Common Variant in *GRN* Is a Genetic Risk Factor for Hippocampal Sclerosis in the Elderly

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Key Words

Alzheimer's disease · Hippocampal sclerosis · Immunohistochemistry · Progranulin gene

Abstract

Background: Hippocampal sclerosis (HpScl) is common in elderly subjects with dementia, either alone or accompanied by other pathologic processes. It is also found in >70% of frontotemporal lobar degeneration with TDP-43 immunoreactive inclusions (FTLD-TDP). TDP-43 inclusions are detected in >20% of Alzheimer disease (AD) and >70% of HpScl cases. The most common cause of FTLD-TDP is mutation in the progranulin gene (*GRN*). Recently, a common genetic variant in the 3' untranslated region (3'UTR) of *GRN* (rs5848; c.*78C>T) located in a microRNA binding site regulated progranulin expression, and the T-allele was increased in FTLD-TDP compared to controls. **Objective:** The goal of this study was to determine if the 3'UTR variant in *GRN* was associated with TDP-43 immunoreactivity in AD with and without HpScl. **Methods:** 644 cases of pathologically confirmed AD, including 57 with HpScl, were screened for TDP-43 immunoreactivity and were genotyped at the *GRN* 3'UTR single-nucleotide polymorphism rs5848 using previously published methods. **Results:** There was a trend ($p = 0.06$) for TDP-43 immunoreactivity, but a very significant ($p = 0.005$) association of HpScl with the variant, with 72% of AD with HpScl carrying a T-allele, compared to 51% of AD without HpScl

carrying a T-allele. **Conclusion:** The results suggest that a genetic variant in *GRN* leading to decreased levels of progranulin may be a risk factor for HpScl in AD, while its role in TDP-43 immunoreactivity in AD remains less certain.

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Introduction

Hippocampal sclerosis (HpScl) is defined by selective neuronal loss and gliosis in CA1 and subiculum sectors of the hippocampus (fig. 1) [1]. HpScl is common in elderly subjects with dementia, either alone or accompanied by other pathologic processes [2]. It is most often associated with pathologic features of frontotemporal lobar degeneration (FTLD) [3, 4] and can be detected in more than 75% of FTLD cases [5]. One of the first studies to draw attention to HpScl in the elderly noted it in more than 20% of demented patients over 80 years of age [1]. The clinical syndrome can be mistaken for amnesic mild cognitive impairment [6, 7], a condition thought to represent prodromal Alzheimer's disease (AD) [8], and in some cases it is the only structural abnormality to explain dementia [9]. It is sometimes associated with tauopathy [10] that resembles argyrophilic grain disease, a medial temporal tauopathy that increases in frequency with age [11] and can also present with amnesic mild cognitive impairment [12]. Focal neuronal loss and gliosis in the

Fig. 1. HpScl is characterized by selective neuronal loss in CA1 with relative preservation of neurons in CA3.

hippocampus can be seen in other degenerative disorders, such as Lewy body disease, but the distribution is different, being most severe in CA2/3 [13, 14].

Immunocytochemistry using a panel of monoclonal antibodies raised to FTLD brain homogenates led to the discovery of TDP-43 as the major constituent of neuronal inclusions in the most common form of FTLD [15] which is now referred to as FTLD-TDP [16]. TDP-43, for transactivation response protein of 43-kDa molecular weight, is an RNA-binding protein involved in transcriptional regulation that has more recently been implicated in other RNA-dependent cellular functions, such as storage, transport and degradation of mRNA [17]. While initially considered to be a specific marker for FTLD-TDP, this has been called into question as TDP-43 immunoreactivity has been found in 30–50% of AD cases [18, 19] and most cases of HpScl [2, 18, 20].

The most common genetic basis of FTLD-TDP is mutation in the gene for progranulin (*GRN*) [21–23], and the pathology in all cases associated with pathogenic mutations in *GRN* is FTLD-TDP [24, 25]. At present, there are over 125 variants reported in *GRN*, but only 66 that are definitely pathogenic (<http://www.molgen.ua.ac.be/FTDMutations>). One of the variants is a single-nucleotide polymorphism (rs5848) in the 3' untranslated region (3'UTR) of *GRN* [26]. Previous studies showed that the T-allele of rs5848 in the 3'UTR of *GRN* was associated with FTLD-TDP [26].

Given that most cases of HpScl are associated with TDP-43 pathology, that many cases of FTLD-TDP have HpScl, and that *GRN* rs5848 is associated with FTLD-TDP, we hypothesized that *GRN* rs5848 would also be associated with HpScl found in AD. As a corollary, if TDP-43 pathology in AD is related to a similar disease process as that seen in FTLD-TDP, the *GRN* rs5848 T-allele might also associate with AD cases that have TDP-43 immunoreactivity. We set out to test these hypotheses by determining the rs5848 genotype in a series of 644 AD cases that were screened for TDP-43 pathology with immunohistochemistry. A subset of cases had HpScl, which would permit assessment of association of *GRN* rs5848 with HpScl, as well.

Methods

We obtained frozen brain tissue for DNA extraction and fixed tissue for immunohistochemistry of 644 cases of pathologically confirmed AD. All cases were from the brain bank at Mayo Clinic, Jacksonville and had been evaluated by previously described methods [27]. *GRN* rs5848 was determined as previously described [26] and TDP-43 immunohistochemistry was also performed as previously described [18]. There were 275 men and 369 women, and the average age at death was 80 ± 9 years; HpScl was detected in 57 cases (9%). Statistical analysis was carried out with SigmaStat for Windows, version 3.0.1a (Systat Software, Richmond, Calif., USA). A significance level was set at 0.05.

Results

TDP-43 immunoreactivity was detected in 219 (34%) of the 644 AD cases. It was present in 44 of 57 (77%) AD cases with HpScl compared to 175 of 587 (30%) AD cases without HpScl (χ^2 49.9, $p < 0.001$). Most often, TDP-43 immunoreactivity was detected in neurons in the limbic distribution, with a smaller subset showing more diffuse distribution, similar to results reported previously [18]. TDP-43 immunoreactivity was not significantly associated with concomitant vascular pathology (Spearman $r = 0.052$), which was detected in 196 (30%) of the AD cases, or with Lewy bodies (Spearman $r = 0.022$), which was detected in 185 (29%) of the AD cases.

Presence of TDP-43 immunoreactivity in AD correlated with older age (Spearman $r = 0.26$, $p < 0.001$), female sex (χ^2 $p = 0.04$) and presence of HpScl (χ^2 $p < 0.001$). A multiple logistic regression analysis showed that age and presence of HpScl were significant predictors of TDP-43 immunoreactivity (age: OR 1.1, 95% confidence interval 1.04–1.10; HpScl: OR 7.0, 95% confidence interval 3.6–13.6). There was a trend for TDP-43 to be associated with *GRN* rs5848 T-allele (χ^2 $p = 0.06$), but when included in a multiple regression analysis with age and HpScl, it was not statistically significant ($p = 0.093$).

The T-allele of *GRN* rs5848 was significantly associated with HpScl in both genotype and allele frequency analyses (table 1). Of the 57 cases of AD and HpScl, 72% carried a T-allele, while only 51% of AD cases without HpScl had a T-allele. The difference in the frequency of the *GRN* rs5848 T-allele in HpScl was greater than chance ($p = 0.005$). The results suggest that a genetic variant in *GRN* may be a risk factor for HpScl in the elderly.

Discussion

Progranulin is encoded by a single gene on chromosome 17q21 (*GRN*), and is a 593-amino acid, cysteine-rich protein with an estimated molecular weight of 68.5 kDa that runs at 90 kDa on standard Western blots due to heavy glycosylation [28]. It contains seven granulin-like domains, which consist of highly conserved tandem repeats of a rare 12 cysteinyl motif [29]. Proteolytic cleavage of the precursor protein by extracellular proteases, such as elastase, gives rise to smaller peptide fragments termed granulins [30]. These fragments range in size from 6 to 25 kDa and have been implicated in a range of biological functions, some with properties similar to proinflammatory cytokines [30]. Progranulin plays a role in embry-

Table 1. *GRN* 3'UTR in AD with and without HpScl

	HpScl	No HpScl	p value (χ^2)
Genotype			0.020
rs5848 CC	16	286	
rs5848 CT	33 (58%)	253 (43%)	
rs5848 TT	8 (14%)	48 (8%)	
Allele			0.005
rs5848 C	65	825	
rs5848 T	49 (43%)	349 (31%)	

onic development, neoplasia, inflammation and wound healing [28], but its function in the central nervous system is not yet understood [31]. It is widely expressed in neurons, glia and endothelial cells. All known mutations in *GRN* have the same effect; they lead to 50% decreased expression of progranulin and mediate their effects through this deficiency, a process known as haploinsufficiency. Whether FTLD-TDP is caused by deficiencies in trophic actions of progranulin or cytokine-like properties of granulins, or both, and whether the effects of *GRN* are primarily on neurons, glia or endothelial cells is unknown, but these are areas of active investigation.

An emerging topic in the genetics of neurodegenerative diseases is that rare variants in genes (i.e. mutations) cause rare familial forms of the disease, but common variants in the same genes may contribute to risk for the disease in common nonfamilial forms of the disease. A number of examples can be cited, i.e. mutations in *SNCA* cause rare familial Parkinson disease [32], while common variants in *SNCA* are a risk factor for common nonfamilial Parkinson disease [33]. Variants in *GRN* are thus candidate genetic risk factors for nonfamilial forms of FTLD-TDP. Of more than 125 variants described in *GRN*, only 66 segregate with disease (<http://www.molgen.ua.ac.be/FTDMutations>). One of these variants is located in the 3'UTR of *GRN* with a sequence consistent with a microRNA binding site [26], and it is associated with sporadic FTLD-TDP [26].

Recent research has drawn attention to the role of diverse functions of small RNAs, in particular a specific subclass of small RNAs, microRNAs, which have the ability to regulate gene translation through repression or promoting messenger RNA cleavage [34, 35]. At present, there are 851 known microRNAs in humans (<http://microrna.sanger.ac.uk>) and many target genes, including *GRN*. The *GRN* sequence has at least one micro-RNA binding site for miR-659, a micro-RNA that is expressed

in the brain [26]. In addition to GRN, there are 974 other targets of miR-659 (<http://microrna.sanger.ac.uk>). The presence of the T-allele at the 3'UTR in GRN favors miR-659 binding, which lowers levels of progranulin expression without affecting the level of messenger RNA for progranulin. The levels of progranulin in the brains of individuals homozygous for the T-allele are intermediate between wild type (CC homozygous) and individuals carrying a disease-causing mutation in GRN [26]. Similar studies have yet to be performed in AD with TDP-43 immunoreactive inclusions or with HpScl.

The association of the rs5848 T-allele with FTLD-TDP suggests that lower progranulin levels may be one of the factors associated with risk of disease. Similarly, in this study we showed that the T-allele was overrepresented in AD cases with HpScl, most of which (77%) had TDP-43 immunoreactivity, which suggests that GRN may also be a risk factor for HpScl. The findings further indicate that HpScl in AD is not merely a reflection of severe neuronal loss due to AD, but rather neuronal loss due to a different mechanism, and perhaps analogous to the mechanism of neuronal loss in FTLD-TDP [36]. While there was a trend for the T-allele to be overrepresented in AD cases with TDP-43 immunoreactive inclusions, in a multivariate

analysis this association was lost when HpScl cases were excluded. In addition to HpScl, advanced age was a risk factor for TDP-43 immunoreactivity in AD. The explanation for the age association is not clear at this time.

Selective neuronal loss in the same distribution as that seen in HpScl is also a feature of hypoxic-ischemic injury to the hippocampus. It was of interest that while there was no association of TDP-43 immunoreactivity with vascular pathology, there was a weak association of HpScl with vascular pathology (Spearman $r = 0.091$, $p = 0.021$). This may indicate that a subset of HpScl in AD may be related to hypoxic ischemic injury [2]. In this series of AD cases, 196 had concomitant vascular pathology with HpScl in 25 cases, 21 of which had TDP-43 immunoreactivity. The 4 cases without TDP-43 immunoreactivity are likely to be those in which hypoxic-ischemic factors may have contributed to hippocampal neuronal loss.

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