

Published in final edited form as:

Neurobiol Aging. 2011 June ; 32(6): 1157–1158. doi:10.1016/j.neurobiolaging.2009.06.006.

No major progranulin genetic variability contribution to disease etiopathogenesis in an ALS Italian cohort

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Abstract

To analyze the contribution of progranulin (PGRN) to the etiopathogenesis of amyotrophic lateral sclerosis (ALS), we performed a PGRN gene screening in 146 Italian patients (12 familial cases) and evaluated the association of two common variants with risk of developing ALS in 239 sporadic cases (SALS). Progranulin mRNA and protein levels were measured in peripheral blood mononuclear cells and serum of a subset of these patients and controls. PGRN sequence analysis revealed a heterozygous change (p.S120Y), previously observed in an independent sporadic ALS-FTD patient. Haplotype analysis showed a conserved PGRN region among these two subjects consistent with possible common ancestor allele. Two non-coding polymorphisms were not associated to increased risk to develop ALS; mRNA and serum levels were not significantly different between cases and controls. Overall, our data argue against the hypothesis of progranulin as a major risk factor for motor neuron dysfunction, at least in Italian population. The p.S120Y variant may characterize rare patients with SALS, although its pathogenetic mechanism remains to be elucidated.

We investigated the role of progranulin as genetic determinant of ALS susceptibility in a large cohort of Italian ALS patients (Table S1). First, we sequenced *PGRN* in 146 ALS patients (12 FALS and 134 SALS) consecutively recruited at the Department of Neurology, Ospedale Maggiore, Milan, Italy, and at the Department of Neurosciences, University of Padua. One change was identified in a 71-year-old female SALS patient (c.359C>A, p.S120Y, Figure S3). This variant has been previously reported in an ALS-FTD patient of European descent (Schymick et al., 2007) and was not present in 181 healthy Italian control subjects screened as part of the current study; the residue S120 is not highly conserved across species. Additional genetic analysis indicated that both patients carrying the S120Y variant share a common haplotype around the PGRN locus (Table S4). A further four rare coding sequence variants that likely represent non-disease-related polymorphisms and four

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Disclosure statement

The authors state that there are no actual or potential conflicts of interest.

frequent non-pathogenic intronic/3' UTR sequence variants were also identified (Table S2). Truncating mutations were not found in our sample set. Next we undertook a case-control association study of rs9897526 and rs5848 variants in the same cohort of 134 sporadic patients plus an additional 105 SALS cases and 181 healthy subjects. No significant association was detected in this analysis under the genotypic model (Table S3 and Figure S2). Quantitative analyses of circulating progranulin were performed (Figure S1): mRNA transcripts and serum levels were not significantly different among ALS patients ($n = 11$ and $n = 30$, respectively) and controls ($n = 9$ and $n = 27$, respectively). In this study, we demonstrated that *PGRN* mutations are not a common cause of ALS in Italy and failed to find a positive association between *PGRN* variability and risk to develop SALS. Loss-of-function mutations within *PGRN* gene (truncating mutations) were the most commonly observed sequence changes in families with ubiquitin-positive frontotemporal lobar degeneration (FTLD-U). Such progranulin mutations lead to haploinsufficiency by lowering *PGRN* levels in FTLD patients rather than the accumulation of mutant protein (Gijssels et al., 2008). Less frequent missense mutations have also been identified in FTLD-U patients, though again these mutations have been nearly uniformly associated with reduced progranulin production and secretion (Shankaran et al., 2008). *PGRN* missense mutations were also described in ALS: a systematic screening of *PGRN* demonstrated the presence of four missense mutations in five Belgian patients (5/230, 2.2%); moreover, common variants (including rs9897528) were significantly associated with a reduction in age of onset and a shorter survival after onset of ALS in Belgian and Dutch populations (Sleegers et al., 2008). In the present study, we detected a single patient carrying a heterozygous missense change (1/146; 0.6%) and two variants which are unlikely to be significantly associated with ALS susceptibility and clinical phenotypes. The variant rs5848 (c.*78C>T), located in the 3' UTR region in a predicted binding site for the human specific miRNA miR-659, has been described to increase significantly the risk of developing FTLD-U, most likely through suppressed translation of *PGRN*: the stronger binding of miR-659 to the *PGRN* mRNA containing the T-allele results in a more efficient inhibition of *PGRN* translation leading to reduced protein expression levels (Rademakers et al., 2008). However another association analysis using SNPs covering the *PGRN* locus failed to demonstrate any effect on disease risk either at the genotype or haplotype level in the Manchester FTLD cohort (Pickering-Brown et al., 2008). Also in Italian ALS patients we observed several common non-disease-related polymorphisms having similar low frequency in cases and controls. According to previously reported data, we found that progranulin is highly expressed in peripheral blood allowing quantification of mRNA levels using a real-time PCR approach (Coppola et al., 2008). This high blood level is consistent with the proposed role for progranulin in wound repair and/or inflammation (Ahmed et al., 2007). However, we observed no difference among ALS patients and healthy subjects in terms of blood progranulin transcript and serum levels. Conversely, increased mRNA levels were reported in spinal cords of ALS patients as detected through a microarray study (Malaspina et al., 2001); an immunohistochemical study has shown that *PGRN* expression is associated with areas of neuronal cell loss in ALS patients (Irwin et al., 2008). Increased blood mRNA levels were reported also in AD patients (Coppola et al., 2008). In FTLD patients, progranulin protein is strongly reduced in plasma and CSF of carriers of genetic defect (Ghidoni et al., 2008). The levels of progranulin in plasma have been suggested as a useful marker for early identification of a risk in asymptomatic subjects. We identified the p.S120Y change in a single case of limb onset sporadic ALS; the mutation has been previously reported in an ALS-FTD patient (Schymick et al., 2007a), and both patients share a common haplotype across the gene suggesting a shared common ancestor for this region of their genome. The identification of an identical mutation in a second individual diagnosed with a rare neurodegenerative disease increases the likelihood that this change is pathogenic. However, it still remains possible that this finding represents a chance occurrence, though the variant was not found in 362 normal chromosomes. Quantitative transcriptional analysis and serum level analysis demonstrated

no effects of p.S120Y on RNA and protein levels (at least in peripheral blood) implying that progranulin transcripts are not degraded through the process of non-sense-mediated mRNA decay, as suggested for null mutations in FTLD patients. Therefore, functional implications of this variation might occur through different mechanisms. Our data clearly indicates that mutations of the *PGRN* gene are, at best, only a rare cause of ALS accounting for less than 1% of cases. Furthermore, it would appear that *PGRN* neither affects risk of developing sporadic ALS nor modifies disease course in this Italian population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The financial support of the following research grant to NB and SC is gratefully acknowledged: Italian Ministry PRIN 2007—“Molecular pathogenesis of motor neuron disorders as a tool for the identification of novel biomolecular and cellular therapeutic agents”. This research was supported (in part) by the Intramural Research Program of the National Institute on Aging (project Z01 AG000949-02).

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