls, emphasizing the role of TREM2 as an early response to neurodegeneration.

Establishing an inflammatory profile has proved difficult in sporadic as well as genetic FTD. Though failing to identify a specific intrathecal inflammatory response in *CHMP2B* mutation carriers, the identification of a circulating inflammatory response to disease progression supports the impression of an inflammatory component in FTD-3.

Keywords

Autosomal Dominant inherited Frontotemporal Dementia

FTD-3

CHMP2B

Inflammation

TREM2

1 Introduction

Frontotemporal Dementia (FTD) is a neurodegenerative disease of behavioral changes, language disorders and a marked cognitive decline. The behavioral variant FTD (bvFTD) presents with personality changes, disinhibition, lack of judgement and loss of empathy and insight [1]. Onset is often early in life, progression is inevitable, and with no available treatment, life expectancy is reduced [2–5]. Familial aggregation is present in 40% of cases, often presenting an autosomal dominant pattern of inheritance [2,6]. Disease causing mutations have been identified in a number of genes including *MAPT*, *GRN*, *C9orf72, VCP*, *FUS*, *TARDBP* and *CHMP2B*, the first three of which accounting for half of all familial cases [7,8].

With cerebrospinal fluid (CSF) providing easily accessible biomarkers, several studies have suggested inflammation as a component of the neuropathological process in FTD [9–13]. However, different studies have provided inconsistent results, and so far no specific intrathecal inflammatory profile for neither sporadic nor genetic FTD has been established.

The *CHMP2B* mediated bvFTD was first described in a large Danish family with an autosomal dominantly inherited dementia, and the cause of disease was identified as a single base mutation in the *CHMP2B* gene on chromosome 3, coining the disease name FTD-3 [14–17]. Since the discovery, diagnostic and presymptomatic testing for the disease causing mutation has been available for at-risk members of the Danish family.

The pathomechanism of *CHMP2B* mediated neurodegeneration appears to be a disruption of endolysosomal degradation causing accumulation and enlargement of endosomes [18–22]. Although

considered an endosome storage disease, previous studies have suggested a possible inflammatory component in the neurodegenerative process; histopathology of the brains of deceased FTD-3 patients have found astrocytic gliosis [15,23], and analyses of mRNA have found a trend towards a proinflammatory profile in patient frontal cortices [24]. In *CHMP2B*$^{intron\ 5}$ mutated transgenic mice, astrogliosis is present at 12 months of age [25], and analyses of mRNA find a significant overexpression of proinflammatory cytokines at 18 months of age [24].

A recent finding of elevated levels of neurofilament light chain (NfL) in CSF of *CHMP2B* mutation carriers demonstrated that the neurodegenerative process is detectable as early as the fourth decade of life [26]. This finding prompted the expectation that an inflammatory process would also be detectable at an early stage. To evaluate the possible inflammatory component of the *CHMP2B* mediated neurodegeneration, this study investigated the CSF and serum of FTD-3 family members, comparing inflammatory biomarkers in *CHMP2B* mutation carriers with non-carriers (controls) in this otherwise genetic homogenous cohort. In CSF and serum, 29 inflammatory cytokines and chemokines were analyzed. In CSF, the soluble Triggering Receptor Expressed on Myeloid cells 2 (sTREM2) was measured as a marker of neuroinflammation and microglia activity [27,28].

As this study was cross-sectional, subsequent analysis correlated biomarkers to disease burden as measured by cognitive assessments in all *CHMP2B* mutation carriers.

2 Materials and Methods

2.1 Study population

The *CHMP2B* mediated FTD has been extensively studied in the large Danish FTD-3 family by the **F**rontotemporal dementia **Re**search in **J**utland **A**ssociation (FReJA) consortium. More than four hundred individuals from this family are alive and at risk of carrying the disease causing mutation in the *CHMP2B* gene. At the Danish Dementia Research Centre, Rigshospitalet, Denmark all family members are offered genetic counselling and presymptomatic testing. When presenting initial symptoms of FTD, family members undergo extensive diagnostic work-up including genotyping and analysis of biomarkers in blood and cerebrospinal fluid. With the consent of the patient, extra serum and CSF samples are stored in the Danish Dementia Biobank for the purpose of research. Additionally, a family contact group updates healthy family members with potential research studies, providing samples from volunteers within the family. The genetic status of those participants is analyzed anonymously, allowing for the inclusion of family members who do not want to know their genetic status.

This study included only members from within the large Danish FTD-3 family, providing a genetically homogenous cohort. Both presymptomatic and clinically affected *CHMP2B* mutation carriers were

included, while family members without the disease causing mutation constituted the control group. All healthy volunteers and most of the clinically affected FTD-3 patients were clinically assessed at the time of sampling. Neuropsychological profiling employed Addenbrooke's Cognitive Examination (ACE) as a standardized and reliable evaluation of global cognitive function [29].

The inclusion of both diagnostically obtained samples and voluntarily submitted samples was approved by the regional Health Research Ethics Committee (protocol number H-1-2012-041). All subjects signed an informed consent form. Participants affected with FTD-3 were included in early stages of disease to ensure that informed consent was legitimate.

Details of the cohort are given in table 1.

2.2 Cerebrospinal fluid and serum

CSF samples were obtained by standard lumbar puncture in the L3/L4 or L4/L5 interspace. Samples were collected in polypropylene tubes, directly placed on ice and within an hour centrifuged at 2000 RPM for 10 minutes, then aliquoted into cryo tubes and stored at -80°C in accordance with consensus criteria [30]. Blood samples were obtained by venipuncture, collected in EDTA tubes and within an hour centrifuged at 2000 RPM for 10 minutes to separate serum and plasma from full blood. Serum and plasma was aliquoted into cryo tubes and stored at -80°C. Samples were thawed hours prior to analysis. Most samples were thawed and analyzed within the first year of storage, although at least two CSF samples were stored longer.

2.2 Immuno assay

Cytokines and chemokines were measured by multiplex, high-sensitivity electrochemiluminescent enzyme-linked immunosorbent assays (Meso Scale Discovery (MSD), Maryland, USA) using V-PLEX panels Proinflammatory Panel 1 Human, Cytokine Panel 1 Human and Chemokine Panel 1 Human. All assays were carried out according to the manufacturer's specifications, read using a MESO QuickPlex SQ 120 and analyzed using DISCOVERY WORKBENCH 4.0 (MSD).

The analyzed biomarkers are given in recent nomenclature (manufacturer's nomenclature in parenthesis) and included CCL11 ('Eotaxin' in manufacturers nomenclature; Lower Limit of Detection (LLOD) 3.26 pg/mL), CCL26 ('Eotaxin-3'; LLOD 1.77 pg/mL), GM-CSF (LLOD 0.14 pg/mL), IFN-γ (LLOD 0.20 pg/mL), IL-1α (LLOD 0.09 pg/mL), IL-1β (LLOD 0.04 pg/mL), IL-2 (LLOD 0.09 pg/mL), IL-4 (LLOD 0.02 pg/mL), IL-5 (LLOD 0.22 pg/mL), IL-6 (LLOD 0.06 pg/mL), IL-7 (LLOD 0.16 pg/mL), CXCL8 ('IL-8'; LLOD 0.04 pg/mL), IL-12 ('IL-12/IL-23p40'; LLOD 0.39 pg/mL), IL-12p70 (LLOD 0.11 pg/mL), IL-13 (LLOD 0.24 pg/mL), IL-15 (LLOD 0.17 pg/mL), sIL-16 (LLOD 2.83 pg/mL), IL-17A (LLOD 0.74 pg/mL), CXCL10 ('IP-10'; LLOD 0.37 pg/mL), CCL2 ('MCP-1'; LLOD 0.09 pg/mL), CCL13 ('MCP-4'; LLOD 1.69 pg/mL), CCL22 ('MDC'; LLOD 1.22 pg/mL), CCL3

('MIP-1α'; LLOD 3.02 pg/mL), CCL4 ('MIP-1β'; LLOD 0.37 pg/mL), CCL17 ('TARC'; LLOD 0.22 pg/mL), TNF-α (LLOD 0.04 pg/mL), LT- α ('TNF-β'; LLOD 0.05 pg/mL) and VEGF (LLOD 1.12pg/mL).
All samples were analyzed as duplicates, and if one or both sample concentrations were below detection range, the observation was discarded. Only if both concentrations were within detection range, mean concentration was calculated and assigned to the observation. As the concentration of many biomarkers in CSF were close to or below detection range, this restrictive inclusion criteria was compensated by applying comparison by ranks in the subsequent analysis (please see section 2.4 Statistical Analysis).
Soluble TREM2 (sTREM2) was measured in CSF only. CSF levels of sTREM2 was measured using an in-house electrochemiluminescent assay on an MSD SECTOR imager 6000 (Meso Scale Discovery (MSD), Maryland, US), using a method adapted from Kleinberger [31]. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay CVs were <10%, and all samples were measured on the same day using the same reagents.

2.4 Statistical analysis

Data were analyzed using SAS® software (Enterprise Guide 7.1, 2014, SAS Institute Inc., Cary, NC, USA). The expected finding that many CSF concentrations were close to or below detection range necessitated the employment of comparison by ranks. Consequently, differences in markers between *CHMP2B* carriers and controls were compared by Kruskal-Wallis test (the NPAR1WAY procedure in SAS®). Normal distribution of biomarkers was assessed by a Kolmogorov-Smirnov goodness-of-fit test (the UNIVARIATE procedure in SAS®). Applying comparison by ranks did not only compensate for the observations below detection range, but also allowed comparison of the few markers not normally distributed.
In subsequent analysis, inflammatory markers normally distributed (or normally distributed after logarithmic transformation) were analyzed in general linear modelling (The GLM procedure in SAS®) testing the influence of age at time of sample, and whether CSF concentrations were correlated to serum levels. Similar analysis including only *CHMP2B* mutation carriers tested the possible correlation between biomarker concentrations and disease progression as measured by ACE. Where ACE was unavailable, observations were omitted in this latter analysis.
Differences were considered significant at the $p<0.05$ level, and all p-values were stated without correction for multiple testing.

3 Results

Serum was available in 18 *CHMP2B* mutation carriers and in 16 controls, and CSF was available in 17 *CHMP2B* mutation carriers and in 15 controls. Serum levels of GM-CSF, IL-1β, IL-4 and LT-α, and CSF levels

of GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-12p70, IL-13, IL-17A and LT-α were below detection range in all samples. The remaining cytokine measurements are summarized in Table 2 stratified by *CHMP2B* mutation status, and with p-values denoting differences between mutation carriers and controls by Kruskal-Wallis test. Serum levels of CCL4 were significantly higher in *CHMP2B* mutation carriers ($H(1)=4.99$, $p=0.026$), although this difference would not hold for correction for multiple comparisons. The finding of a significantly higher serum level of IL-1α in controls ($H(1)=6.01$, $p=0.014$) merely reflected the fact that no serum levels reached the detection limit in *CHMP2B* mutation carriers, while six controls had levels above LLOD.

Serum levels of CXCL8 and CCL13 and CSF levels of IL-15 and sTREM2 were significantly correlated to age (GLM, $F(1,31)=6.34$, $p=0.017$; $F(1,31)=6.64$, $p=0.015$, $F(1,30)=6.97$, $p=0.013$ and $F(1,30)=6.53$, $p=0.016$ respectively). When including age as covariate, serum levels of CCL4 were still significantly increased in *CHMP2B* mutation carriers (GLM, $F(2,29)=6.02$, $p=0.020$), as illustrated in Figure 1. CSF levels of sTREM2 tended to increase more rapidly with age in *CHMP2B* carriers than in controls (Figure 2), but this difference was not significant (GLM, $F(2,29)=2.83$, $p=0.104$).

CSF levels of cytokines IL-12, sIL-16, CCL13, CCL4 and CCL17 were significantly correlated to serum levels (GLM, $F(1,26)=4.43$, $p=0.043$; $F(1,27)=4.75$, $p=0.038$; $F(1,2)=35.77$, $p=0.027$; $F(1,26)=12.16$, $p=0.002$; $F(1,13)=18.02$, $p=0.001$, respectively), but including serum levels as covariates in GLM analysis did not yield any significant differences between *CHMP2B* mutation carriers and controls (data not shown), except for CSF levels of CCL11 (GLM, $F(2,12)=3.31$, $p=0.045$).

When including only *CHMP2B* mutation carriers, differences between presymptomatic carriers and clinically affected FTD-3 patients became apparent. CSF levels of IL-15 and IL-1α and serum levels of IL-15, CCL22, CCL4 and TNF-α were increased in clinically affected FTD-3 patients (p-values of Kruskal-Wallis test in Table 3). The observed differences in serum CCL13 levels should be attributed to the differences in age between the two groups.

Additionally, serum levels of IL-15, CXCL10, CCL22 and TNF-α were significantly correlated to disease burden as measured by ACE (GLM, $F(1,14)=7.05$, $p=0.019$; $F(1,14)=5.01$, $p=0.042$; $F(1,14)=5.89$, $p=0.029$; $F(1,14)=22.94$, $p<0.001$, respectively). As low ACE score indicates a high disease burden, Figure 3 illustrates the positive correlation between disease burden and the inflammatory markers. The significant correlation to serum TNF-α levels holds for correction for multiple comparison. No CSF levels of TNF-α were within detection range in *CHMP2B* mutation carriers.

Discussion

In the effort to identify an inflammatory profile in *CHMP2B* mediated bvFTD, this cross-sectional study evaluated 30 biomarkers in the serum and CSF of FTD-3 family members, comparing both presymptomatic and clinically affected *CHMP2B* mutation carriers to controls. No specific differences were found in CSF, while a systemic inflammatory response occurring during the neurodegenerative process was apparent in serum.

The failure to detect an intrathecal inflammatory response was surprising because earlier studies on *CHMP2B* mediated neurodegeneration had identified neuroinflammation in patient brains and animal models of FTD-3 [15,23–25]. Further, the recent findings of a neurodegenerative process as early as the fourth decade of life in *CHMP2B* mutation carriers [26] would imply that an early neuroinflammatory response should be detectable.

Establishing an intrathecal inflammatory profile in FTD has previously proven difficult; one early study of presumed sporadic FTD reported elevated CSF levels of TNF-α [9], while another study reported decreased CSF levels of IL-12 [32]. Smaller studies reported elevated CSF levels of CXCL8 and IL-15 [33,34]. All of these findings were however negated in a later study [13]. Reports of elevated CSF levels of TGF-β and IL-11 [9,11] have not yet been reproduced nor negated, while elevated CSF levels of CCL2 in sporadic FTD have been identified in two studies [13,33].

With the identification of FTD causing genes, focus has shifted to genetic homogenous FTD cohorts. As the progranulin protein seems involved in neuroinflammation and microglia activation [10,35,36], the *GRN* mediated FTD appeared promising in terms of identifying an inflammatory component of FTD. In a small cohort of *GRN* mutation carriers, an apparent CSF profile of elevated CXCL10 and decreased levels of TNF-α, IL-15 and CCL5 has been described [13].

An interesting and consistent finding is the elevated CSF levels of CHI3L1 (YKL-40) in pathomechanistic different FTD subtypes [37,38]. However, as an unspecific biomarker of neuroinflammation and astrogliosis, CHI3L1 is increased in other types of dementia as well, offering no specific profiling of FTD. A recent study of the Danish FTD-3 family found no differences in CSF levels of CHI3L1 between *CHMP2B* mutation carriers and controls [26], challenging the assumption of neuroinflammation in FTD-3.

The finding that sTREM2 concentrations are positively correlated to age is in accordance with earlier similar findings [28]. The tendency that sTREM2 levels increased more rapidly in *CHMP2B* mutation carriers compared to controls could support an inflammatory component in the development of FTD-3. The finding did not reach the level of significance, and reassessment in a larger cohort is required.

The role of TREM2 in FTD is complex. Although a rare cause of disease, homozygous carriers of mutations in the *TREM2* gene develop an FTD-like phenotype [39], and heterozygous carriers of pathogenic *TREM2*

polymorphisms have been identified in cohorts of FTD patients [40]. In carriers of pathogenic *TREM2* mutation, CSF levels of sTREM2 are decreased, suggesting a loss-of-function as the pathomechanism [41]. Interestingly, one study also found CSF levels of sTREM2 decreased in a larger cohort of patients with FTD, including *GRN* and *C9orf72* mediated FTD [31].

In the context of inflammation, TREM2 encodes a receptor expressed on immune cells regulating phagocytosis, and TREM2 levels increase with inflammatory stimuli in a number of tissues. In the brain, TREM2 is expressed exclusively by microglia [42], and sTREM2 levels have been suggested as a marker of microglia activity and neuroinflammation [27].

Recent studies have suggested TREM2 as an early response to neurodegeneration [28]. In the cohort of *CHMP2B* mutations carriers, this response could very well be reflected in the increase of sTREM2 levels around the age of onset of clinical FTD-3 (Figure 2). As sTREM2 levels did not correlate to disease burden as measured by ACE, the early response did not accelerate further with the progression of disease.

In serum, the inflammatory marker of CCL4 was elevated in *CHM2B* mutation carriers early and throughout life, apparently peaking around the time of symptom onset in the sixth decade (Figure 1). Most well described in patients infected with human immunodeficiency virus type 1 (HIV-1), CCL4 is involved in the activation of neutrophilic inflammation and cytokine release. Increased intrathecal levels have been associated with dementia caused by HIV [43], and the secretion of CCL4 have been induced by microglia [44]. Although this latter association was induced by the HIV-1 transcriptor protein Tat, it is intriguing that the neuroinflammatory process can be mirrored in the serum of *CHMP2* mutation carriers.

Serum levels of IL-1α were undetectable in *CHMP2B* mutation carriers, while present in controls. An early and acute cytokine in the pathway to TNF-α activation, IL-1α would expectedly be increased in a neuroinflammatory process, but this was not demonstrated in neither this nor earlier studies [45].

Blood samples are more easily accessible than CSF, but studies of circulating inflammatory biomarkers in FTD patients are nevertheless scarce and have proven equally incoherent; an early study found levels of IL-6 increased in *GRN* mediated FTD when compared to presymptomatic *GRN* carriers, suggesting an inflammatory response at the onset of symptomatic FTD [46]. The same study found IL-6 unaltered in sporadic FTD. That this pathomechanism should be exclusive in *GRN* mediated FTD was later challenged by the finding that plasma levels of IL-6 were elevated in all FTD patients compared to controls, with no differences between sporadic FTD and genetic (*GRN*, *MAPT* and *C9orf72*) mediated FTD [47]. And while the first study found TNF-α levels unaltered in both *GRN* and sporadic FTD [46], a later although smaller study found TNF-α levels elevated in *GRN* mediated FTD when compared to controls [35]. Recently, a small but

comprehensive study of sporadic and *GRN* mediated FTD found no alterations in the serum levels of 27 circulating inflammatory biomarkers when compared to normal controls, including measurements of IL-6 and TNF-α [13].
Conclusively, the efforts of establishing an inflammatory profile in FTD have produced incoherent results.

In a novel approach, this cross-sectional study of the *CHMP2B* cohort correlated biomarkers with disease burden as measured by ACE.
A circulating inflammatory response was suggested by the increased serum levels of TNF-α , IL-15, CCL22 and CCL4 in clinically affected FTD-3 patients when compared to presymptomatic *CHMP2B* mutation carriers. This possible response was substantiated by the observation that the first three of these biomarkers along with CXCL10 were positively correlated to disease burden (Figure 3), suggesting an increasing inflammatory response to the advancing neurodegenerative process.
In this respect, IL-15 and TNF-α are especially interesting; it has been hypothesized that they share a common biological pathway with TNF-α inducing IL-15 transcription via the NF-κB complex. TNF-α is regulatory of the entry of immune cells into the brain [48]. It has been the subject of earlier studies in FTD, and increased levels of TNF-α have been identified in CSF and serum of FTD patients [9,35]. However, other studies have negated this in *GRN* mediated as well as sporadic FTD [13,46].
The peripheral inflammatory response identified in the *CHMP2B* mutation carriers could very well reflect an intrathecal neuroinflammatory process. Unfortunately, only one measurement of TNF-α in the CSF of *CHMP2B* mutation carriers were within detection range, making it impossible to identify an intrathecal TNF-α response to disease burden. A more sensitive analysis of TNF-α in CSF would be required to further explore this possibility.

The intention of comparing *CHMP2B* mutation carriers to non-carriers in an otherwise genetic homogenous cohort was achieved by including only members of the Danish FTD-3 family. This, on the other hand, limited the sample size. Small cohorts and an overall scarcity of CSF are common circumstances when studying FTD; most of the mentioned reports on CSF included less than twenty patients with FTD, reflecting the difficulties in obtaining coherent results when cohorts are small.
The failure to identify an intrathecal inflammatory profile in *CHMP2B* mediated FTD may also in part be due to the low number of observations in which biomarkers in CSF were within detection range. This limitation was compensated by the primary comparisons by ranks, but was not compensated in the subsequent correlation of biomarkers to disease burden. Thus, possible associations could have been overlooked. No post-hoc correction for multiple comparisons was performed, and p-values were stated as is.

Comparing presymptomatic carriers of FTD causing mutations with clinically affected FTD patients have previously provided valuable insight into the pathomechanisms at disease onset [13,32]. As proven here, this comparison can be expanded by adding clinical information of disease burden (in our case as measured by ACE). Given the apparent correlation between disease burden and inflammatory response recognized here, we suggest this approach applied in future studies of inflammation in FTD.

Conclusion

Previous identifications of an inflammatory component in the disease process of *CHMP2B* mediated neurodegeneration was supported by the findings of a circulating inflammatory response correlated to disease burden in FTD-3. The methods applied here failed to identify an intrathecal inflammatory profile of FTD-3, possibly due to the small cohort and the low sensitivity of the measured biomarkers in CSF.
The findings add to the assumption that TREM2 is an early response to neurodegeneration.
We suggest measures of disease burden incorporated in future studies of the correlation between inflammation and neurodegeneration.

Acknowledgements

This work has been possible only due to the continued dedication and support from FTD-3 family members. This has been facilitated through more than twenty years by the FReJA collaboration. This work has been granted financial support from Aase Crones Estate, The Jascha Foundation, Aase & Ejnar Danielsens Foundation and Desiree & Niels Ydes Foundation.
FReJA consortium: Gydesen S, Brown J, Isaacs A, Collinge J, Gade A, Englund E, Fisher E, Stokholm J, Thusgaard T, Holm I.

Conflicts of interest

None.

Words

Abstract: 247 words

Text: 3459 words

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Figures and tables

Table 1: Characteristics of participants.

	Controls	Presymptomatic *CHMP2B* mutation carriers	Affected FTD-3
CSF N	15	7	10
Serum N	16	10	8
Sample Age Mean (range)	60 (38 – 71)	52 (32 – 68)	63 (53 – 73)
ACE Mean (range)	94 (87 – 100)	90 (81 – 96)	71 (41 – 89)

Table 2: Mean levels of biomarkers in serum and CSF, stratified by *CHMP2B* mutation status.

Serum				CSF			
Analyte	Controls	*CHMP2B* mutation carriers	P-value	Analyte	Controls	*CHMP2B* mutation carriers	P-value
CCL11	68.17	84.31	0.280	CCL11	5.42	4.42	0.397
CCL26	9.70	13.95	0.640	CCL26	NA	29.46	0.177
IFN-γ	8.24	12.19	0.471				
IL-1α	3.33	NA	0.014	IL-1α	1.08	1.11	0.552
IL-2	NA	5.36	0.332				
IL-5	0.72	0.78	1.000	IL-5	1.19	1.07	0.120
IL-6	0.86	0.97	1.000	IL-6	1.65	1.40	0.375
IL-7	19.52	18.66	0.428	IL-7	0.84	0.95	0.199
CXCL8	15.32	17.41	1.000	CXCL8	46.14	46.05	0.865
IL-10	0.68	0.36	0.798	IL-10	0.24	0.33	0.576
IL-12	105.28	113.02	0.914	IL-12	4.10	5.43	0.230
IL-12p70	0.27	0.42	1.000				
IL-13	0.60	0.63	0.588				
IL-15	3.04	3.02	1.000	IL-15	2.96	2.86	0.720
IL-16	203.78	195.61	0.829	IL-16	7.94	7.08	0.234
IL-17	6.93	4.04	0.639				
CXCL10	56.55	61.07	0.407	CXCL10	389.00	419.53	0.985
CCL2	66.91	65.96	0.692	CCL2	363.97	304.38	0.206
CCL13	72.77	73.60	0.857	CCL13	4.19	5.16	0.895
CCL22	925.94	946.12	0.428	CCL22	17.43	26.17	0.696
CCL3	12.79	4.62	0.231	CCL3	8.21	8.39	0.329
CCL4	12.53	19.44	0.026	CCL4	8.65	9.33	0.637
CCL17	100.28	72.33	0.349	CCL17	3.16	2.57	0.063
TNF-α	3.00	3.43	0.885	TNF-α	0.26	0.29	0.964
VEGF	160.61	212.22	0.183	VEGF	1.86	1.86	0.836
				sTREM2	5160.00	6494.00	0.193

Table 3: Mean levels of biomarkers in serum and CSF of *CHMP2B* mutation carriers, stratified by clinical status.

Serum				CSF			
Analyte	Presymptomatic carriers	Affected FTD-3	P-value	Analyte	Presymptomatic carriers	Affected FTD-3	P-value
CCL11	64.62	106.47	0.149	CCL11	4.20	4.55	0.673
CCL26	21.60	5.34	0.290	CCL26	17.75	41.17	0.862
IFN-γ	7.34	17.66	0.923				
IL-1α	NA	NA	1.000	IL-1α	1.11	NA	0.009
IL-2	5.36	NA	0.346				
IL-5	0.90	0.73	0.563	IL-5	1.18	1.02	0.702
IL-6	0.90	1.00	0.216	IL-6	1.04	1.65	0.064
IL-7	16.60	20.97	0.178	IL-7	NA	0.95	0.066
CXCL8	16.20	18.78	0.124	CXCL8	37.62	51.95	0.064
IL-10	0.31	0.41	0.532	IL-10	0.36	0.29	0.728
IL-12	96.88	131.17	0.336	IL-12	5.39	5.47	0.205
IL-12p70	0.42	NA	0.346				
IL-13	0.63	NA	0.169				
IL-15	2.51	3.59	0.003	IL-15	2.62	3.03	0.032
IL-16	161.23	234.28	0.068	IL-16	6.52	7.47	0.329
IL-17	4.04	NA	0.169				
CXCL10	55.50	67.33	0.773	CXCL10	459.46	391.58	0.495
CCL2	58.17	74.73	0.211	CCL2	284.60	318.21	0.626
CCL13	54.63	94.95	0.021	CCL13	NA	5.16	0.223
CCL22	796.07	1114.92	0.034	CCL22	34.76	17.58	0.512
CCL3	4.84	4.39	0.910	CCL3	11.34	7.80	0.254
CCL4	13.93	25.63	0.009	CCL4	8.37	10.00	0.329
CCL17	57.19	89.36	0.124	CCL17	2.86	2.42	0.732
TNF-α	2.76	4.19	0.009	TNF-α	NA	0.29	0.403
VEGF	201.13	224.69	0.847	VEGF	1.86	1.86	1.000
				sTREM2	5646.00	7087.00	0.435

Figure 1: Serum CCL4 levels are increased in *CHMP2B* carriers throughout life, peaking at the mean age of symptom onset in FTD-3.

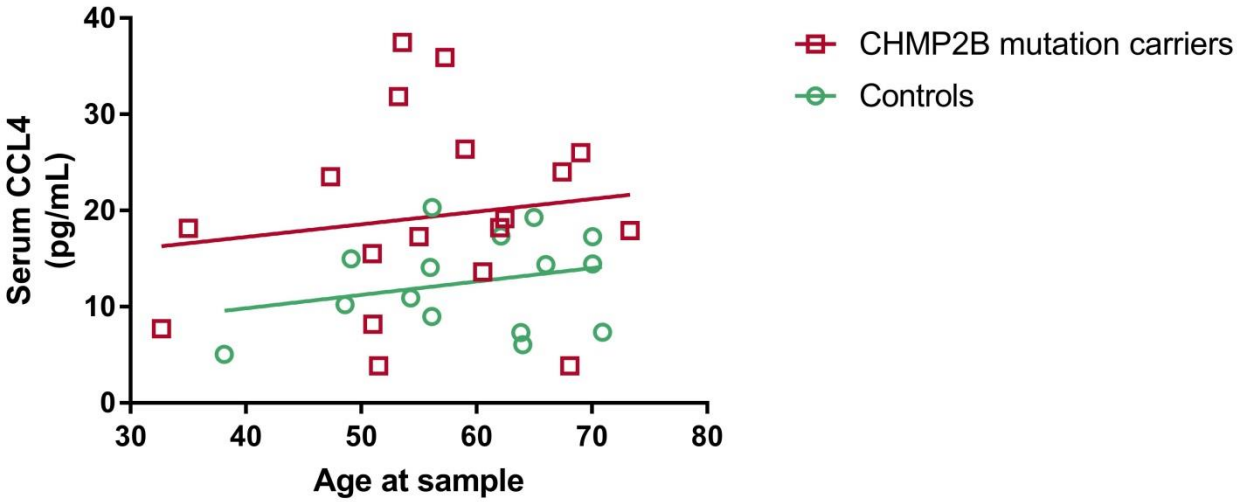

Figure 2: CSF levels of sTREM appear to increase more rapidly in *CHMP2B* mutation carriers than in controls.

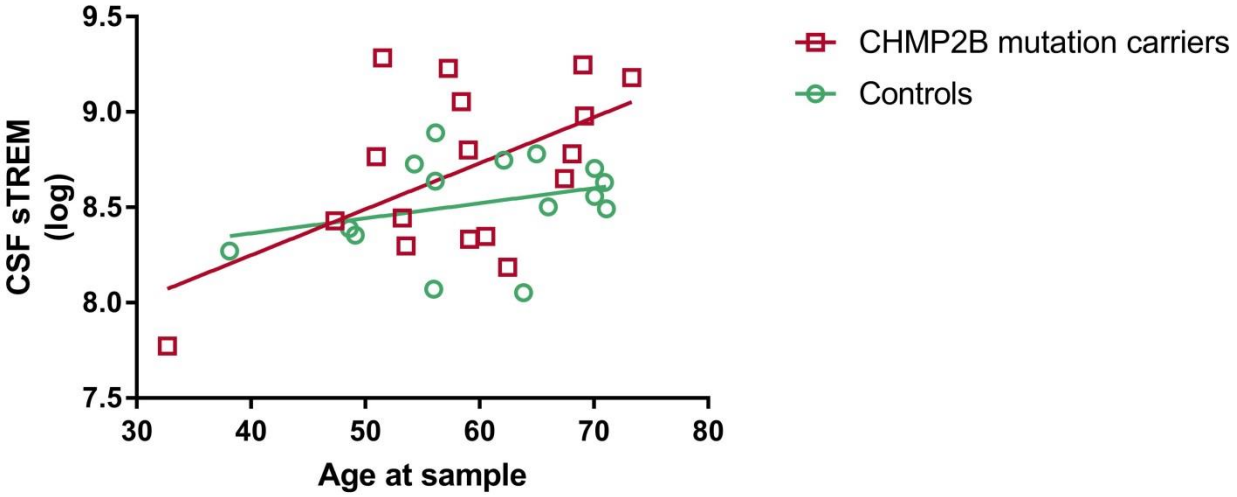

Figure 3: Serum levels of biomarkers IL-15, CXCL10, CCL22 and TNF-α are positively correlated to disease burden measured by Addenbrooke's Cognitive Examination (Low ACE; high disease burden).