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PRION PROTEIN CODON 129 POLYMORPHISM MODIFIES AGE AT ONSET OF FRONTOTEMPORAL DEMENTIA WITH THE C. 709-1G>A PROGRANULIN MUTATION

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Abstract

Frontotemporal lobar degeneration due to mutations in the progranulin gene (PGRN) presents a high variability both in the clinical phenotype and age of onset of disease. Factors that influence this variability remain largely unknown. The aim of our study was to determine whether selected genetic variables modify age at onset of disease in our series of 21 patients with a single splicing mutation (c.709-1G>A) in the PGRN gene, all of whom were of Basque descent. In our analysis, we included the following genetic variables: PGRN rs5848 and rs9897526 polymorphisms, APOE and MAPT genotypes and PRNP codon 129 polymorphism. We found no association between PGRN polymorphisms, APOE and MAPT genotypes and age at onset of the disease; while we report evidence for an association between PRNP codon 129 polymorphism and age at onset of disease in frontotemporal dementia-PGRN(+) patients. MM homozygous carriers presented onset of disease on average 8.5 years earlier than patients who carried at least one valine on their PRNP codon 129 (MV or VV). The biological justification for this association remains speculative.

Keywords

APOE; PRNP codon 129; frontotemporal dementia; frontotemporal lobar degeneration; progranulin; prion protein gene

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INTRODUCTION

Progranulin (PGRN) gene mutations are a frequent genetic cause of frontotemporal lobar degeneration (FTLD). Despite the suggested common pathogenic mechanism of haploinsufficiency for all the PGRN mutations, there is a huge variability both in age at onset and initial symptoms of disease.

Genetic or environmental factors that influence the phenotypic variability and the age at onset remain unknown. Of the genetic factors studied, the haplotype of microtubule-associated protein tau (MAPT) seems to have no effect on the age at onset^{1,2}, although one recent study has shown that H1H2 subjects carrying the Leu271LeufsX10 mutation in PGRN had an earlier disease onset than H2H2 individuals³. The presence of at least one APOE ε4 allele has been associated with a delay in onset in some studies^{1,4}, but not in others². One international study found that patients with the Arg493X mutation that carried the A-allele at rs9897526, a polymorphism on their wild-type PGRN allele, have delayed symptom onset². Although homozygosity for the T-allele at rs5848 could act as a risk factor for frontotemporal dementia (FTD), no effect of this common genetic variant as a modifier factor in PGRN mutation carriers has been proved².

Our group identified the c.709-1G>A (Ala237Trpfsx4) mutation in the PGRN gene in patients with FTD^{5,6}. The objective of this study was to determine the potential role of some selected genetic factors in the age at onset of the disease in our series of patients with this single splicing mutation in PGRN. We included PRNP codon 129 analysis in our study because this polymorphism has been suggested as a possible disease-modifying factor in various neurodegenerative diseases.

METHODS

Study population

Patients were recruited from the “Cognitive Disorders Unit” at Donostia Hospital, a tertiary referral centre. We included patients with the c.709-1G>A mutation in PGRN. All patients were diagnosed with a frontotemporal dementia complex syndrome and were of Basque origin. The clinical and neuropsychological features of the series have been previously published^{5,6}. We considered age at onset as being when principal caregivers reported having observed the first symptoms of disease in a detailed interview.

Genotyping

DNA was extracted from blood cells by standard procedures after written informed consent was obtained from patients or relatives. PGRN gene sequencing procedures used in our laboratory have been published elsewhere⁵. APOE and MAPT genotypes were analyzed as described in Blazquez et al⁷ and Baker et al⁸, respectively. In order to analyze the PRNP 129 codon position a fragment of 385bp of PRNP was amplified using primers designed by us (PRNP Fw 5′-GCCAAAAACCAACATGAAGC-3′ and PRNP Rev primer 5′-CATGCTCGATCCTCTCTGG-3′). The fragment obtained was digested by BsaAI endonuclease which cuts if there is a valine at codon 129.

Statistical analysis

Categorical variables are described by absolute and relative frequencies and compared with the Chi-squared test. The Pearson correlation coefficient was used in order to study the linear association between continuous variables, and, for each group considered, the normal distribution of such variables was checked using the Shapiro-Wilks test. ANOVA test, or the Welch test as required, was used in order to analyze mean equalities. The Kernel density

estimator was used to estimate the probability function. P-values lower than 0.05 were considered significant. Statistical analysis was performed using R statistical software, version R 2.6.1.

RESULTS

Twenty-one patients with the c.709-1G>A mutation from thirteen families were included in the genetic analysis. Fourteen were women and seven men and their age at onset of disease ranged from 42 to 70 years (Mean $\pm$ SD = 59.2 ± 7.2). This variable showed a fairly symmetrical distribution with a median of 61 years. No significant difference was found in the age at onset between men and women (men, 57.4 ± 6.8 vs. women, 60.1 ± 7.5 , $p = 0.432$) or between different initial diagnostic groups ($p = 0.22$).

The distribution of the studied polymorphisms and haplotypes is summarized in Table 1. Age at onset of dementia was not associated with the rs5848 ($p = 0.591$) or the rs9897526 ($p = 0.654$) polymorphisms of the PGRN gene; nor was age at onset affected by MAPT genotype or APOE status when we compared the group that carried at least one APOE $\epsilon 4$ allele with other APOE variants (APOE $\epsilon 4$ carriers, 61 ± 7.4 years vs. APOE $\epsilon 4$ non carriers, 59.9 ± 6.2 years, $p = 0.756$).

Analyses with the ANOVA test showed significant differences among the three groups of patients classified according to their PRNP codon 129 status (MM, MV and VV, $p = 0.043$). Specifically, the MV group and VV group were comparable regarding age at onset of disease (MV, 61.7 ± 6.28 years vs. VV, 61.7 ± 5.85 years). Given these results we compared the group of patients who had at least one valine on their PRNP codon 129 (MV and VV) with the MM group. The MM homozygous patients, on average, were found to present 8.5 years earlier than patients who carried at least one valine on their PRNP codon 129 (MM, 53.2 ± 7.11 years vs. MV-VV, 61.7 ± 5.9 years, $p = 0.011$) (Fig. 1).

DISCUSSION

Clinical descriptions of FTD-PGRN(+) patients show significant variability in the age at onset of the disease. Our study included patients with a single mutation in the PGRN gene (c.709-1G>A) and all patients were of Basque descent, with a homogeneous genetic background.

In this paper, we report the influence of the PRNP codon 129 status on the age at onset of FTD due to PGRN haploinsufficiency. We observed an earlier age at onset of the disease for the PRNP codon 129 MM homozygous group. No significant differences were found between PRNP codon 129 distribution in our patients and in a sample of 119 Basque healthy subjects (MM: 40.3%, MV: 47.1%, VV: 12.6%, $p > 0.05$, data not published). The pathogenic mechanism that connects this polymorphism and some degenerative diseases like Alzheimer's disease or primary progressive aphasia may reflect a general response to cellular stress rather than specific co-operation in aggregation of other proteins⁹.

The function of the cellular prion protein (PrP^c) is uncertain, though it has been implicated in several potential functions, including intracellular transport of copper, cellular resistance to oxidative stress or normal synaptic functioning. A relationship between TDP-43, the main constituent of FTLD-PGRN(+) associated ubiquitin-positive inclusions, and PrP aggregates in human prion diseases has not been demonstrated¹⁰.

Our study has a number of limitations; one of them is the small sample size. Although our sample is relatively large, taking into account the prevalence of the disease and the inclusion of only patients carrying a single mutation in PGRN, it could be argued that it is too small to

establish statistically robust differences. However, the estimated statistical power of our study is 0.763, close to the usually recommended 0.8 value in most study designs. We tried to replicate our finding in a sample of 54 patients with a clinical diagnosis of FTD, but we did not find significant influence of codon 129 polymorphism on the age at onset of disease (data not shown). These results are not surprising since this is a heterogeneous sample based on clinical diagnosis. Codon 129 PRNP influence could be undermined by the stronger influence of other genetic or environmental factors in a group of patients with probably different subjacent pathological diseases.

Our findings warrant further replication in larger FTD-PGRN(+) series in order to avoid any spurious associations. An international effort should be encouraged to bring together patients into international cooperative studies.

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Figure 1.

Distribution of age of onset of PRNP codon 129 MM homozygous cases compared with MV and VV groups. Age at onset density curves show that MM homozygous patients at codon 129 of PRNP presented on average 8.5 years earlier than patients who carried at least one valine on their PRNP codon 129. Both curves show a fairly symmetrical and normal distribution.

Table 1

Distribution of genotypes analyzed, diagnostic groups and age at onset of the disease.

Analyzed variables	Genotype	N	Age at onset (Mean $\pm$ SD)
rs5848	CT	9	58.2 $\pm$ 6.2
	CC	12	60 $\pm$ 8.1
rs9897526	GG	19	59 $\pm$ 7.5
	GA	2	61.5 $\pm$ 6.4
APOE	ϵ 2 ϵ 3	1	70
	ϵ 3 ϵ 3	15	59.3 $\pm$ 5.7
	ϵ 3 ϵ 4	4	61 $\pm$ 7.4
MAPT	H1H1	9	56.9 $\pm$ 8.3
	H1H2	11	60.3 $\pm$ 5.6
	H2H2	1	70
PRNP 129	MM	6	53.2 $\pm$ 7.1
	MV	9	61.7 $\pm$ 6.3
	VV	6	61.7 $\pm$ 5.8
Initial diagnosis	bvFTD	11	57 $\pm$ 7.51
	PNFA	5	62.8 $\pm$ 6.72
	AD	3	64 $\pm$ 5.19
	PD	1	60
	Gerstmann syndrome	1	52

AD: Alzheimer disease; bvFTD: behavioral variant frontotemporal dementia; PD: Parkinson's disease PNFA: progressive nonfluent aphasia.