Genetic Background Predicts Poor Prognosis in Frontotemporal Lobar Degeneration

B. Borroni a M. Grassi f S. Archetti b A. Papetti a R. Del Bo g C. Bonvicini c G.P. Comi g M. Gennarelli c d G. Bellelli e M. Di Luca h A. Padovani a

a Center for Aging Brain and Dementia, Department of Neurology, University of Brescia, b Department of Laboratories, Brescia Hospital, c IRCCS Fatebenefratelli, d Department of Biomedical Sciences and Biotechnology, University of Brescia, and e Department of Rehabilitation and Alzheimer’s Evaluation Unit, Cremona and Geriatric Research Group, Brescia, f Department of Health Sciences, Section of Medical Statistics and Epidemiology, University of Pavia, Pavia, g Dino Ferrari Center, Department of Neurological Sciences, University of Milan, IRCCS ‘Foundation Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena’, and h Center of Excellence for Neurodegenerative Disorders, University of Milan, Milan, Italy

Key Words
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Abstract

Background: Ruling out predictors of survival in frontotemporal lobar degeneration (FTLD) is a clinical challenge for defining disease outcomes and monitoring therapeutic interventions. Little is known about determinants of survival in FTLD. Objective: The aim of the present study was to identify whether genetic determinants are key, not only as risk factors but as predictors of survival in FTLD. Methods: Ninety-seven FTLD patients were considered in the present study. A clinical evaluation and a standardized assessment were carried out. Each patient underwent blood sampling for genetic testing, and mutations within the progranulin (PGRN) gene, microtubule-associated protein tau (MAPT) haplotype, apolipoprotein E (APOE) genotype and 4 vascular endothelial growth factor (VEGF) polymorphisms were evaluated. Discrete-time survival models were applied. Results: Monogenic FTLD due to PGRN mutations [odds ratio (OR) = 3.62, 95% confidence interval (CI) = 1.12–11.7; p = 0.032], and MAPT *H2 haplotype (OR = 3.23, 95% CI = 1.08–9.69; p = 0.036) were associated with an increased hazard risk of poor outcome. Conversely, APOE genotype, and VEGF polymorphisms were not associated with survival risk in the FTLD sample. Conclusions: Genetic background is not only crucial in disease pathogenesis, but it also modulates disease course. Genetic factors influencing prognosis should be taken into account to include homogeneous groups in future clinical trials and to monitor efficacy of future interventions.

Introduction

Frontotemporal lobar degeneration (FTLD) is the second most common form of neurodegenerative dementias in adulthood, after Alzheimer’s disease [1]. The improving knowledge of neuropathological hallmarks and disease mechanisms suggests new possible therapeutic targets to be tested in the near future [2]. However, to assess
the beneficial effect of therapeutic interventions and to include homogeneous patients in clinical trials, a better definition of natural disease course and factors related to higher mortality risk is mandatory [3].

The common experience of clinicians who follow patients with FTLD is the existence of large heterogeneity in the course of the disease not merely explained by clinical phenotypes [4]. A subgroup of these patients shows a benign prognosis over years, others progress to institutionalization with a malignant course. Previous studies demonstrated that FTLD progresses to death faster than Alzheimer’s disease [5], but the current literature has still not recognized significantly associated factors at the time of diagnosis to be used in clinical practice as markers of survival risk. Demographic characteristics, age at symptom onset, or family history of dementia do not help in identifying patients with higher risk of mortality [5–11]. More recently, it has been suggested that a positive family history and older age at onset might be predictive of worse prognosis [12]. In the same way, clinical presentation, except for motor neuron disease, is not associated with different patterns of progression over time [5, 6, 8, 13]. Notwithstanding, patients with definite FTLD have a worse prognosis if language deficits are also present [14].

The few available works on survival risk in FTLD have not considered certain variables yet. In this view, as FTLD is recognized as having a stronger genetic background compared to other neurodegenerative dementias, the role of genetic factors should inevitably be analyzed further.

FTLD may present an autosomal dominant pattern of inheritance, and mutations within progranulin (PGRN) [15, 16] and microtubule-associated protein tau (MAPT) [17] are recognized as the most common forms of known genes.

However, despite several efforts to identify monogenic causes of FTLD, less interest has been shown in the understanding of the genetic risk factors associated with the disease, and no study has tested the effect of genetic variations of possible candidate genes on disease survival.

It has been demonstrated that MAPT H1/H2 haplotype influences clinical FTLD presentation [18] and short-term prognosis in FTLD spectrum [11]; on the other hand, apolipoprotein E (APOE) genotype, beyond being the most recognized genetic risk factor for late-onset Alzheimer’s disease, might modulate the FTLD clinical picture [19, 20]. More recently, vascular endothelial growth factor (VEGF) polymorphisms have been associated with increased risk of FTLD [21].

With these caveats in mind, we sought to test whether genetic background, namely PGRN mutations and genetic variations within MAPT, APOE and VEGF (–2578C/A, –1190G/A, –1154G/A, and –634G/C), might be associated with higher risk of mortality in FTLD. To this end, we applied discrete-time survival models, and we considered demographic characteristics and disease presentation as possible contributors.

**Methods**

**Subjects**

This study is part of an ongoing research program aimed at evaluating the core feature of FTLD and predictors of prognosis at the Center for Aging Brain and Neurodegenerative Diseases, University of Brescia, Italy, between 2001 and 2010. Ninety-seven subjects who fulfilled the Neary and McKhann criteria for FTLD [22, 23], including behavioral frontotemporal dementia, semantic dementia, and progressive nonfluent aphasia variants, and who met inclusion/exclusion criteria were considered in the present study.

Specifically, inclusion criteria were: (a) full consensus agreement by at least 2 experienced reviewers on FTLD diagnosis; (b) follow-up for at least 2 years after diagnosis, and diagnosis confirmation; (c) blood sampling and genetic test availability. Stringent exclusion criteria were applied as follows: (a) cerebrovascular disorders, previous stroke, hydrocephalus, and intracranial mass documented by MRI; (b) a history of traumatic brain injury or other neurological diseases; (c) significant medical problems (e.g. poorly controlled diabetes or hypertension; cancer within the past 5 years; clinically significant hepatic, renal, cardiac, or pulmonary disorders); (d) major depressive disorder, bipolar disorder, schizophrenia, substance abuse disorder, or mental retardation according to criteria of the DSM-IV; (e) FTLD with motor neuron disease.

**Variables**

All subjects underwent a somatic and neurological evaluation, and routine laboratory examinations, a brain structural MRI and/or brain functional study by single photon emission tomography. The diagnostic assessment involved a review of full medical history, a semi-structured neurological examination, and a complete mental status evaluation.

All patients underwent a cognitive, behavioral and functional examination according to a standardized FTLD assessment, as previously published [11].

In the present study, age at onset, clinical features, and genetic variables were considered. Briefly, the age at onset of symptoms was based on a family report of the earliest persistently abnormal clinical feature in the domains of language, social function or personality change, or executive functioning. Patients considered to have a positive family history were those who had a first-degree relative with dementia. In regard to comorbidities, history of hypertension, diabetes mellitus, hypercholesterolemia and cardiomyopathy were assessed in each subject. Hypertension was considered present either if systolic blood pressure was >140 mm Hg and diastolic pressure >90 mm Hg in more than 3 separate measurements, or if the subject was treated with antihypertensive drugs before recruitment. The diagnosis of diabetes mellitus was
established according to WHO criteria. Hypercholesterolemia was considered present either if cholesterol serum levels were >220 mg/dl or if the subject was under treatment with cholesterol-lowering drugs. The presence of atrial fibrillation, ischemic cardiomyopathy or hypertensive cardiomyopathy was also considered according to common clinical criteria.

Blood sampling was carried out after informed consent for genetic analyses. In each patient, mutations within PGRN gene were analyzed, as well as MAPT haplotype, APOE genotype and 4 different VEGF polymorphisms (–2578C/A, –1190G/A, –1154G/A, and –634G/C). FTLD patients were screened for MAPT mutations; in the present analysis, MAPT pathogenetic mutations were not considered, because only 1 patient carried monogenic MAPT disease.

All participants were made fully aware of the research goals, and the signature of an informed consent was required from all subjects. The work was conducted in accordance with local clinical research regulations and in conformity with the Declaration of Helsinki.

**Genetic Analyses**

Total genomic DNA was prepared from peripheral blood according to standard procedures.

**PGRN Sequencing**. All the 12 exons plus exon 0 of PGRN and at least 30 base pairs (bp) of their flanking introns were evaluated by polymerase chain reaction (PCR). PCR primers were designed to optimize denaturing high-performance liquid chromatography (dHPLC) conditions, following previously provided primer pairs, as previously published [24]. The dHPLC method was adopted to screen, and samples with an altered dHPLC profile were purified with Microcon centrifugal filter devices (Amicon Bioseparations – Millipore) and sequenced. Sequencing was performed in duplicate from purified PCR on the 310 DNA sequencer ABI Prism (Applera Biosystems, Italy), according to the manufacturer’s instructions. Sequences were compared with those available in public databases.

**MAPT Haplotype Analysis**. MAPT haplotype was evaluated by saitojin gene (STH) amplification using the forward primer 5’ GCA AGT TCA GTT GCC ATC TTC 3’ and reverse primer 5’ CTC TTT TGC ATG CAC CTA GT 3’. PCR product consisted of a fragment of 795 bp. PCR was performed using 200 ng of genomic DNA in 50 μl of reaction mixture consisting of 0.2 mM of each primer, 5 mM of deoxynucleotide triphosphate, 2.5 mM of MgCl₂, 5 nl of 10X PCR buffer and 2.5 U of Taq polymerase. After initial denaturation at 95°C for 5 min, the reaction mixture was subjected to 35 cycles of 40 seconds denaturation at 95°C, 2-min annealing at 56°C, 1-min extension at 72°C, followed by a final 10-min extension step at 72°C. Genotyping of the STH single nucleotide polymorphism was performed by digesting the PCR product with Hinf I restriction enzyme (New England Biolabs). The STH*Q allele was characterized by 5 fragments of 261, 243, 194, 54, and 30 bp, while the STH*R allele was characterized by 2 fragments of 97 bp, a fragment of 261 bp and 1 of 243 bp.

**APOE Genotyping**. Genetic variation at the APOE locus was determined by restriction isotyping using PCR amplification and subsequent digestion with Hha I (Qiogene). The nucleotide substitutions that result in Arg-Cys interchange at position 112 and 158 alter Hha I cleavage sites: each genotype can be distinguished by unique combinations of Hha I fragment sizes in all homozygotic and heterozygotic combinations.

**VEGF Genotyping**. Two genomic DNA regions containing portions of the VEGF promoter were amplified by PCR. The amplification protocol was as follows: 5 min at 94°C for the first cycle, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s for the subsequent 35 cycles, and a final extension at 72°C for 5 min. To detect the polymorphism C(–2578)A, fragments were directly electrophoresed on a 3% agarose gel because of an 18-nucleotide insertion always associated with the –2578A allele, whereas CC homozygotes do not contain this insertion. –1190G/A and –1154G/A variants were detected through direct sequencing analysis of PCR fragments using Big Dye Terminator protocol on an automated 3100 ABI Prism Genetic Analyzer (Applied Biosystem, Foster City, Calif., USA). The polymorphism G(–634)C (rs2010963) was detected by allelic discrimination through a TaqMan SNP genotyping assay (ID: C_8311614_10) on an ABI Prism 7000 instrument (Applied Biosystem).

**Follow-Up Outcomes**

Each patient was followed up over a 9-year period from the time of the study enrollment/diagnosis, and the survival times were recorded in years from the age at onset of symptoms. The binary endpoint was determined as the entry to nursing home or other long-term care facility (institutionalization) and death (outcome = 1), and otherwise (no institutionalization/death, outcome = 0). Endpoints were determined by clinical periodic follow-up when possible, or by a semi-structured telephone interview.

**Statistical Analysis**

Results are given as means ± standard deviation (SD). All statistical tests were 2-sided, and p values less than 0.05 were considered statistically significant. FTLD groups were compared by means of one-way ANOVA or χ² test, as appropriate.

Since the continuous times were replicated in many patients, and the number of time values is less than 20, discrete-time survival analysis models (for an overview, see Singer and Willett [25]) were performed using six time intervals of the follow-up period: 1–2, 3–4, 5–6, 7–8, 9–10, and >10 years.

Only time-independent covariates have been considered, thus only covariates whose values remain constant during follow-up. The survival analysis investigated demographic features (age at onset, and gender), clinical variables (diagnosis, family history, history of hypertension, hypercholesterolemia, diabetes, and cardiomyopathy) and genetic background (PGRN mutation and MAPT haplotype, APOE genotype, VEGF polymorphisms). Univariate models (single predictor entered in the multiple models) and one multivariate model (all predictors entered in a single model) were fitted. In the multivariate model, total number of comorbidities (from 0 to 4), and total number of VEGF polymorphisms (from 0 to 4) were considered for reducing multicollinearity.

The maximum likelihood estimates of model parameters (= hazard odds ratios, OR), 95% confidence intervals (95% CI), and p values of the t test (= parameter estimate/standard error) using ‘robust’ standard error were reported.

Data analysis and discrete-time survival modeling were performed using the SPSS (version 15.0) software (www.spss.com), and Mplus (version 6.0) software (www.mplus.com), respectively.
Results

Among 180 FTLD patients, 97 subjects fulfilling inclusion and exclusion criteria and with genetic testing available were considered in the present analysis.

Demographic, clinical and genetic features according to clinical diagnosis are reported in table 1. FTLD patients were homogeneous across clinical diagnoses. The average time delay between age at diagnosis and age at onset of symptoms was equal to 2 years (SD = 1.7 years). Seven out of 97 patients carried PGRN mutations (7.2%), namely T272SfsX10 (n = 6) and Q341X (n = 1). Forty percent of patients carried at least one MAPT*H2 allele (H1/H2 or H2/H2), and 29% the APOE*ε4 allele. Thirty-two out of 97 FTLD patients had been institutionalized or died throughout the follow-up observation. In the overall group, the average survival time from the onset of symptoms was 6.2 years (SD = 2.9 years), and the failure rate was about 5 events per 100 person-years at risk.

The univariate (crude) and multivariate (adjusted for) hazard OR estimates from discrete-time survival models are reported in table 2. There was no significant evidence to suggest that gender, positive family history of dementia, and comorbidities, i.e. history of hypertension, hypercholesterolemia, diabetes, and cardiomyopathy, significantly correlated with the rate of survival. Clinical diagnoses and age at onset of symptoms predicted the survival rate in FTLD only when either the crude OR or multivariate model were considered, respectively.

Conversely, as shown in table 2, genetic background significantly predicted the survival rate in FTLD patients in both the univariate and multivariate models. In particular, monogenic cases due to PGRN mutations (OR = 5.11, 95% CI = 1.83–14.2, p = 0.002 for univariate model, and OR = 3.62, 95% CI = 1.12–11.7, p = 0.032 for multivariate model), and patients carrying the MAPT*H2 haplotype (OR = 2.67, 95% CI = 1.10–6.46, p = 0.029 for

| Table 1. Demographic and clinical characteristics of included FTLD patients |
|-----------------------------|-------------|-------------|-------------|-------------|-------------|
|                             | FTLD overall | bvFTD (n = 80) | SD (n = 8) | PNFA (n = 9) | p value |
| Age at onset, years         | 63.6 ± 7.8   | 63.5 ± 7.8   | 68.6 ± 9.0 | 60.5 ± 7.5   | 0.094     |
| Age at diagnosis, years     | 65.6 ± 7.8   | 65.6 ± 7.5   | 70.3 ± 9.0 | 63.1 ± 8.6   | 0.146     |
| Female gender, %            | 51.5 (50)    | 48.8 (39)    | 75.0 (6)   | 55.6 (5)     | 0.335     |
| Family history, %           | 42.3 (41)    | 43.8 (35)    | 25.0 (2)   | 44.4 (4)     | 0.587     |
| Clinical variables          |             |             |             |             |           |
| Hypertension, %             | 36.6 (34)    | 37.7 (29)    | 28.6 (2)   | 33.3 (3)     | 0.872     |
| Hypercholesterolemia, %     | 39.4 (37)    | 42.9 (33)    | 29.5 (2)   | 22.2 (2)     | 0.334     |
| Diabetes, %                 | 12.9 (12)    | 14.3 (11)    | 0.0        | 11.1 (1)     | 0.612     |
| Cardiomyopathy, %           | 16.0 (15)    | 17.9 (14)    | 0.0        | 11.1 (1)     | 0.424     |
| Comorbidities, n            | 1.05 ± 0.98  | 1.13 ± 0.98  | 0.50 ± 0.54| 0.77 ± 1.28  | 0.218     |
| Genetic background          |             |             |             |             |           |
| PGRN mutations, n           | 7.2 (7)      | 5.0 (4)      | 0.0        | 33.3 (3)     | 0.006     |
| APOE*ε4, %                  | 29.0 (27)    | 30.3 (23)    | 37.3 (3)   | 11.1 (1)     | 0.380     |
| MAPT*H2 haplotype, %        | 40.0 (36)    | 37.3 (28)    | 71.4 (5)   | 37.5 (3)     | 0.210     |
| VEGF –2578 C/A, AA, %       | 22.6 (19)    | 23.9 (17)    | 14.3 (1)   | 16.7 (1)     | 0.790     |
| –1190 G/A, AA, %            | 25.0 (21)    | 28.2 (20)    | 0.0        | 16.7 (1)     | 0.230     |
| –1154 G/A, AA, %            | 17.9 (15)    | 19.7 (14)    | 0.0        | 16.7 (1)     | 0.428     |
| –634 G/C, CC, %             | 10.0 (8)     | 9.0 (6)      | 14.3 (1)   | 16.7 (1)     | 0.771     |
| VEGF polymorphisms, n       | 0.78 ± 1.00  | 0.85 ± 1.04  | 0.28 ± 0.48| 0.66 ± 0.81  | 0.353     |

bvFTD = Behavioral variant of frontotemporal dementia; SD = semantic dementia; PNFA = progressive nonfluent aphasia. Number of subjects are given in parentheses.

1 Differences in the total are due to missing values: smoking habits = 81 cases, APOE and MAPT = 90 cases, VEGF polymorphisms = 84 cases available.

2 F test of one-way ANOVA or χ² test, as appropriate.
univariate model, and OR = 3.23, 95% CI = 1.08–9.69, p = 0.036 for multivariate model) had a 3-fold increased hazard risk of mortality/early institutionalization compared to the reference (‘wild’) genotypes. Conversely, APOE genotype and those VEGF polymorphisms previously associated with increased risk for FTLD [21] were not associated with survival in the FTLD sample.

Figure 1 displays the estimated discrete-time survival probabilities of the multivariate model for the four PGRN × MAPT groups at the mean value for age at onset (= 64 years), and at the reference category (= 0) for the other covariates.

**Discussion**

The evaluation of predictors of survival in FTLD is essential for a number of reasons. First of all, it allows the identification of factors that can be targeted to reduce the mortality risk, and to define faster decliners eligible for therapeutic interventions. This would allow clinicians to...
meet caregivers’ and patients’ expectations and counsel on disease course and disease progression. Moreover, the estimate of mortality risk might be of help in reducing the number of patients needed in clinical trials, and in establishing the effects of a disease-modifying drug within a reasonable time frame.

Up to now, only a few studies on natural disease course and survival predictors are available. It is well demonstrated that neither demographic characteristics, nor co-morbidities, nor family history of dementia can help clinicians to predict the mean survival rate [5, 6, 10, 26]. It has previously been suggested that a data-driven approach on neuropsychological data might be of help in identifying patients at higher risk of progression [10]. On the other hand, no data on the role of genetic background in modulating survival rate are available.

In the present work, we suggest that genetic background is not only a significant determinant in mono- and polygenic FTLD, but that it influences disease course. Both pathogenic PGRN mutations and MAPT *H2 haplotype were significantly associated with poor prognosis over time.

Only one previous study has reported the effect of PGRN mutations on survival in FTLD patients, arguing that patients bearing PGRN mutations had a shorter survival (5 years) than patients without PGRN mutation-associated disorder [27]. Accordingly, it has been demonstrated that patients carrying PGRN mutations have more generalized atrophy and smaller brains at postmortem compared to patients without PGRN mutations, therefore suggesting that PGRN results in a more rapid, ‘malignant’ form of FTLD [28].

Beyond the key role of autosomal dominant inherited disorder, our findings also demonstrated a significant association between the presence of *H2 allele within MAPT gene and worse prognosis. The mechanism by which MAPT gene polymorphisms contribute to the modulation of FTLD in these patients is currently unclear; it could be related to an effect on tau expression or to an association with other disease-modifying factors. In fact, H1 and H2 alleles have different transcriptional activity in human cell lines with H1 being more efficient at driving MAPT gene expression than H2 allele [29]. In the same way, functional brain imaging studies have supported the claim of a more severe frontal impairment in *H2 compared to H1 carriers in FTLD, thus leading to a faster progression [30, 31].

These findings add to the growing body of literature that argues that the clinical course of FTLD is heterogeneous. The observations of the present study might be of help in clinical practice, but we recognize that they entail some limitations. It is noteworthy that the results of this study need to be replicated in other work, and neuropathological confirmation would be necessary to further confirm the present findings. Furthermore, one of the drawbacks of the present study is that we considered both institutionalization and death as an outcome measure, this being able to influence the final results.

In conclusion, the present work argues that genetic background is not only crucial in disease pathogenesis and disease onset, but it modulates disease course. Establishing disease-modifiable and nonmodifiable factors of poor prognosis is mandatory to define homogeneous patient groups to include in future clinical trials and to monitor efficacy of future interventions. Future studies comparing prognosis in the different autosomal dominant FTLDs would be of interest.

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References


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