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Molecular signature of disease onset in Granulin mutation carriers: a gene expression analysis study

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ABSTRACT

Mutations within Granulin (*GRN*) gene are causative of autosomal dominant frontotemporal lobar degeneration (FTLD). Though *GRN* mutations are inherited at birth, the disease onset usually occurs in the sixth decade of life. The objective of this study was to identify new genetic pathways linked to inherited *GRN* disease and involved in the shift from asymptomatic to symptomatic stages. Microarray gene expression analysis on leukocytes was carried out on 15 patients carrying *GRN T272SfsX10* mutation, and their asymptomatic siblings with ($n = 14$) or without ($n = 11$) *GRN* mutation. The results were then validated by real-time polymerase chain reaction, and compared with those obtained in a cohort of FTLD without GRN mutation ($n = 16$). The association between candidate genes and damage of specific brain areas was investigated by voxel-based morphometry on magnetic resonance imaging scans (family-wise error-corrected). Leukocytes mRNA levels of *TMEM40* and *LY6G6F* and other genes mainly involved in inflammation were significantly higher in patients carrying *GRN* mutations compared with asymptomatic carriers and other FTLD. The higher the levels of *TMEM40* the greater is the damage of parietal lobule; the higher the *LY6G6F* gene expression the greater is the atrophy in superior frontal gyrus. Enhanced inflammation associated with the onset of *GRN* disease might be either related to disease pathogenetic mechanism leading to neurodegeneration or to a compensatory pathway that counteracts disease progression. The identification of specific molecular targets of *GRN-FTLD* disease is essential when considering future disease-modifying therapies.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a highly heterogeneous neurodegenerative disorder, characterized by behavioral abnormalities, language impairment, and deficits of executive functions (McKhann et al., 2001; Neary et al., 1998). A strong genetic background is recognized, with approximately 50% of patients showing a positive family history of dementia (Rademakers and Rovelet-Lecrux, 2009). In the past 15 years a number of genes have been identified as causative of autosomal dominant FTLD (Baker et al., 2006; Borroni et al., 2010a; Cruts et al., 2006; DeJesus-Hernandez et al., 2011; Renton et al., 2011; Spillantini and Goedert, 2000; Van Langenhove et al., 2010), and Microtuble Associated Protein Tau (*MAPT*), *Granulin* (*GRN*) and *C9orf72* genes are currently considered the key players of inherited disease, responsible for the greater number of cases with known genetic defect. Pathogenetic mutations within these causative genes lead to different clinical phenotypes, with inter- and intra-disease variability and wide range of age at symptom onset.

Among others, *GRN* mutations are expected to induce a loss of 50% of progranulin, with a mechanism of haploinsufficiency, and the presence of ubiquitinated TAR-DNA binding protein (TDP)-43 protein is the neuropathologic hallmark (Baker et al., 2006; Cruts et al., 2006; Mackenzie et al., 2011). The physiologic role of PGRN, and the effect of its reduction in the brain, is still largely unknown, although it has been recently suggested that progranulin might be involved in inflammatory pathways, innate immunity (Tang et al., 2011; Toh et al., 2011), and acts as a neurotrophic factor

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(Van Damme et al., 2008). The behavioral variant of frontotemporal dementia (bvFTD) and the agrammatic variant of primary progressive aphasia (avPPA) are the most typical presentations in *GRN* mutation carriers, with neuroimaging data indicating greater frontal and parietal damage than other FTLD cases (Le Ber et al., 2008; Masellis et al., 2006; Rohrer et al., 2010; Snowden et al., 2006). One of the most intriguing issues of the current literature is the identification of molecular pathways leading to neurodegeneration of specific brain areas, and to the clinical onset in adulthood, when the disease is inherited at birth. Defining the mechanisms responsible for disease transition from presymptomatic to symptomatic stages is crucial in order to identify targets of intervention for this devastating condition.

A combined approach of advanced biologic and neuroimaging techniques might be useful to evaluate the molecular and structural brain modifications moving from preclinical to clinical stages of the disease. High-throughput techniques are stimulating the discovery of new genes associated with different pathologies, and microarray gene expression studies in leukocytes have been demonstrated to represent a good peripheral model reflecting the mechanisms occurring in the brain (Sullivan et al., 2006).

In addition, magnetic resonance imaging (MRI) has become an increasingly popular tool for human brain investigation in vivo. MRI has the unique ability to provide quantitative information of brain tissue structure, and voxel-based morphometry (VBM) is currently regarded as a robust magnetic resonance technique suitable for assessing structural gray matter modifications in an unbiased fashion (Gorno-Tempini et al., 2004; Whitwell et al., 2012). Our group has previously characterized a genetically related group of families carrying the same *GRN Thr272fs* mutation in Italian patients with FTLD (Borroni et al., 2008a, 2010b) and has demonstrated that these families have a common ancestor (Borroni et al., 2011). Considering the symptomatic and asymptomatic carriers of the same GRN mutation, our objective was to investigate the molecular pathways associated with *GRN* mutation, in particular to disease onset by a data-driven gene expression analysis. When biological markers were identified, the association between candidate genes and damage of specific brain areas was investigated by VBM on MRI scans.

2. Methods

2.1. Subjects

Two-hundred individuals, recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, at University of Brescia (Brescia, Italy), were enrolled for the current study. For the aim of the work, subjects' recruitment followed the strategy summarized in Fig. 1. From a large pool of almost 200 patients with FTLD (all genetically characterized for the presence/absence of *GRN* and *MAPT* mutations, and *C9orf72* expansion), the 15 identified as carriers of *GRN Thr272fs* mutation were enrolled in the present study. Their asymptomatic siblings were also invited, this implying for them to undergo the genetic assessment of mutation for *GRN Thr272fs* and blood sampling for biological study. Among 25 available asymptomatic siblings, 14 of them were found to be *GRN Thr272fs* mutation carriers. The remaining 11 (noncarriers of mutation) were enrolled as control group. Asymptomatic mutation carriers and noncarriers were part of a genetically homogeneous population, because they all came from the same 6 families. This latter aspect makes our experimental design well controlled, because the presence/absence of *GRN Thr272fs* mutation is the only critical variable between the groups.

For the second part of the present study, 16 FTLD patients (taken from the same pool of 200 FTLD), noncarriers of *GRN Thr272fs* mutation, were also enrolled in the study, matched for age, sex, and phenotype with the group of FTLD *GRN Thr272fs* mutation carriers. Further, in these patients, *MAPT*, TAR-DNA Binding Protein (*TARDBP*), *C9orf72* genes were screened and mutations excluded.

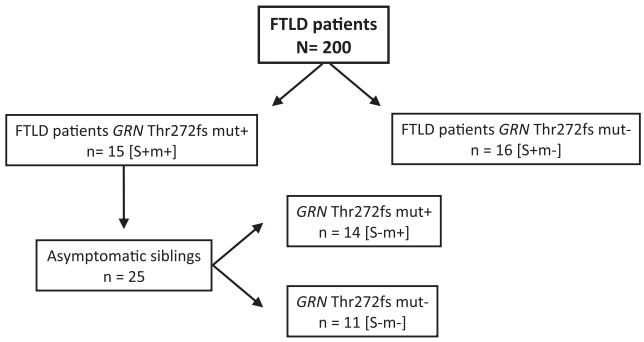

Fig. 1. Recruitment strategy used for the current study. From a pool of almost 200 genetically characterized frontotemporal lobar degeneration (FTLD) patients (S+), we recruited 15 patients carriers of *GRN Thr272fs* mutation. From their families, we recruited 25 asymptomatic siblings, 14 of them carriers for *GRN Thr272fs* mutation (m+), and 11 noncarriers (m−). From the same pool of 200 patients, we also recruited 16 individuals with FTLD noncarriers for *GRN Thr272fs* mutation.

The included subjects underwent blood sampling for biological analyses, and divided in the following experimental groups: (1) FTLD patients, carriers of *GRN Thr272fs* mutation ($n = 15$) (S+m+); (2) FTLD patients, noncarriers of *GRN Thr272fs* mutation ($n = 16$) (S+m−); (3) asymptomatic at-risk individuals, carriers of *GRN Thr272fs* mutation ($n = 14$) (S−m+); and (4) asymptomatic individuals, nonmutation carriers ($n = 11$) (S−m−). Main demographic, genetic, and clinical characteristics of the entire population of subjects who were considered are summarized in Table 1.

All FTLD patients met current clinical diagnostic criteria for bvFTD (18 cases) (Rascovsky et al., 2011) and avPPA (13 cases) (Gorno-Tempini et al., 2011), with a similar distribution between carriers and noncarriers of *GRN Thr272fs* mutation (see Table 1). To increase as much as possible the confidence of a correct diagnosis of FTLD in patient noncarriers of *GRN Thr272fs* mutation, they had to be clinically and neuropsychologically followed-up for at least 2 years, at the time of recruitment. In all FTLD patients and

Table 1
Main demographic, clinical characteristics, and serum progranulin dosage of studied subjects

Variable	S+m+	S−m+	S−m−	S+m−
	$n = 15$	$n = 14$	$n = 11$	$n = 16$
Age at evaluation, y	62.1 ± 6.6	42.0 ± 10.1	40.7 ± 10.2	65.6 ± 10.8
Sex, female, % (n)	73 (11)	43 (6)	81 (9)	38 (6)
Age at onset, y	59.2 ± 6.6	—	—	63.3 ± 10.8
Phenotype	8 bvFTD; 7 avPPA	—	—	10 bvFTD; 6 avPPA
Disease duration, y	2.14 ± 1.8	—	—	2.0 ± 0.97
Education, y	7.4 ± 3.5	10.9 ± 3.1	10.5 ± 3.8	7.5 ± 3.3
FH, % (n)	86.7 (13)	—	—	12.5 (2)
Serum PGRN, pg/ml	46.2 ± 23.3	39.8 ± 10.6	123.9 ± 23.0	146.2 ± 54.4

No significant differences were found between patients with FTLD m+ and FTLD m−, with the exception of FH ($p < 0.001$) and serum progranulin levels ($p < 0.001$). Asymptomatic m+ and asymptomatic m− individuals differed for sex distribution ($p = 0.021$) and serum progranulin levels ($p = 0.001$). Group comparisons were performed by Mann–Whitney test or χ^2 test (statistical threshold: $p \leq 0.05$). See text for further details.
Key: avPPA, agrammatic variant of primary progressive aphasia; bvFTD, behavioral variant of frontotemporal dementia; FH, family history; m+/−, presence/absence of *GRN Thr272fs* mutation; PGRN, progranulin; S+/−, presence/absence of frontotemporal lobar degeneration.