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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000368
Article Type:	Research
Date Submitted by the Author:	21-Oct-2011
Complete List of Authors:	Lunau, Line; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Mouridsen, Kim; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Rodell, Anders; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Østergaard, Leif; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Nielsen, Jørgen; Copenhagen University, Panum Institute, Section of Neurogenetics, Institute of Cellular & Molecular Medicine Isaacs, Adrian; UCL Institute of Neurology, Department of Neurodegenerative Disease Johannsen, Peter; Rigshospitalet, Copenhagen University Hospital, Memory Disorder Research Group
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Mental health, Radiology and imaging
Keywords:	Neurodegenerative, Dementia < NEUROLOGY, CEREBRAL BLOOD FLOW, FUNCTIONAL IMAGING, Genetics < TROPICAL MEDICINE, MRI

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

Short title: CBF in presymptomatic FTD-3.

Lunau L[a], Mouridsen K[a], Rodell A[a], Østergaard L[a], Nielsen JE[b+c], Isaacs AM[d], Johannsen P[b] & The FReJA Consortium.

a. Center of Functionally Integrative Neuroscience, Aarhus University Hospital, Aarhus, Denmark.

b. Memory Disorder Research Group, Rigshospitalet, Copenhagen University Hospital, Denmark.

c. Section of Neurogenetics, Institute of Cellular & Molecular Medicine , The Panum Institute, University of Copenhagen, Copenhagen; Denmark.

d. Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK.

Keywords: FTD-3, *CHMP2B*, CBF, MRI.

Corresponding author
Peter Johannsen, MD, PhD
Memory Clinic, section 6702
Rigshospitalet
Blegdamsvej 9
2100 Copenhagen
Denmark
fax: +45 3545 2446
email: peterj@dadlnet.dk

Short title: CBF in presymptomatic FTD-3.

Abstract

Background: Frontotemporal dementia linked to chromosome 3 (FTD-3) is an autosomal dominantly inherited neurodegenerative disease caused by a truncating mutation in *CHMP2B*. The disease is characterized by insidious and progressive changes in personality, behaviour and cognition.

The purpose of the study is to assess functional change in the presymptomatic stage. Local functional changes in brain tissue perfusion was measured as cerebral blood flow (CBF) with two different MR techniques, gradient echo (GRE) and Spin Echo (SE), focusing on CBF in all cerebral vessels and cerebral capillaries, respectively.

Methods: Presymptomatic *CHMB2B* mutation carriers and first-degree related non-carriers were MRI-scanned twice with an interval of 15 months. Perfusion images were co-registered to structural T1-images (6). Perfusion data were extracted from 7 regions-of-interest (ROIs), normalized to white matter, and statistically compared between carriers and non-carriers.

Results: We included 11 carriers and 7 first-degree related family non-carriers. 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.

Short title: CBF in presymptomatic FTD-3.

Conclusion: Significantly decreased cerebral blood flow 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.

Short title: CBF in presymptomatic FTD-3.

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 cerebral blood flow (CBF) in the presymptomatic stage of *CHMP2B* mutation carriers compared to first-degree related non-carriers.
- CBF was measured gradient echo (GRE) MR techniques focusing on CBF in all cerebral vessels, and with Spin Echo (SE) technique focusing on cerebral capillaries.

Key messages:

- Eleven presymptomatic mutation carriers were compared to 7 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:

- Limitations of the study are the relative few participants and the short follow-up time compared to the disease duration.

Short title: CBF in presymptomatic FTD-3.

- 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.

Short title: CBF in presymptomatic FTD-3.

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 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 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 generalized cortical and central atrophy and often widening of the posterior lateral ventricles. No white matter

changes are seen. In presymptomatic carriers, localized cortical (7) and more generalized atrophy (8) has been demonstrated.

$H_2{}^{15}O$-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 and perfusion measured by MRI can be performed by two different types of perfusion weighted imaging (PWI): 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 purpose of this study was to assess changes in rCBF measured with MRI in the presymptomatic stage of subjects with *CHMP2B* mutation compared to first-degree relatives without the mutation.

Based on previous PET measurements of rCBF in symptomatic FTD-3 cases, we hypothesized that a truncation mutation in CHMP2B leads to preclinical functional changes in the cerebral blood flow

Methods:

The study fulfills 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.

Short title: CBF in presymptomatic FTD-3.

Subjects

18 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, DK (JN), and resulted in 11 being carriers and 7 non-carriers.

Clinical interview and neurological examination was carried out by an experienced neurologist specialized 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. 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 5ml/sec, followed by infusion of 30 ml saline with the same injection rate. In plane resolution was 128x128.

Image analysis

Structural T2-weighted and perfusion images were linearly co-registered to structural T1-weighted images using a 6 parameter rigid-body transformation (3 translation, 3 rotation, scale=1.0), and a mutual information similarity measure (14),(15). 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 using a modification of the linear and nonlinear method (16). Using this approach we achieved a population specific average model, which is referred to as the FTD-3 standard brain.

The FTD-3 standard brain was segmented using a model-based approach (17). The resulting ROIs in the FTD-3 standard brain were finally individualized using the previously determined nonlinear transform between subject T1 native space and the FTD-3 standard space.

The selected ROI's included all major lobes; see Table 2 for a complete list. As no asymmetry has been shown neither in the pathology nor on previous structural scans in FTD-3 the ROI's include both left- and right hemispheric regions.

On the raw perfusion images, arterial input functions were selected semi-automatically (18). Maps of relative CBF and CBV were calculated on a voxel-by-voxel basis, using singular value decomposition with a block-circulant

deconvolution matrix (18). Mean transit time (MTT) 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" (www.r-project.org).

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

On boxplots (Figure 2 and 3), there is a trend towards more pronounced group differences on follow-up compared to baseline, though this could not be confirmed through the statistical analysis.

Discussion

Presymptomatic *CHMP2B* mutation carriers showed significantly lower cerebral blood flow in 4 of the 7 pre-selected ROIs when compared to 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 hypoperfusion 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 mesotemporal region, amygdala and anterior cingulum, compared to healthy controls (19).

A few other studies have assessed primarily structural brain change in presymptomatic mutations carriers of other types of frontotemporal dementia. These studies describe early and presymptomatic structural changes, measured both as decreased whole-brain volume (20,21) and as reduced fractional anisotropy in white matter (21). 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 (22), the global pattern of atrophy (7,8) and finally the changes in cerebral blood flow 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 to 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 counseling and testing.

Secondly, 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 (PVE), 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 to SPECT and PET, the resolution in perfusion-weighted MRI is smaller, which decreases the PVE-problem.

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.

Short title: CBF in presymptomatic FTD-3.

Acknowledgement

MR technician Dora Ziedler, CFIN Aarhus University Hospital, Denmark is thanked for her help with the scans.

Contributorship

Concept and protocol development: PJ, LL, JN, LØ, AMI.

Data collection: PJ, JEN, AMI.

Data analysis and interpretation: LL, KM, AR, PJ.

Manuscript initial writing: LL, PJ.

Manuscript revision and approval: all authors.

Funding statement

The study was supported by the Danish MRC: grant 22-04-0458 and the Danish National Research Foundation (general research grant to LØ).

Short title: CBF in presymptomatic FTD-3.

References

1. Skibinski G, Parkinson NJ, Brown JM, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet. 2005;37:806-8.

2. Urwin H, Authier A, Nielsen JE, et al. Disruption of endocytic trafficking in frontotemporal dementia with *CHMP2B* mutations. Hum Mol Genet. 2010;19:2228-38.

3. Filimonenko M, Stuffers S, Raiborg C, et al. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol. 2007;179:485-500.

4. Lee JA, Beigneux A, Ahmad ST, et al. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. Curr Biol. 2007;17:1561-7.

5. van der Zee J, Urwin H, Engelborghs S, et al. *CHMP2B* C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. Hum Mol Genet. 2008;17:313-22.

6. Gydesen S, Brown JM, Brun A, et al. Chromosome 3 linked frontotemporal dementia (FTD-3). Neurology. 2002;59:1585-94.

7. Eskildsen SF, Ostergaard LR, Rodell AB, et al. Cortical volumes and atrophy rates in FTD-3 *CHMP2B* mutation carriers and related non-carriers. Neuroimage. 2009;45:713-21.

8. Rohrer JD, Ahsan RL, Isaacs AM, et al. Presymptomatic generalized brain atrophy in frontotemporal dementia caused by *CHMP2B* mutation. Dement Geriatr Cogn Disord. 2009;27:182-6.

Short title: CBF in presymptomatic FTD-3.

9. Holm IE, Isaacs AM, Mackenzie IR. Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the *CHMP2B* gene. Acta Neuropathol. 2009;118:719-20.

10. Holm IE, Englund E, Mackenzie IR, et al. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. J Neuropathol Exp Neurol. 2007;66:884-91.

11. Mackenzie IR, Neumann M, Bigio EH, et al. Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol. 2009;117:15-8.

12. Ostergaard L. Principles of cerebral perfusion imaging by bolus tracking. J Magn Reson Imaging. 2005;22:710-7.

13. Boxerman JL, Hamberg LM, Rosen BR, et al. MR contrast due to intravascular magnetic susceptibility perturbations. Magn Reson Med. 1995;34:555-66.

14. Collins DL, Neelin P, Peters TM, et al. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 1994;18:192-205.

15. Collingnon A, Maes F, Delaere D, et al. Automated multi-modality image registration based on information theory. IPMI. 1995:263-74.

16. Grabner G, Janke, Budge M, et al. Symmetric Atlasing and Model Based Segmentation: An Application to the Hippocampus in Older Adults. Medical Image Computing and Computer-Assisted Intervention 2006:58-66.

17. Collins DL, Peters TM, Dai W, et al. Model-based segmentation of individual brain structures from MRI data. Proc SPIE. 1992;1808:10-23.

18. Wu O, Ostergaard L, Weisskoff RM, et al. Tracer arrival timing-insensitive technique for estimating flow in MR perfusion-weighted imaging using singular value decomposition with a block-circulant deconvolution matrix. Magn Reson Med. 2003;50:164-74.

19. Luckhaus C, Flub MO, Wittsack HJ, et al. Detection of changed regional cerebral blood flow in mild cognitive impairment and early Alzheimer's dementia by perfusion-weighted magnetic resonance imaging. Neuroimage. 2008;40:495-503.

20. Boeve BF, Tremont-Lukats IW, Waclawik AJ, et al. Longitudinal characterization of two siblings with frontotemporal dementia and parkinsonism linked to chromosome 17 associated with the S305N tau mutation. Brain 2005;128:752-72.

21. Janssen JC, Schott JM, Cipolotti L, et al. Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry. 2005;76:162-8.

22. Urwin H, Ghazi-Noori S, Collinge J, et al. The role of CHMP2B in frontotemporal dementia. Biochem Soc Trans. 2009;37:208-12.

Short title: CBF in presymptomatic FTD-3.

Table 1.

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

	Carriers	Non-carriers
N	11	7
Females	2	2
Mean Age (sd)	56 yrs (5.9)	55 yrs (6.2)

Short title: CBF in presymptomatic FTD-3.

Table 2.

Differences (contrasts) in normalized rCBF between *CHMP2B* mutation carriers and non-carriers.

	GRE ("all vessels")	SE ("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.

Short title: CBF in presymptomatic FTD-3.

Figure 1.

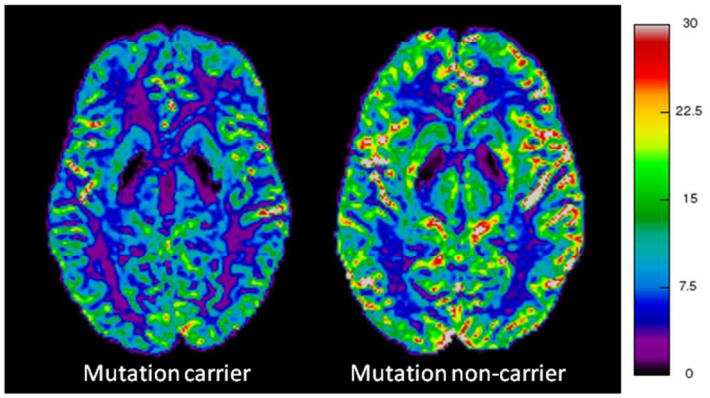
Representative images of transaxial maps of capillary rCBF (SE-sequence; arbitrary values normalised to WM) in a pre-symptomatic mutation carrier (left), and a mutation non-carrier (right).

Figure 2.

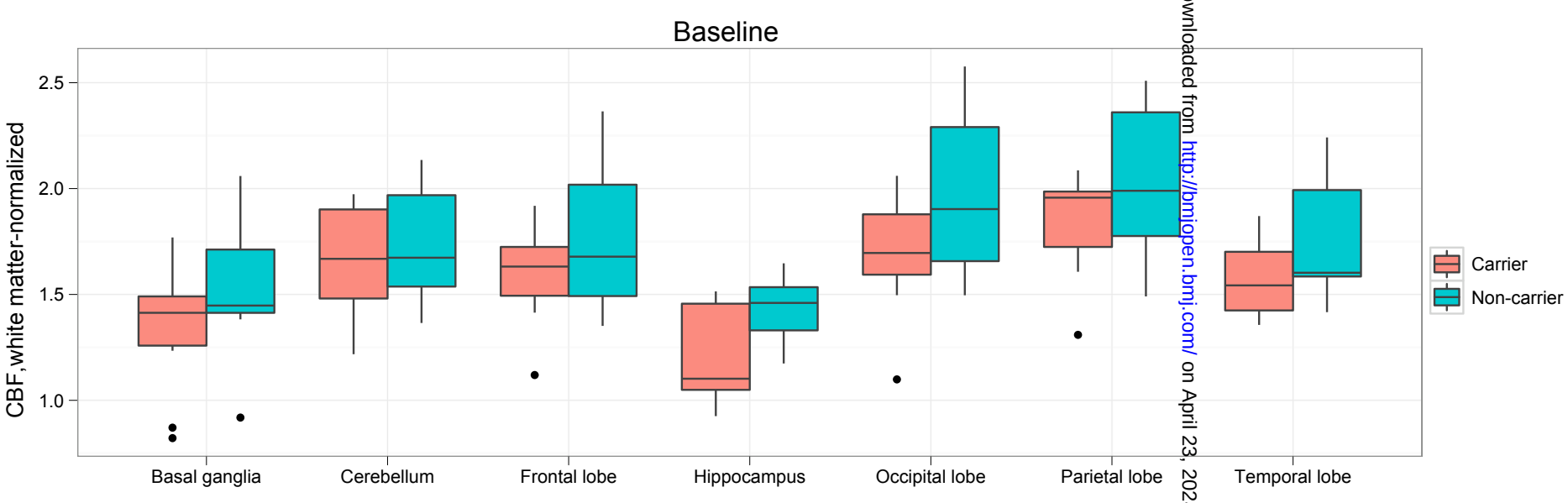
Boxplots showing capillary (SE) rCBF differences at baseline between pre-symptomatic mutation carriers and non-carriers. ("dots" = outliers).

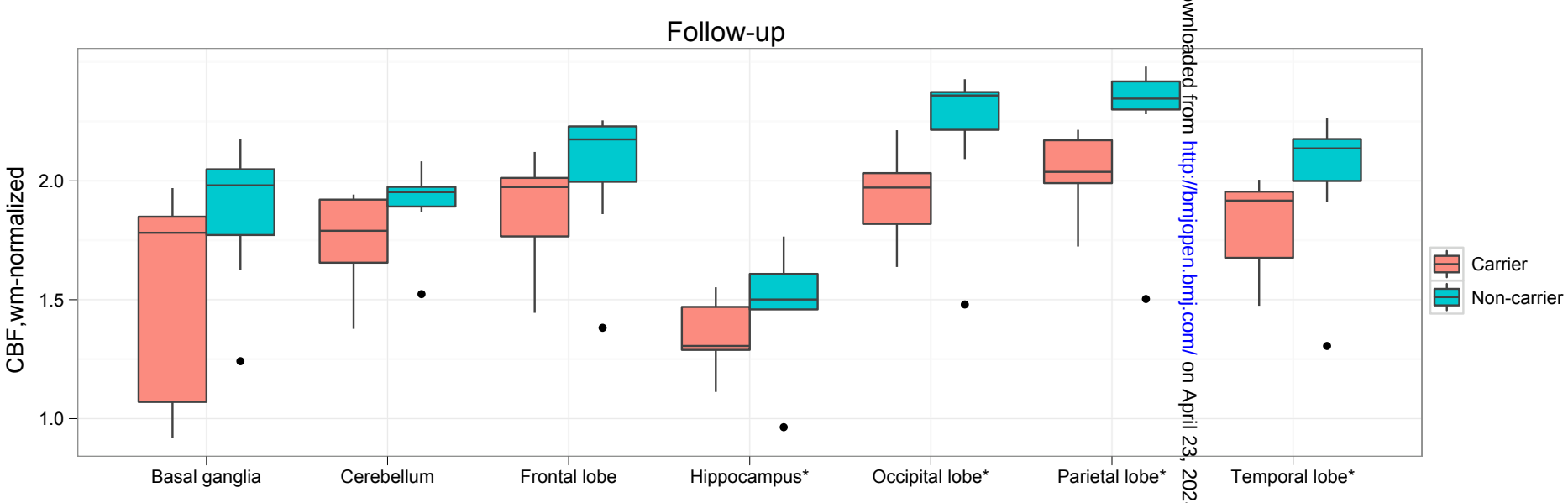
Figure 3.

Boxplots showing capillary (SE) rCBF differences at follow-up between pre-symptomatic mutation carriers and non-carriers. ("dots" = outliers; *p<0.05 on the likelihood ratio test).



Representative images of transaxial maps of capillary rCBF (SE-sequence; arbitrary values normalised to WM) in a pre-symptomatic mutation carrier (left), and a mutation non-carrier (right).
254x190mm (96 x 96 DPI)





STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*9-10*
Bias	9	Describe any efforts to address potential sources of bias	9-10, 13
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	Na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	9-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 2 and 3
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000368.R1
Article Type:	Research
Date Submitted by the Author:	06-Jan-2012
Complete List of Authors:	Lunau, Line; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Mouridsen, Kim; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Rodell, Anders; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Østergaard, Leif; Aarhus University Hospital, Center of Functionally Integrative Neuroscience Nielsen, Jørgen; Copenhagen University, Panum Institute, Section of Neurogenetics, Institute of Cellular & Molecular Medicine Isaacs, Adrian; UCL Institute of Neurology, Department of Neurodegenerative Disease Johannsen, Peter; Rigshospitalet, Copenhagen University Hospital, Memory Disorder Research Group
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Mental health, Radiology and imaging
Keywords:	Neurodegenerative, Dementia < NEUROLOGY, CEREBRAL BLOOD FLOW, FUNCTIONAL IMAGING, Genetics < TROPICAL MEDICINE, MRI

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

Short title: CBF in presymptomatic FTD-3.

Lunau L[a], Mouridsen K[a], Rodell A[a], Østergaard L[a], Nielsen JE[b+c], Isaacs AM[d], Johannsen P[b] & The FReJA Consortium.

a. Center of Functionally Integrative Neuroscience, Aarhus University Hospital, Aarhus, Denmark.

b. Memory Disorder Research Group, Rigshospitalet, Copenhagen University Hospital, Denmark.

c. Section of Neurogenetics, Institute of Cellular & Molecular Medicine , The Panum Institute, University of Copenhagen, Copenhagen; Denmark.

d. Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK.

Keywords: FTD-3, *CHMP2B*, CBF, MRI.

Corresponding author
Peter Johannsen, MD, PhD
Memory Clinic, section 6702
Rigshospitalet
Blegdamsvej 9
2100 Copenhagen
Denmark
fax: +45 3545 2446
email: peterj@dadlnet.dk

Short title: CBF in presymptomatic FTD-3.

Abstract

Background: Frontotemporal dementia linked to chromosome 3 (FTD-3) is an autosomal dominantly inherited neurodegenerative disease caused by a truncating mutation in *CHMP2B*. The disease is characterized by insidious and progressive changes in personality, behaviour and cognition.

The purpose of the study is to assess functional change in the presymptomatic stage. Local functional changes in brain tissue perfusion was measured as cerebral blood flow (CBF) with two different MR techniques, gradient echo (GRE) and Spin Echo (SE), focusing on CBF in all cerebral vessels and cerebral capillaries, respectively.

Methods: Presymptomatic *CHMB2B* mutation carriers and first-degree related non-carriers were MRI-scanned twice with an interval of 15 months. Perfusion images were co-registered to structural T1-images. Perfusion data were extracted from 7 regions-of-interest (ROIs), normalized to white matter, and statistically compared between carriers and non-carriers.

Results: We included 11 carriers and 7 first-degree related family non-carriers. 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.

Short title: CBF in presymptomatic FTD-3.

Conclusion: Significantly decreased cerebral blood flow 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.

Short title: CBF in presymptomatic FTD-3.

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 cerebral blood flow (CBF) in the presymptomatic stage of *CHMP2B* mutation carriers compared to first-degree related non-carriers.
- CBF was measured gradient echo (GRE) MR techniques focusing on CBF in all cerebral vessels, and with Spin Echo (SE) technique focusing on cerebral capillaries.

Key messages:

- Eleven presymptomatic mutation carriers were compared to 7 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:

- Limitations of the study are the relative few participants and the short follow-up time compared to the disease duration.

Short title: CBF in presymptomatic FTD-3.

- 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.

Short title: CBF in presymptomatic FTD-3.

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 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).

Short title: CBF in presymptomatic FTD-3.

Structural neuroimaging with CT and MRI show generalized cortical and central atrophy and often widening of the posterior lateral ventricles. No white matter changes are seen. In presymptomatic carriers, localized cortical (7) and more generalized atrophy (8) has been demonstrated.

$H_2{}^{15}O$-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 and perfusion measured by MRI can be performed by two different types of perfusion weighted imaging (PWI): 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 to first-degree relatives without the mutation.

Based on previous PET measurements of rCBF in symptomatic FTD-3 cases, we hypothesized that a truncation mutation in CHMP2B leads to preclinical functional changes in the cerebral blood flow

Short title: CBF in presymptomatic FTD-3.

Methods:

The study fulfills 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

18 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, DK (JN), and resulted in 11 being carriers and 7 non-carriers.

Clinical interview and neurological examination was carried out by an experienced neurologist specialized 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, behavior 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 5ml/sec, followed by infusion of 30 ml saline with the same injection rate. In plane resolution was 128x128.

Image analysis

Structural T2-weighted and perfusion images were linearly co-registered to structural T1-weighted images using a 6 parameter rigid-body transformation (3 translation, 3 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 method used was a modification of the linear and nonlinear 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 Montreal Neurological Institute (18, 19). Existing ROI's in (MNI) ICBM 152_linear space were first transformed to the FTD-3 standard space using a linear and nonlinear registration, but without tissue classification. The

Short title: CBF in presymptomatic FTD-3.

resulting ROIs in the FTD-3 standard space were then individualized using the previously determined nonlinear transform between each subjects T1 native space and the FTD-3 standard space.

The ROI's were selected based on previous PET-CBF studies in symptomatic FTD-3 cases (partly described in (6)) 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 ROI's 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 (MTT) 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" (www.r-project.org).

On average, there was a non-significant tendency to higher CBF estimates in the second scan compared to 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.

Short title: CBF in presymptomatic FTD-3.

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

On boxplots (Figure 2 and 3), there is a trend towards more pronounced group differences on follow-up compared to 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 amongst mutation carriers in Figure 2 is the same subject in all 4 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 cerebral blood flow in 4 of the 7 pre-selected ROIs when compared to 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 hypoperfusion 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 mesotemporal region, amygdala and anterior cingulum, compared to healthy controls (21).

In the present study we have used a contrast enhanced MRI technique to measure CBF. Arterial spin label (ASL) MRI is a promising other type of MRI technique that can be used for CBF measurements where an *i.v.* contrast infusion is not necessary, and therefore is easier to apply and with a lower cost. The drawback is that the ASL 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 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 cerebral blood flow 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 to 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 counseling and testing.

Secondly, a VOI based approach has the disadvantage of the a priori definition of VOIs compared to 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 patophysiological process in FTD-3 seems more widespread in the cerebrum, than indicate 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 (PVE), 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 to SPECT and PET, the resolution in perfusion-weighted MRI is smaller, which decreases the PVE-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.

Acknowledgement

MR technician Dora Ziedler, CFIN Aarhus University Hospital, Denmark is thanked for her help with the scans.

Contributorship

Concept and protocol development: PJ, LL, JN, LØ, AMI.

Data collection: PJ, JEN, AMI.

Data analysis and interpretation: LL, KM, AR, PJ.

Manuscript initial writing: LL, PJ.

Manuscript revision and approval: all authors.

Funding statement

The study was supported by the Danish MRC: grant 22-04-0458 and the Danish National Research Foundation (general research grant to LØ).

Short title: CBF in presymptomatic FTD-3.

Conflicts of interests

No, there are no competing interests

Data sharing

The corresponding author can be contacted for exchange of data.

Short title: CBF in presymptomatic FTD-3.

References

1. Skibinski G, Parkinson NJ, Brown JM, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet. 2005;37:806-8.

2. Urwin H, Authier A, Nielsen JE, et al. Disruption of endocytic trafficking in frontotemporal dementia with *CHMP2B* mutations. Hum Mol Genet. 2010;19:2228-38.

3. Filimonenko M, Stuffers S, Raiborg C, et al. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol. 2007;179:485-500.

4. Lee JA, Beigneux A, Ahmad ST, et al. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. Curr Biol. 2007;17:1561-7.

5. van der Zee J, Urwin H, Engelborghs S, et al. *CHMP2B* C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. Hum Mol Genet. 2008;17:313-22.

6. Gydesen S, Brown JM, Brun A, et al. Chromosome 3 linked frontotemporal dementia (FTD-3). Neurology. 2002;59:1585-94.

7. Eskildsen SF, Ostergaard LR, Rodell AB, et al. Cortical volumes and atrophy rates in FTD-3 *CHMP2B* mutation carriers and related non-carriers. Neuroimage. 2009;45:713-21.

8. Rohrer JD, Ahsan RL, Isaacs AM, et al. Presymptomatic generalized brain atrophy in frontotemporal dementia caused by *CHMP2B* mutation. Dement Geriatr Cogn Disord. 2009;27:182-6.

Short title: CBF in presymptomatic FTD-3.

9. Holm IE, Isaacs AM, Mackenzie IR. Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the *CHMP2B* gene. Acta Neuropathol. 2009;118:719-20.

10. Holm IE, Englund E, Mackenzie IR, et al. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. J Neuropathol Exp Neurol. 2007;66:884-91.

11. Mackenzie IR, Neumann M, Bigio EH, et al. Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol. 2009;117:15-8.

12. Ostergaard L. Principles of cerebral perfusion imaging by bolus tracking. J Magn Reson Imaging. 2005;22:710-7.

13. Boxerman JL, Hamberg LM, Rosen BR, et al. MR contrast due to intravascular magnetic susceptibility perturbations. Magn Reson Med. 1995;34:555-66.

14. Gonzalez RG, Fischman AJ, Guimaraes AR, Carr CA, Stern CE, Halpern EF, et al. Functional MR in the evaluation of dementia: correlation of abnormal dynamic cerebral blood volume measurements with changes in cerebral metabolism on positron emission tomography with fludeoxyglucose F 18. AJNR Am J Neuroradiol. 1995;16:1763-70.

15. Yoshiura T, Mihara F, Kuwabara Y, Ogomori K, Kaneko K, Tanaka A, et al. MR relative cerebral blood flow mapping of Alzheimer disease: correlation with Tc-99m HMPAO SPECT. Acad Radiol. 2002; 9: 1383-7

16. Collins DL, Neelin P, Peters TM, et al. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 1994;18:192-205.

17. Collingnon A, Maes F, Delaere D, et al. Automated multi-modality image registration based on information theory. IPMI. 1995:263-74.18. Grabner G, Janke, Budge M, et al. Symmetric Atlasing and Model Based Segmentation: An Application to the Hippocampus in Older Adults. Medical Image Computing and Computer-Assisted Intervention 2006:58-66.

19. Collins DL, Peters TM, Dai W, et al. Model-based segmentation of individual brain structures from MRI data. Proc SPIE. 1992;1808:10-23.

20. Wu O, Ostergaard L, Weisskoff RM, et al. Tracer arrival timing-insensitive technique for estimating flow in MR perfusion-weighted imaging using singular value decomposition with a block-circulant deconvolution matrix. Magn Reson Med. 2003;50:164-74.

21. Luckhaus C, Flub MO, Wittsack HJ, et al. Detection of changed regional cerebral blood flow in mild cognitive impairment and early Alzheimer's dementia by perfusion-weighted magnetic resonance imaging. Neuroimage. 2008;40:495-503.

22. Boeve BF, Tremont-Lukats IW, Waclawik AJ, et al. Longitudinal characterization of two siblings with frontotemporal dementia and parkinsonism linked to chromosome 17 associated with the S305N tau mutation. Brain 2005;128:752-72.

23. Janssen JC, Schott JM, Cipolotti L, et al. Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry. 2005;76:162-8.

24. Urwin H, Ghazi-Noori S, Collinge J, et al. The role of CHMP2B in frontotemporal dementia. Biochem Soc Trans. 2009;37:208-12.

Short title: CBF in presymptomatic FTD-3.

Table 1.

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

	Carriers	Non-carriers
N	11	7
Females	2	2
Mean Age (sd)	56 yrs (5.9)	55 yrs (6.2)

Short title: CBF in presymptomatic FTD-3.

Table 2.

Differences (contrasts) in normalized rCBF between *CHMP2B* mutation carriers and non-carriers.

	GRE ("all vessels")	SE ("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.

Short title: CBF in presymptomatic FTD-3.

Figure 1.

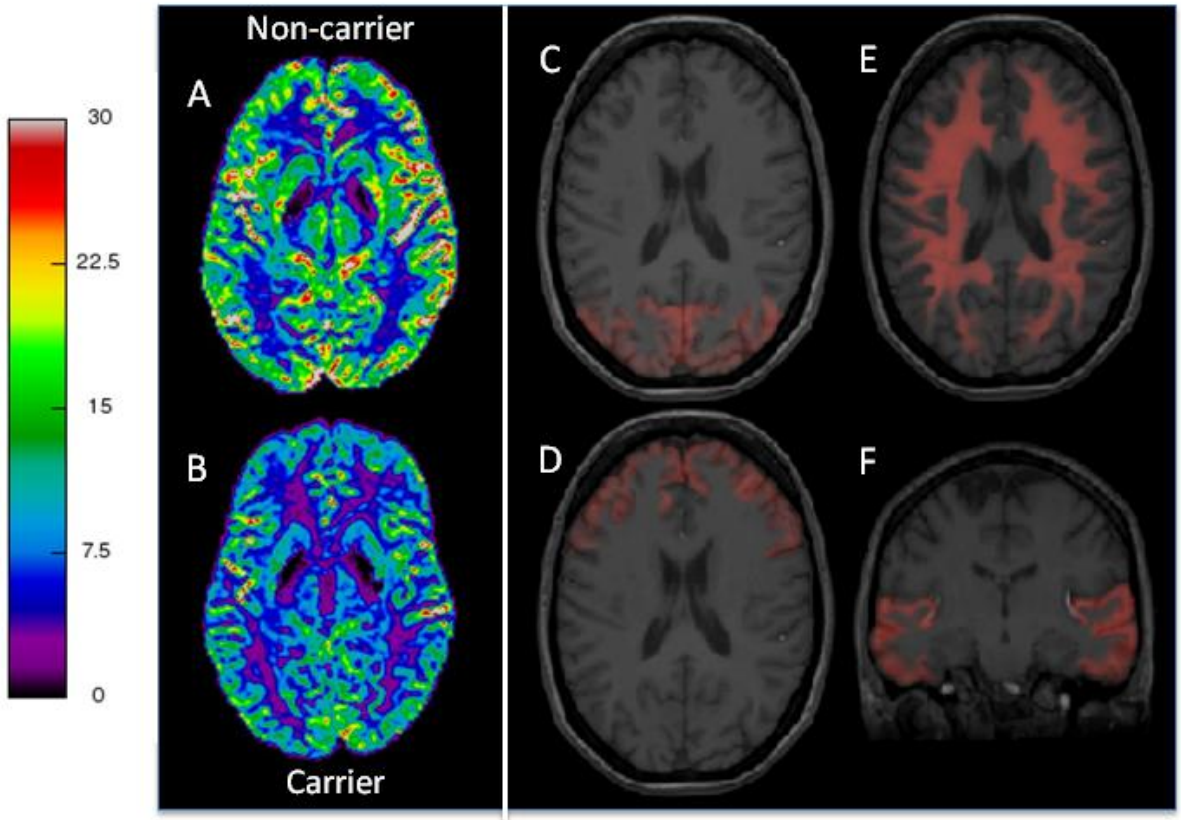
Representative images of transaxial maps of capillary rCBF (SE-sequence; arbitrary values normalised to WM) in a pre-symptomatic mutation non-carrier (A), and a mutation carrier (B). Examples of some of the ROIs in red color used for the analyses (C: occipital cortex; D: frontal cortex; E: white matter; and F: temporal cortex).

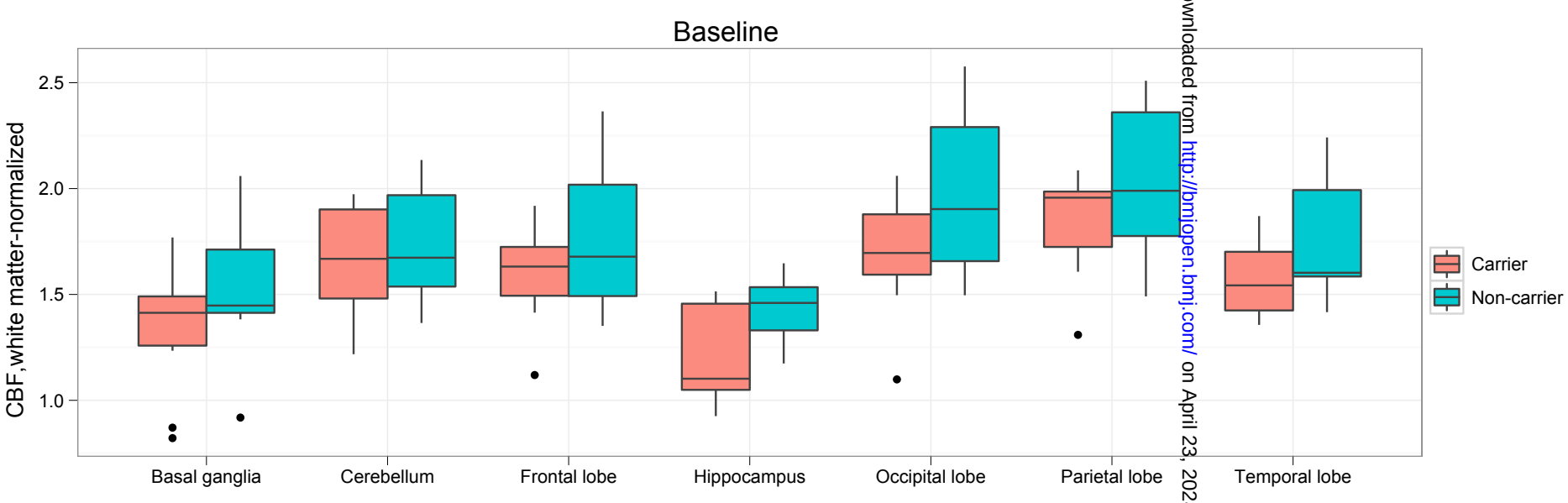
Figure 2.

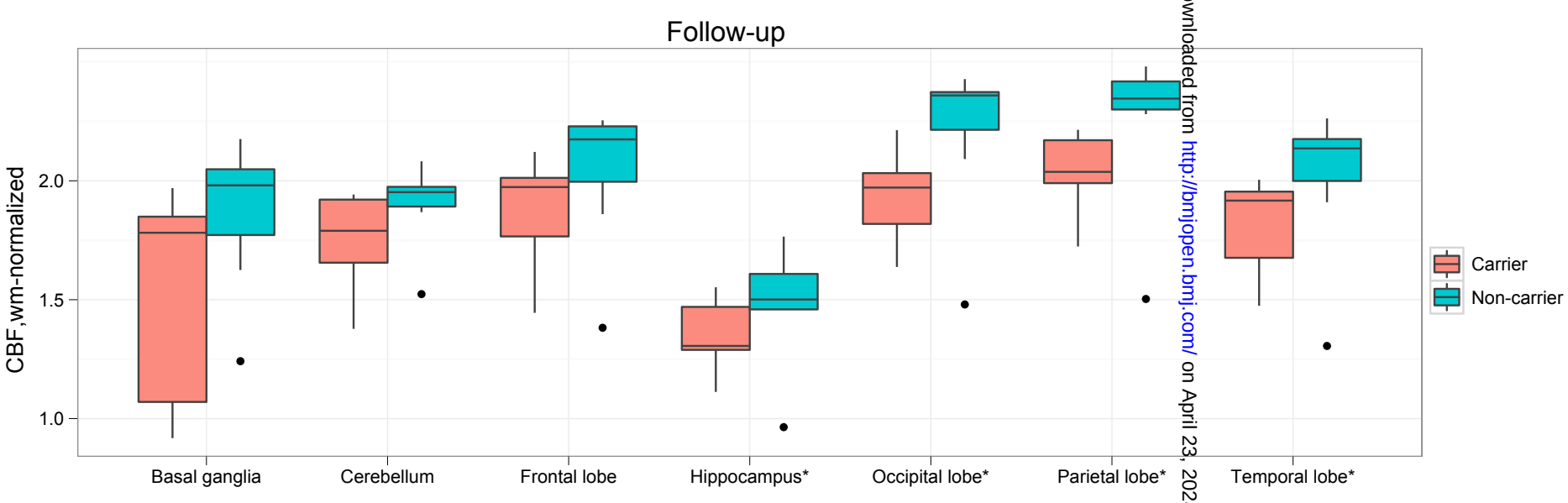
Boxplots showing capillary (SE) rCBF differences at baseline between pre-symptomatic mutation carriers and non-carriers. ("dots" = outliers).

Figure 3.

Boxplots showing capillary (SE) rCBF differences at follow-up between pre-symptomatic mutation carriers and non-carriers. ("dots" = outliers; *p<0.05 on the likelihood ratio test).







STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*9-10*
Bias	9	Describe any efforts to address potential sources of bias	9-10, 13
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	Na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	9-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 2 and 3
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.