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Understanding phenotype variability in frontotemporal lobar degeneration due to granulin mutation

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ABSTRACT

Phenotype in patients with *granulin (GRN)* mutations is unpredictable, ranging from behavioral variant frontotemporal dementia (bvFTD) to agrammatic variant of primary progressive aphasia (avPPA). To date the wide clinical variability of FTLD-GRN remains unexplained. The aim of the study was to identify genetic pathways differentiating phenotypic expression in patients carrying *GRN* mutations. Patients carrying the same *GRNT272SfsX10* mutation were enrolled, a careful clinical assessment was carried out, and the diagnosis of either bvFTD ($n = 10$, age $= 63.9 \pm 9.4$) or avPPA ($n = 6$, age $= 58.8 \pm 4.7$) was done. Microarray gene expression analysis on leukocytes was performed. Genes differentially expressed between the groups were validated by real time polymerase chain reaction considering an age-matched healthy controls group ($n = 16$, age $= 58.4 \pm 10.7$). We further considered a group of FTD with no *GRN* mutations (GRN-) ($n = 21$, 13 bvFTD, and 8 avPPA) for comparisons. Real-time polymerase chain reaction (PCR) confirmed a significant decrease in leukocytes mRNA messenger RNA (mRNA) levels of *RAP1GAP* in bvFTD patients as compared with avPPA ($p = 0.049$). This finding was specific for patients with *GRN* mutations, as we did not observe this pattern in FTD *GRN*-patients ($p = 0.99$). The alteration of RAP1GAP mRNA levels may explain the clinical variability of GRN-FTLD patients. This is the first report linking a molecular pathway to specific phenotype expression in FTLD-GRN. To understand the clinical relevance of our early results it will be mandatory to extend the observation to other clinical and neuropathological series.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a clinically, pathologically, and genetically heterogenous disorder. Three prototypical variants have been described, namely the behavioral variant frontotemporal dementia (bvFTD), the semantic variant of primary progressive aphasia, and the agrammatic variant of primary progressive aphasia (avPPA) (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). In most cases, at autopsy either FTLD with tau-positive inclusions or FTLD with TDP-43-positive aggregates may be variably found over the clinical spectrum of FTLD cases (Mackenzie et al., 2009, 2011).

Despite the consistent progress in carefully characterizing the clinical phenotypes and in defining neuroimaging correlates, there is no unique correspondence with neuropathological substrates.

The mismatch between neuropathological and clinical features is still evident when monogenic FTLD is considered. *Granulin (GRN)* gene has been identified as a major cause of autosomal dominant FTLD, leading to TDP-43 inclusions by a haploinsufficiency mechanism (Cruts et al., 2006; Toh et al., 2011). FTLD patients carrying *GRN* mutations clinically present unpredictable phenotypic variability even within families carrying the same mutation (Beck et al., 2008; Bruni et al., 2007; Chen-Plotkin et al., 2011; Kelley et al., 2009; Larner, 2012; Le Ber 2008; Li et al., 2008; Moreno et al., 2009; Pickering-Brown et al., 2008; Rademakers et al., 2007; van Swieten and Heutink, 2008; Yu et al., 2010), and bvFTD and avPPA represent the most frequent pictures.

Aim of the present study was to dissect clinical heterogeneity in patients carrying the same *GRN* mutation. To this, a coalescent cohort of patients with *GRN Thr272fs* mutation was considered, and whole gene expression analysis on leukocytes was carried

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out to compare the expression profiles in the different clinical phenotypes (bvFTD vs. avPPA).

2. Methods

2.1. Subjects

Two hundred fifty FTLD patients, recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, at University of Brescia (Brescia, Italy), were screened for pathogenic *GRN* and *MAPT* mutations and *C9orf72* expansion; those carrying *GRN Thr272fs* mutation were enrolled in the present study. In these patients, diagnosis of either bvFTD or avPPA was made according to the current clinical criteria (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). All FTLD patients carrying *GRN Thr272fs* mutation were part of a genetically homogenous population, as these patients harbor a common ancestor as previously reported (Borroni et al., 2011), making our experimental design well controlled.

All patients were subjected to an extensive neurologic and neuropsychological evaluation, as previously described (Borroni et al., 2010), a routine laboratory examination, and a conventional brain magnetic resonance imaging before entering this study. We further enrolled a group of 21 FTD patients without GRN mutations, matched for age and gender, for comparisons and we sub-grouped them according to clinical phenotype, that is, 8 avPPA (age 69.4 ± 9.6) and 13 bvFTD (age 67.1 ± 6.2). A group of healthy controls, matched for age and gender was also considered (see Table 1).

FTD patients, with or without GRN mutations, and healthy controls did not report significant medical problems. None was under treatment for cognitive disturbances, but 7 (3 FTD GRN and 4 GRN-) were under psychotropic drugs for behavioral disturbance control.

Each subject underwent venous blood sampling for biological analyses.

Written informed consent (from the subject or from the responsible guardian if the subject was incapable) was obtained, for each procedure. The work was conformed to the Helsinki Declaration and approved by local ethics committee.

2.2. Granulin sequencing

Genomic DNA was extracted from peripheral blood using a standard procedure. All the 12 exons plus exon 0 of *GRN*, and at least 30 base pairs of their flanking introns were evaluated by polymerase chain reaction (PCR) and subsequent sequencing. *GRN Thr272fs (g.1977_1980 delCACT)* was tested as previously described (Borroni et al., 2008).

2.3. RNA isolation and microarray gene expression procedures

Blood samples were obtained by venipuncture using PaxGene Tubes (Qiagen, Manchester, UK). RNA isolation was performed by PaxGene Blood RNA Kit (Qiagen) according to the manufacturer protocols. RNA quality and integrity were assessed using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). Total RNA (250 ng) from each sample were reverse transcribed to complementary DNA (cDNA), followed by overnight in vitro transcription to generate complementary RNA, which was reverse transcribed, and the 7.5 μg of sense cDNA were fragmented and labeled. The quality of cDNA and fragmented cDNA was assessed using Agilent bioanalyzer (Agilent Technology, TX, USA). Hybridization was performed on human gene 1.1 ST array strips (Affymetrix, Inc, Santa Clara, CA). The reactions of hybridation, fluidics, and imaging were performed on the Affymetrix Gene Atlas instrument according to the manufactured protocol.

2.4. Microarray data analysis

Gene expression data were imported from the Gene Atlas instrument into Partek Genomics Suite 6.0 (Partek, St Louis, Mo) software as CEL files using default parameters. Principal-component analysis was performed to identify outliers. Differential expression analysis between bvFTD and avPPA phenotypes was performed by the analysis of variance (ANOVA) and a gene list was created using a cutoff of $p < 0.05$ and 1.6-fold change.

Table 1
Demographic and clinical characteristics of FTLD-*GRN* patients according to clinical diagnosis

Variable	FTD-GRN all (N = 16)	bvFTD-GRN (N = 10)	avPPA-GRN (N = 6)	Controls (N = 16)	*p* (bvFTD vs. avPPA)
Age at evaluation, years	64.2 ± 8.5	65.8 ± 9.9	61.5 ± 4.9	58.4 ± 10.7	0.35
Gender, female%	62.5	60	66	56	0.78
Family history, %	100	100	100	0	1.00
Age at onset, years	59.3 ± 8.6	61.4 ± 9.3	55.8 ± 6.4	-	0.04
Education, years	6.8 ± 2.2	6.1 ± 2.3	8.0 ± 1.7	8.4 ± 3.8	0.03
Serum PGRN, pg/ml	48.2 ± 21.4	50.6 ± 23.6	43.4 ± 18.5	141.7 ± 42.5	0.47
FTLD-modified CDR	7.3 ± 5.6	8.4 ± 6.3	5.9 ± 4.8		0.37
MMSE	22.5 ± 5.4	22.6 ± 4.5	22.3 ± 6.1		*0.85*
UPDRS-III	10.0 ± 16.7	14.2 ± 20.1	3.0 ± 4.3		*0.04*
Short story	8.2 ± 5.5	8.7 ± 6.2	6.6 ± 3.8		*0.77*
Raven matrices	15.9 ± 7.2	14.2 ± 4.5	17.2 ± 9.2		*0.15*
Rey figure, copy	18.5 ± 10.1	16.0 ± 8.2	22.1 ± 12.5		*0.14*
Rey figure, recall	6.4 ± 4.8	4.0 ± 3.2	9.3 ± 5.1		*0.09*
Phonological fluency	14.0 ± 10.5	17.3 ± 10.5	9.6 ± 9.6		*0.12*
Semantic fluency	20.3 ± 5.4	20.6 ± 4.9	20.0 ± 6.6		*0.85*
Digit span, backward	4.3 ± 0.6	4.3 ± 0.5	4.2 ± 0.8		*0.83*
Token test	24.5 ± 5.8	25.2 ± 4.9	23.1 ± 7.7		*0.57*
Trail making test, A	71.0 ± 25.6	85.0 ± 16.4	52.3 ± 25.5		*0.16*
Trail making test, B	380.7 ± 162.0	350 ± 166.7	456.0 ± 62.4		*0.16*
NPI, total score	18.5 ± 13.0	20.8 ± 13.3	14.8 ± 12.7		*0.57*
FBI, total score	18.0 ± 11.1	21.8 ± 12.1	12.2 ± 7.1		*0.18*

Key: avPPA, agrammatic variant of primary progressive aphasia; bvFTD, behavioral variant of frontotemporal dementia; FBI, frontal behavioral inventory; FTD-GRN, frontotemporal patients carrying Granulin Thr272fs mutation; FTLD-modified CDR, clinical dementia rating scale modified for frontotemporal dementia, sum of boxes values; MMSE, Mini Mental State Examination; NPI, neuropsychiatric inventory; UPDRS-III, Unified Parkinson's Disease rating Scale.