NeuroImage: Clinical 18 (2018) 591–598

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

Gray matter changes in asymptomatic *C9orf72* and *GRN* mutation carriers

Karteek Popuri[a], Emma Dowds[b], Mirza Faisal Beg[a], Rakesh Balachandar[a], Mahadev Bhalla[a], Claudia Jacova[c], Adrienne Buller[a], Penny Slack[b], Pheth Sengdy[b], Rosa Rademakers[d], Dana Wittenberg[b], Howard H. Feldman[e], Ian R. Mackenzie[f], Ging-Yuek R. Hsiung[b,*]

[a] School of Engineering Science, Simon Fraser University, Canada
[b] Division of Neurology, Department of Medicine, University of British Columbia, Canada
[c] School of Professional Psychology, Pacific University, Hillsboro, OR, USA
[d] Department of Neuroscience, Mayo Clinic Jacksonville, FL, USA
[e] Department of Neurosciences, University of California San Diego, CA, USA
[f] Department of Pathology and Laboratory Medicine, University of British Columbia, Canada

ARTICLE INFO

Keywords:
Frontotemporal dementia
Magnetic resonance imaging
C9orf72 mutation
Granulin mutation
Cortical thickness
Subcortical volumes

ABSTRACT

Frontotemporal dementia (FTD) is a neurodegenerative disease with a strong genetic basis. Understanding the structural brain changes during pre-symptomatic stages may allow for earlier diagnosis of patients suffering from FTD; therefore, we investigated asymptomatic members of FTD families with mutations in *C9orf72* and granulin (*GRN*) genes. Clinically asymptomatic subjects from families with *C9orf72* mutation (15 mutation carriers, *C9orf72*+; and 23 non-carriers, *C9orf72*−) and *GRN* mutations (9 mutation carriers, *GRN*+; and 15 non-carriers, *GRN*−) underwent structural neuroimaging (MRI). Cortical thickness and subcortical gray matter volumes were calculated using FreeSurfer. Group differences were evaluated, correcting for age, sex and years to mean age of disease onset within the subject's family. Mean age of *C9orf72*+ and *C9orf72*− were 42.6 ± 11.3 and 49.7 ± 15.5 years, respectively; while *GRN*+ and *GRN*− groups were 50.1 ± 8.7 and 53.2 ± 11.2 years respectively. The *C9orf72*+ group exhibited cortical thinning in the temporal, parietal and frontal regions, as well as reduced volumes of bilateral thalamus and left caudate compared to the entire group of mutation non-carriers (NC: *C9orf72*− and *GRN*− combined). In contrast, the *GRN*+ group did not show any significant differences compared to NC. *C9orf72* mutation carriers demonstrate a pattern of reduced gray matter on MRI prior to symptom onset compared to *GRN* mutation carriers. These findings suggest that the preclinical course of FTD differs depending on the genetic basis and that the choice of neuroimaging biomarkers for FTD may need to take into account the specific genes involved in causing the disease.

1. Introduction

Frontotemporal Dementia (FTD) is a heterogeneous clinical syndrome characterized by progressive alterations in behavior, personality, language, and motor function (McKhann et al., 2001; Snowden et al., 2005). Approximately half of affected individuals have a positive family history, with alterations in three genes responsible for the majority of the inherited cases (Snowden et al., 2002; Seelaar et al., 2008; Feldman et al., 2003). Mutations in the gene encoding the microtubule associated protein tau (*MAPT*) are responsible for familial FTD associated with tau pathology, while mutations in the granulin (*GRN*) and chromosome 9 open reading frame 72 (*C9orf72*) genes cause FTD with transactive response DNA binding protein M_r 43 kDa (TDP-43) pathology (Snowden et al., 2002; Warren et al., 2013; Pan and Chen, 2013; Renton et al., 2011; Gijselinck et al., 2012; DeJesus-Hernandez et al., 2011; Baker et al., 2006; Cruts et al., 2006). Since each of these mutations cause an autosomal dominant inheritance with a high degree of penetrance, asymptomatic mutation carriers could be considered "preclinical" and are appropriate subjects for investigating the pre-symptomatic stages of FTD.

Studies that have investigated the structural brain changes in fully affected FTD patients with the *C9orf72* mutation have reported symmetric atrophy of frontal lobes, followed by temporal and parietal lobes (Boxer et al., 2011; Mahoney et al., 2012; Whitwell et al., 2012a; Sha et al., 2012). In contrast, symptomatic *GRN* mutation carriers have been reported to have asymmetric atrophy involving the left inferior frontal, temporal and parietal cortices (Mahoney et al., 2012; Whitwell et al., 2012a; Rohrer et al., 2010; Whitwell et al., 2009). Similar findings have

* Corresponding author at: Division of Neurology, Department of Medicine, University of British Columbia, S151 – 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada.
E-mail address: hsiung@mail.ubc.ca (G.-Y.R. Hsiung).

https://doi.org/10.1016/j.nicl.2018.02.017
Received 21 November 2017; Received in revised form 25 January 2018; Accepted 16 February 2018
Available online 17 February 2018

been reported in asymptomatic *GRN* mutation carriers, displaying asymmetric gray matter atrophy involving frontal, temporal and parietal lobes (Cruchaga et al., 2009; Pievani et al., 2014). A recent large scale study from the "Genetic Frontotemporal dementia Initiative (GENFI)" reported heterogeneous patterns of structural changes associated with *C9orf72* and *GRN* gene mutations in combined samples of symptomatic and asymptomatic subjects (Rohrer et al., 2015). The findings of the GENFI study suggest that structural brain changes could be detected many years prior to disease onset.

Most studies to date have employed symptomatic carriers or a combination of symptomatic and asymptomatic subjects to investigate the structural brain changes associated with *C9orf72* and *GRN* mutations (Mahoney et al., 2012; Whitwell et al., 2012a; Rohrer et al., 2010; Whitwell et al., 2009; Rohrer et al., 2015). As the aim of our study is to investigate brain changes prior to onset of clinical symptoms, we placed the focus on asymptomatic subjects in our current analysis. We hypothesize that *C9orf72* and *GRN* mutation carriers (*C9orf72*+ and *GRN*+, respectively) would exhibit regional atrophy compared to family members who do not carry the mutations (*C9orf72*− and *GRN*−) and that the patterns of atrophy would be different between the *C9orf72*+ and *GRN*+ groups.

2. Materials and methods

2.1. Subjects

Participants were recruited through the University of British Columbia (UBC) hospital clinic for Alzheimer's disease and related disorders. Each subject was a member of a family with autosomal dominant FTD caused by a mutation in either the *C9orf72* or *GRN* gene, and each was considered at risk for FTD by virtue of having an affected first-degree relative. All subjects underwent genetic analysis to establish whether they were carriers of their family's mutation; however, the subjects and the investigators performing the clinical assessments remained blinded to the genetic results. Some features of the cohort have been described previously (Jacova et al., 2013; Hallam et al., 2014). Participants underwent a detailed neurological examination and were screened for cognitive deficits with various scales including the mini mental state examination (MMSE) (Folstein et al., 1975; Folstein et al., 1983), Frontal Assessment Battery (FAB) (Dubois et al., 2000), and the Frontal Behavioral Inventory (FBI) (Kertesz et al., 2000). None of the subjects in the current analysis demonstrated any abnormal motor finding or reflexes, or any significant differences in the ALS motor scales. Patients who fulfilled the diagnostic criteria for possible or probable behavioral variant FTD (bvFTD) (Rascovsky et al., 2011), primary progressive aphasia (PPA) (Gorno-Tempini et al., 2011) or amyotrophic lateral sclerosis (ALS) (Brooks et al., 2000) were excluded. Participants who were free from neurological and clinical cognitive deficits and classified as being asymptomatic were included in the current analysis. Demographic information and family history were shown in Table 1. Years prior to expected disease onset was calculated by subtracting the subjects age from the mean age of disease onset in their respective family. The study was reviewed by institutional research ethics board, and written informed consent was obtained from the participants prior to data collection.

2.2. Genetic status

Genetic analysis was performed at the Mayo Clinic, Jacksonville, Florida, on DNA extracted from peripheral blood using standard protocols (DeJesus-Hernandez et al., 2011; Baker et al., 2006). Subjects were screened for the mutation known to cause FTD in their family and each was classified as being a mutation carrier (*C9orf72*+ or *GRN*+) or non-carrier (neither *C9orf72*− nor *GRN*−). All the *C9orf72*+ subjects in our cohort had repeat size in the clearly positive (> 100) range.

2.3. MRI image acquisition

All subjects underwent structural T1-weighted MRI scanning using a 1.5T GE Signa scanner. The MRI scans were 256 × 256 × 256 in resolution with isotropic 1 mm^3 voxels, and were acquired using the following parameters: Localizers (0:25 min): sagittal/coronal and axial, TR/TE = 5.4/1.6 ms, FOV = 22 cm, matrix size = 256 × 128; 3D T1-Fast Spoiled gradient echo IR Prepped (8:35 min): axial, TR/TE = 10.3/4.8 ms, inversion time = 450 ms, FOV = 25 cm, matrix size = 256 × 256, number of slices = 170 contiguous slices, slice thickness = 1 mm and flip angle = 8^o.

2.4. Cortical thickness and subcortical volume measurements

The T1 MRI images were processed using FreeSurfer neuroimage analysis suite (https://surfer.nmr.mgh.harvard.edu) to generate surface-based cortical thickness maps for each subject. FreeSurfer's cortical reconstruction pipeline (Dale et al., 1999; Fischl et al., 1999) first extracts and tessellates the outer pial (gray matter-cerebrospinal fluid boundary) and the inner white (gray-white matter boundary) surfaces of the cortical mantle. Afterwards, cortical thickness is computed at each vertex of the tessellation as the shortest distance between the pial and white matter surfaces (Fischl and Dale, 2000). The automatic cortical reconstruction outputs were passed through a rigorous manual quality control procedure to identify and correct for any errors according to the troubleshooting guidelines provided by FreeSurfer. To facilitate statistical group analysis, the cortical thickness surface maps for each subject were mapped into the standard MNI ICBM 152 nonlinear average T1 template space (Grabner et al., 2006) (http://nist.mni.mcgill.ca/?p=858) and smoothed using a 15 mm full width at half maximum Gaussian kernel. This resulted in cortical thickness maps defined on a common surface tessellation containing 297,800 vertices and 64 labeled cortical regions of interest. Moreover, the volumetric segmentation pipeline (Fischl et al., 2002) in FreeSurfer was used to extract the volumes of the deep subcortical gray matter structures. Further, the cerebellar sub-region volumes were calculated by transferring the cerebellar sub-region labels from the SUIT atlas [cite Diedrichsen et al., 2009 and 2011] onto the subject's MRI using the large deformation diffeomorphic metric mapping (LDDMM) non-rigid registration method [cite: Beg et al., 2005]. The subcortical and cerebellar sub-region volumes were normalized using the intracranial volume (ICV) to correct for the brain size of the subject.

2.5. Statistical analysis

Group-wise comparison of the clinical and demographic variables were performed using the MATLAB 2016b (MathWorks, Inc., Natick, MA) numerical computing environment. Normality of the data was verified using Anderson-Darling test. Group difference was performed using independent *t*-test for normally distributed data, else the Wilcoxon rank sum test was used. Chi-square test was used for comparing the group differences in the distribution of categorical variables (sex). Results were considered statistically significant at p values < 0.05.

Vertex-wise group differences among the generated cortical thickness surface maps were explored using the SurfStat toolbox (www.math.mcgill.ca/keith/surfstat/) for MATLAB. A generalized linear model (GLM) approach was performed controlling for the effects of age, sex, and years prior to mean age of illness onset. The cluster significance threshold was set at $p < 0.05$, using random field theory (RFT) correction (Chung et al., 2010) for multiple comparisons, and only significant clusters consisting of > 100 vertices were reported. Similarly, GLM analysis was performed to evaluate the group differences in ICV normalized subcortical volumes correcting for the influences of age, sex and years prior to mean age of illness onset. Group wise comparisons were made between each of the carrier groups