Presymptomatic cerebral blood flow changes in *CHMP2B* mutation carriers of familial frontotemporal dementia (FTD-3), measured with MRI

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ABSTRACT

Objectives: To assess functional changes measured by cerebral blood flow (CBF) in the presymptomatic stage of frontotemporal dementia linked to chromosome 3 (FTD-3) caused by a truncating mutation in *CHMP2B*.

Design: Case–control study.

Setting: A memory clinic and tertiary referrals centre for dementia and inherited neurodegenerative disorders.

Participants: The authors included 11 presymptomatic *CHMP2B* mutation carriers and seven first-degree-related family non-carriers. Participants were MRI scanned twice with an interval of 15 months.

Primary and secondary outcome measures: Local functional changes in brain tissue perfusion were measured as CBF with two different MR techniques, gradient echo (GRE) and spin echo (SE), focusing on CBF in all cerebral vessels (GRE) and cerebral capillaries (SE), respectively. As planned, data analysis included co-registration of perfusion images to structural T1 images. Perfusion data were then extracted from seven regions-of-interest, normalised to white matter and statistically compared between carriers and non-carriers.

Results: For SE, contrasts between carriers and non-carriers showed significant differences in temporal, occipital and parietal lobes and in hippocampus. There was no evidence of changes from baseline to follow-up. For GRE, there were no significant differences between carriers and non-carriers.

Conclusions: Significantly decreased CBF was found in presymptomatic *CHMP2B* mutation carriers in occipital-and parietal lobes. Comparing SE with GRE, data indicate that FTD-3 vascular pathology might primarily affect brain capillaries.

INTRODUCTION

Frontotemporal dementia linked to chromosome 3 (FTD-3) is an autosomal dominant inherited neurodegenerative disease first described in a large Danish family. In 2005, a truncating mutation in the *CHMP2B* gene

ARTICLE SUMMARY

Article focus

- Frontotemporal dementia linked to chromosome 3 (FTD-3) is an autosomal dominantly inherited neurodegenerative disease caused by a truncating mutation in *CHMP2B*.
- The study assesses change in CBF in the presymptomatic stage of *CHMP2B* mutation carriers compared with first-degree-related non-carriers.
- CBF was measured with GRE MR techniques focusing on CBF in all cerebral vessels and with SE technique focusing on cerebral capillaries.

Key messages

- Eleven presymptomatic mutation carriers were compared with seven first-degree-related family non-carriers and scanned twice with 15 months in between.
- For capillary measurements (SE), contrasts between carriers and non-carriers showed significant differences in temporal, occipital and parietal lobes and in the hippocampus. There was no difference for the measurements over all vessels (GRE) and no difference over time.
- Comparison of SE with GRE, data indicate that FTD-3 vascular pathology primarily affect brain capillaries.

Strength and limitations of this study

- Limitations of the study are the relative few participants and the short follow-up time compared with the disease duration.
- The strength is that all 11 patients carry the exact same mutation and that the non-mutation carriers all are first-degree relatives, thus reducing the genetic variability.

on chromosome 3 was identified,¹ affecting one of the proteins in the endosomal ESCRTIII complex leading to disruption of the endosomal transport and degradation of proteins.^{1–4} A second, distinct, truncating *CHMP2B* mutation was subsequently identified in a familial Belgian frontotemporal

dementia patient.⁵ The clinical symptoms of the disease begin with subtle personality changes. Patients become disinhibited with lack of empathy and inappropriate emotional responses. Some develop hyperorality with, for instance, chain smoking. Other cortical cognitive deficits like dyscalculia have been seen as an early symptom. Neuropsychological testing most often demonstrate that also posteriorly located cognitive functions like visuospatial function are affected. The patients often have little to no insight. In the initial phase cranial nerves, pyramidal and extrapyramidal functions are in most instances normal, although some patients have developed features of motor neuron involvement. The average age of onset is 57 years with a very wide range from 46 to 67 years of age, although exact age of onset is difficult to determine in the individual case, as the onset in many cases are subtle and slowly progressive behavioural changes.

The disease is steadily progressive. In later stages, the patients are often either predominantly apathetic or exhibit more aggressive behaviour. Some patients develop parkinsonian features, dystonia, pyramidal signs or myoclonia. In terminal stages, the patients are bedridden. The average duration of disease is approximately 8 years with a large variation.⁶

Structural neuroimaging with CT and MRI show generalised cortical and central atrophy and often widening of the posterior lateral ventricles. No white matter changes are seen. In presymptomatic carriers, localised cortical⁷ and more generalised atrophy⁸ has been demonstrated.

H₂¹⁵O-positron emission tomography (PET) scanning of regional cerebral blood flow (rCBF) in symptomatic individuals showed severe and widespread rCBF deficits, most prominent in frontal, parietal and temporal lobes and normal rCBF only in primary visual cortex, thalami and basal ganglia.⁶

FTD-3 is characterised pathologically by the presence of ubiquitin and p62 inclusions that are negative for both TDP-43 and FUS.^{9–11}

Cerebral blood flow (CBF) and perfusion measured by MRI can be performed by two different types of perfusion-weighted imaging: gradient echo (GRE) and spin echo (SE). GRE is equally sensitive to signals in all vessel sizes, whereas the SE sequence reflects signals mainly from the capillary bed, as signals from larger vessels are effectively refocused in SE.^{12–13} The MRI method has been validated against PET CBF measurements in Alzheimer's disease showing a very good correlations between the two methods.^{14–15}

The purpose of this study was to assess changes in rCBF measured with MRI in the presymptomatic stage of subjects with *CHMP2B* mutation compared with first-degree relatives without the mutation.

Based on previous PET measurements of rCBF in symptomatic FTD-3 cases, we hypothesised that a truncation mutation in *CHMP2B* leads to preclinical functional changes in the CBF.

METHODS

The study fulfils the Helsinki II declaration and was approved by the regional research ethics committee. Subjects were recruited through a family contact group. All participants gave written informed consent prior to participation.

Subjects

Eighteen first-degree-related family members without clinical disease were recruited from the Danish FTD-3 family. All subjects were anonymously tested for the *CHMP2B* mutation, as some subjects did not want to know their genetic status. Neither the subjects nor any member of the research group, who has contact with the subjects have been informed of the genetic status of individual participants. Genetic testing was performed at the Institute of Neurology, London, UK (AI), and at the Section of Neurogenetics, Copenhagen, Denmark (JEN), and resulted in 11 being carriers and seven non-carriers.

Clinical interview and neurological examination was carried out by an experienced neurologist specialised in dementia and FTD (PJ), and for each subject, a close relative, usually the spouse, was interviewed in a semi-structured manner. None of the participants fulfilled criteria for FTD, and the interviews and testing did not indicate symptomatic onset of clinical FTD-3 disease. There was no change in reported health, behaviour or clinical status between the first and the second assessment. Hence, all participants were presymptomatic all through the study period. They were either working full time or retired due to age.

Image acquisition

All individuals were scanned twice on a 3T Signa Excite MRI-scanner, a baseline and a follow-up scan. The average scan interval time was 15 months.

T1 axial images were acquired with (TE/TR=2.89/6.396). Standard dynamic susceptibility contrast MRI was performed with GRE (TE/TR=30/1500) and SE (TE/TR=60/1500) following intravenously administered contrast agent (gadobutrol) 10 ml/kg and 5 ml/kg, respectively, for GRE and SE. Injection rate was 5 ml/s, followed by infusion of 30 ml saline with the same injection rate. In plane, resolution was 128×128.

Image analysis

Structural T2-weighted and perfusion images were linearly co-registered to structural T1-weighted images using a six-parameter rigid-body transformation (three translation, three rotation, scale=1.0) and a mutual information similarity measure.^{16–17} Representative images of transaxial maps of capillary rCBF from the SE sequence are shown in figure 1.

The T1 images for all subjects were iteratively registered to a common mean of the population using the Montreal Neurological Institute (MNI) ICBM 152_linear average brain as initial reference space. The registration

Figure 1 Representative images of transaxial maps of capillary regional cerebral blood flow (spin echo sequence; arbitrary values normalised to white matter) in a presymptomatic mutation non-carrier (A) and a mutation carrier (B). Examples of some of the regions-of-interest in red colour used for the analyses (C: occipital cortex; D: frontal cortex; E: white matter and F: temporal cortex).

method used was a modification of the linear and non-linear method for model-based segmentation.¹⁸ Using this approach, we achieved a population-specific average model, which is referred to as the FTD-3 standard brain (FTD-3 standard space).

The FTD-3 standard brain was segmented using a model-based approach developed at MNI.^{18 19} Existing regions-of-interest (ROIs) in (MNI) ICBM 152_linear space were first transformed to the FTD-3 standard space using a linear and non-linear registration but without tissue classification. The resulting ROIs in the FTD-3 standard space were then individualised using the previously determined non-linear transform between each subjects T1 native space and the FTD-3 standard space.

The ROIs were selected based on previous PET CBF studies in symptomatic FTD-3 cases (partly described in⁶) and included all major lobes, see table 2 and figure 1 for a complete list. As no asymmetry has been shown neither in the pathology nor on previous structural scans in FTD-3, the ROIs include both left- and right-hemispheric regions.

On the raw perfusion images, arterial input functions were selected semi-automatically.²⁰ Maps of relative CBF and CBV were calculated on a voxel-by-voxel basis, using singular value decomposition with a block-circulant deconvolution matrix.²⁰ Mean transit time was calculated, using the central volume theorem, as the ratio between CBV and CBF.¹³

Statistics

Data analysis was carried out in the program ‘R’ (<http://www.r-project.org>).

On average, there was a non-significant tendency to higher CBF estimates in the second scan compared with the first scan. Therefore, the following statistical model

was chosen to account for this. A linear model (M0) was fitted, which included the interaction between the carrier and scan effects (from baseline to follow-up). Another model (M1) was fitted without this interaction. To accommodate the correlation between successive scans, both models included a random effect for subjects. A likelihood ratio test was performed for the reduction from M0 to M1 in order to test whether carrier status was independent of scan time for each ROI.

Contrasts between groups and scan times, respectively, were also estimated.

RESULTS

Demographics of the subjects are shown in table 1. Three dropped out, thus only providing a baseline scan. As some of the participants did not want to be informed of the genetic carrier status, information on carrier status at a subject level cannot be stated. Due to a technical error during one scan, one subject’s baseline SE image was excluded.

On SE, the likelihood ratio test showed significant differences in rCBF between carriers and non-carriers in four out of seven ROIs (table 2). On GRE, there were no significant differences between the two groups.

The likelihood ratio test showed no significant interactions between carrier and scan effects, neither for SE

Table 1 Demographics of *CHMP2B* mutation carriers and first-degree family non-carriers

	Carriers	Non-carriers
N	11	7
Women	2	2
Mean age (SD)	56 years (5.9)	55 years (6.2)

Table 2 Differences (contrasts) in normalised regional cerebral blood flow between *CHMP2B* mutation carriers and non-carriers

	Gradient echo (all vessels)	Spin echo (capillaries)
Frontal	0.11	0.16
Temporal	0.09	0.19*
Hippocampus	−0.01	0.18*
Parietal	0.15	0.28*
Occipital	0.02	0.21*
Cerebellum	−0.09	0.11
Basal ganglia	0.02	0.24

*p<0.05 on the likelihood ratio test.

nor GRE, except for GRE for the hippocampus ROI. This lack of interaction suggests that the differences in rCBF between carriers and non-carriers are unchanged from baseline to follow-up.

On boxplots (figures 2 and 3), there is a trend towards more pronounced group differences on follow-up compared with baseline, though this could not be confirmed through the statistical analysis. One of the non-carriers had a significantly lower CBF than all other subjects in the basal ganglia but not in other regions. There is no explanation for this result. In the non-carriers, there was a tendency to higher estimates of CBF in the second scan for both SE and GRE data. The outlier among mutation carriers in figure 2 is the same subject in all four indicated ROIs. An analysis of the data without this subject did not change the consistency of the results in all regions; although with the relative small group sizes, this subject contributes significantly to the statistical p value.

DISCUSSION

Presymptomatic *CHMP2B* mutation carriers showed significantly lower CBF in four of the seven pre-selected ROIs when compared with first-degree relatives without the mutation. The rCBF reduction was seen in SE

measurements but not in GRE measurements. As the SE sequences are sensitive to signal from capillaries and the GRE sequence to the whole vascular network, the results indicate an involvement of the capillaries in the pathophysiology of FTD-3.

In severely affected symptomatic FTD-3 cases, we have previously, with $H_2^{15}O$ -PET rCBF measurements, found pronounced and widespread decreased CBF in most of the brain sparing the visual cortex, basal ganglia and cerebellum.⁶

These data cannot distinguish between a primary involvement of the capillaries leading to a flow reduction or whether the flow reduction is primarily related to a decrease in the metabolic activity of neurons and glia leading to a flow reduction. As the function of *CHMP2B* protein and the ESCRT complex encompasses transport and degradation of proteins, it might also be a partly independent and parallel pathobiological process in neurons and in the vessel walls.

Development of a mouse model would facilitate studies of vessel involvement in both brain and other tissues that may quantify the involvement of capillary hyperperfusion in the disease process.

This present finding, demonstrating pathological changes in a presymptomatic stage of a neurodegenerative disease, has similarities with a study in the prodromal pre-dementia phase of Alzheimer's disease, which demonstrated 10%–23% significant decrease in CBF in the mesiotemporal region, amygdala and anterior cingulum, compared with healthy controls.²¹

In the present study, we have used a contrast-enhanced MRI technique to measure CBF. Arterial spin-labelled MRI is a promising other type of MRI technique that can be used for CBF measurements where an intravenous contrast infusion is not necessary and therefore is easier to apply and with a lower cost. The drawback is that the arterial spin label method does not measure the microvasculature and therefore with the present findings would not have been suitable for this study.

A few other studies have assessed primarily structural brain change in presymptomatic mutations carriers of

Figure 2 Boxplots showing capillary (spin echo) regional cerebral blood flow differences at baseline between presymptomatic mutation carriers and non-carriers ('dots' = outliers). CBF, cerebral blood flow.

Figure 3 Boxplots showing capillary (spin echo) regional cerebral blood flow differences at follow-up between presymptomatic mutation carriers and non-carriers ('dots' = outliers; * $p < 0.05$ on the likelihood ratio test). CBF, cerebral blood flow.

other types of frontotemporal dementia. These studies describe early and presymptomatic structural changes, measured both as decreased whole-brain volume^{22, 23} and as reduced fractional anisotropy in white matter.²³ Thus subtle structural and functional brain changes can be demonstrated in subjects very likely on the course to a clinical manifestation of a neurodegenerative disease.

Further research is needed to elucidate the relation between the altered function of the *CHMP2B* gene, the affected cellular processes,²⁴ the global pattern of atrophy^{7, 8} and finally the changes in CBF which are presented here.

There are limitations of this study. First, the number of subjects is low, but although the Danish FTD-3 family is large, not all are willing to participate and the number of subjects in a relevant age of up to 15 years before average onset is lower giving a natural limitation of group sizes. A strength of this study is that the control group are first-degree relatives only. Thus, some variance is eliminated compared with a control group of unrelated subjects. There is also an ethical argument for using first-degree relatives in order to be able to keep clinicians and the family blinded to the genetic status of the individuals as only some of the participants have chosen to go through the clinical process of genetic counselling and testing.

Second, a Volume-Of-Interest (VOI)-based approach has the disadvantage of the a priori definition of VOIs compared with a voxel-based approach. This may have affected the findings. The voxel-based approach was chosen after comparison of PET CBF studies in symptomatic FTD-3 cases and results indicating that changes in mutation carriers probably are more global than focal in nature.^{6, 8} As discussed by Rohrer *et al.*,⁸ the pathophysiological process in FTD-3 seems more widespread in the cerebrum than indicated by the phenotype. The locations of the ROIs were therefore chosen based on the changes seen in symptomatic cases. The volume of the ROIs chosen is relatively small, as this analysis was specifically focused on delineating the cortex. The reason for choosing cortical volumes was to increase the

sensitivity of picking up changes, as it is likely that the early changes are most likely cortical. Contrarily, there might be a problem with partial volume effects, where brain atrophy mimics perfusion reduction as consequence of the cortical atrophy, since we have already previously shown decreased cortical thickness in this group.⁷ However, compared with SPECT and PET, the resolution in perfusion-weighted MRI is smaller, which decreases the partial volume effects problem. The strength of the results is the consistent findings in all assessed regions in the two scan series.

Very few studies have assessed changes of physiological brain processes in a presymptomatic stage of familial dementia. Knowledge about functional changes such as reduced blood flow in relation to capillary involvement in presymptomatic mutation carriers will have implications for both studies of the FTD-3 animal models as well as for the clinical studies of FTD-3 patients. Ultimately, it may have implications for early detection and possible future treatment regimes in FTD and other neurodegenerative disease.

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Competing interests None.

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