

The Association between Frontotemporal Lobar Degeneration and Bullous Pemphigoid

Kasper Katisko^a, Nina Kokkonen^{b,c}, Johanna Krüger^{c,d}, Päivi Hartikainen^{a,e}, Anne M Koivisto^{a,e}, Seppo Helisalmi^a, Ville E Korhonen^g, Merja Kokki^h, Jussi Tuusa^{b,c}, Sanna-Kaisa Herukka^{a,e}, Eino Solje^{a,e}, Annakaisa Haapasalo^f, Kaisa Tasanen^{b,c}, Anne M Remes^{a,c,d,e}

Affiliations:

a = Institute of Clinical Medicine – Neurology, University of Eastern Finland, Kuopio, Finland

b = PEDEGO Research Unit, Dermatology, University of Oulu, Oulu, Finland

c = Medical Research Center, Oulu University Hospital, Oulu, Finland

d = Research Unit of Clinical Neuroscience, Neurology, University of Oulu, Oulu, Finland

e = Neurocenter, Neurology, Kuopio University Hospital, Kuopio, Finland

f = A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland

g = Neurocenter, Neurosurgery, Kuopio University Hospital, FI-70029, Kuopio, Finland

h = Anesthesia and Operative Services, Kuopio University Hospital, Kuopio, Finland

E-mails:

kasperk@uef.fi, nina.kokkonen@oulu.fi, johanna.kruger@fimnet.fi, paivi.hartikainen@kuh.fi, anne.koivisto@kuh.fi, seppo.helisalmi@uef.fi, villekori@student.uef.fi, merja.kokki@kuh.fi, jussi.tuusa@oulu.fi, sanna-kaisa.herukka@uef.fi, eino.solje@uef.fi, annakaisa.haapasalo@uef.fi, kaisa.tasanen-maatta@oulu.fi

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Corresponding author:

Anne M. Remes
Professor, MD, PhD
Unit of Clinical Neuroscience, Neurology
University of Oulu
P.O. Aapistie 7, FI 90014, Oulu Finland
E-mail: anne.remes@oulu.fi
Tel: +358 505330090

Abstract

Recent studies have shown an epidemiological and immunological association between bullous pemphigoid (BP) and several neurological or psychiatric diseases. Here, our aim was for the first time to specify whether an association exists between BP and frontotemporal lobar degeneration (FTLD). Medical histories of FTLD patients (N=196) were screened for clinical comorbidity and BP180 and BP230 autoantibodies were analyzed in the sera of FTLD patients (N=70, including 24 *C9orf72* repeat expansion carriers) by BP180-NC16A-ELISA and BP230-ELISA. One FTLD patient (*C9orf72* repeat expansion carrier) had a comorbid diagnosis of BP. Increased levels of serum BP180 autoantibodies (cutoff value >9U/ml) were detected more often in FTLD patients (10.0%) than in controls (4.9%). Moreover, elevated levels of both BP180 and BP230 autoantibodies were found more often in *C9orf72* repeat expansion-carrying FTLD than non-carrying patients or controls. However, none of these differences reached a statistical significance likely due to our limited cohort size. In conclusion, our findings suggest that subset of FTLD patients especially with the *C9orf72* repeat expansion may have an immunological association with BP.

Keywords

Frontotemporal dementia, *C9orf72*, Bullous Pemphigoid, autoantibody, immunology, dementia, comorbidity

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a heterogeneous group of clinical syndromes comprising four major clinical subtypes. The most common clinical subtype of FTLD is behavioral variant frontotemporal dementia (bvFTD) characterized by prominent behavioral and executive deficits. The other three clinical subtypes of FTLD are language variants with different phenotypes of primary progressive aphasia; nonfluent variant of primary progressive aphasia (nfvPPA), semantic variant of primary progressive aphasia (svPPA) and logopenic variant of primary progressive aphasia (lvPPA) [1,2].

The most common genetic cause for FTLD is the hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (*C9orf72*) [3,4]. The prevalence of the *C9orf72* repeat expansion in FTLD patients is exceptionally common in Scandinavia and particularly in Finland, whereas the other known mutations causing FTLD are extremely rare in Finnish population [5–7]. However, the exact mechanisms underpinning FTLD pathogenesis remain thus far unclear. Recent studies have associated FTLD with possible dysfunctions in the immune system [8–11]. For example, *C9orf72* knock-out murine models show increased levels of cytokines and autoantibodies and develop fatal autoimmune disease phenotypes [8,9]. In addition, a genome wide association study revealed that FTLD in general is associated with a specific HLA locus 6p21.3 [10]. These data altogether suggest that immune system processes may be involved in FTLD pathogenesis.

Several epidemiological studies have demonstrated that patients with neurological or psychiatric diseases, such as multiple sclerosis, Parkinson's disease or different types of dementias have an increased risk for developing bullous pemphigoid (BP) [12–16]. BP is the most common autoimmune blistering skin disease, which manifests as severe pruritus, blisters, erosions and crusts in people aged

70 and over [17]. Diagnosis of BP is based on typical clinical criteria, direct immunofluorescence (IF) microscopy analysis of a perilesional skin biopsy and serological assays including BP180-NC16A enzyme-linked immunosorbent assay (ELISA) and indirect IF analysis [17]. The pathogenesis of BP, compared to many other autoimmune diseases, is quite well understood: BP patients have circulating IgG autoantibodies targeting BP180 (also known as collagen XVII), a hemidesmosomal protein required for the integrity of cutaneous basement membrane [17,18]. The non-collagenous 16A (NC16A) domain of BP180 represents the main immunodominant epitope. The BP autoantibodies target also BP230 (also known as dystonin-e). Both BP180 and BP230 are expressed in the central nervous system and this, together with the strong epidemiological association, has led to an assumption that neurodegeneration or neuroinflammation could lead to a cross-reactive immunoresponse between neural and cutaneous antigens [19,20]. So far, circulating BP180 antibodies have been found in a subpopulation of patients with Parkinson's disease, Alzheimer's disease or unspecified dementia, but at the moment the functional significance of BP autoantibodies in patients with neurological disease is still largely unknown [20–23]

The aim of our current study was for the first time to analyze the association between FTLD and BP by screening medical histories of almost 200 patients with FTLD for the evidence of clinical BP comorbidity. The FTLD cohort contained a high proportion of *C9orf72* repeat expansion carriers. In addition, we measured the levels of circulating BP180 and BP230 IgG autoantibodies in the sera of 70 FTLD patients and 61 control subjects.

2. Materials and methods

2.1 Ethical considerations:

The study was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from the participants. The study protocol was approved by the research ethics committees of Northern Savo hospital district, Kuopio Finland or Northern Ostrobothnia hospital district, Oulu Finland.

2.2 Study participants:

Medical histories for a comorbid diagnosis of BP were screened from a total of 196 patients with FTLD (Table 1). FTLD clinical subtypes with at least a probable diagnosis of bvFTD, nvPPA or svPPA were determined according to the latest criteria [1,2] by an experienced neurologist specialized in memory diseases. The *C9orf72* repeat expansion was analyzed from 173/196 patients. The *C9orf72* repeat expansion was detected in 56 patients and 117 patients did not carry this mutation. DNA sample for genetic analyzes was not available for 11.7% (23/196) of the patients. Six of them had neuropathological examination and were pathologically confirmed as FTLD (five with TDP-43 neuropathology and one with tau-neuropathology). Total of 62 patients fulfilled a definite and 134 patients a probable FTLD diagnosis according to the latest diagnostic criteria [1,2].

Control group (N=61) consisted of 21 healthy subjects without any neurological diseases and of 40 participants that were recruited among patients scheduled for knee replacement surgery at the Kuopio University Hospital [23].

2.3 Serum samples

Serum samples for BP-autoantibody analyses were available for 70 FTLD patients (including 24 *C9orf72* repeat expansion carriers) and 61 controls. Serum samples were collected at Kuopio University Hospital and Oulu University Hospitals and stored at -80°C until measurement.

2.4 Genetic analyses

The *C9orf72* repeat expansion status was analyzed using the repeat-primed polymerase chain reaction assay [3]. Six patients of the *C9orf72* repeat expansion group carried an intermediate expansion (20-30 repeats) and the rest (N=50) carried a full expansion (>30 repeats). All the *C9orf72* repeat expansion non-carriers had less than five repeats.

2.5 BP180-NC16A-ELISA and BP230-ELISA

Autoantibodies against BP180 and BP230 were analyzed from serum samples with commercially available ELISA kits (Medical and Biological Laboratories Co Ltd, Nagoya, Japan) according to manufacturer's instructions. As a cut-off value, a concentration of 9 U/ml was used for both autoantibodies, and serum levels of >9 U/ml were considered as a positive result. The cut-off value of 9 U/ml is widely acknowledged as the normal limit and it has been calculated by Kobayashi et al. in 2002 and Sakuma-Oyama et al. in 2004 [24,25]. In a meta-analysis, this cut-off value had sensitivity of 87% (CI 0.85-0.89) and specificity of 98% (CI 0.98-0.99) [26]. BP180-NC16A-ELISA was performed for each sample as duplicate and the mean concentration from the duplicates was used for further analysis. BP230-ELISA was performed once for each sample.

2.6 Statistical analyses

All statistical analyses were performed by using SPSS statistic version 23 (International Business Machines corporation, Armonk, New York, USA). Fisher's exact test was used for categorical data (dichotomous variable describing either negative or positive BP-autoantibody result). A p-value of < 0.05 was considered statistically significant.

3. Results

3.1 FTLD patients

Characteristics of the study population of the 196 FTLD patients are described in Table 1. Careful examination of the patient records of all the FTLD cases from hospital databases revealed that one out of 196 (a carrier of the *C9orf72* repeat expansion) had a clinical diagnosis of BP. The patient was clinically diagnosed to have nvPPA at the age of 64 years. Gradual word finding problems had started approximately one year before the diagnosis, leading finally to mutism in three years. The patient also had behavioral symptoms, including anxiety and a tendency to run away. Gradually the patient began to have walking problems and in the neurological examination the patient presented with symmetrical rigidity in upper and lower limbs as well as axially. The extrapyramidal symptoms worsened during four years, leading finally to total immobility. At the age of 67 years, the patient was referred to the Department of Dermatology, Oulu University Hospital due to localized cutaneous blistering which had started six months earlier. Localized erythema and erosions were detected in a 10-cm diameter skin area at the right buttock, but no blisters were visible. The patient had seborrheic eczema on her face, but otherwise the skin was healthy. Direct IF staining showed linear deposits of IgG and complement C3 at the cutaneous basement membrane and indirect IF was positive (1:1250) confirming a diagnosis of BP. BP180-NC16A-ELISA assay was not available at that time. The patient was successfully treated with topical betamethasone. History of other immunological diseases of this entire FTLD cohort is described in our previous study [27].

3.2 BP180-NC16A-ELISA and BP230-ELISA assays

Serum samples from the patients with FTLD (N=70) and neurologically healthy control subjects (N=61 in BP180-NC16A-ELISA, N=40 in BP230-ELISA) were tested for the levels of BP180 and BP230 IgG autoantibodies using a well-established, commercially available ELISA analysis (Table 2). Serum sample of the patient with a clinical diagnosis of BP was not available for this assay. It was observed that 10.0% (7/70) of the FTLD samples and 4.9% (3/61) of the control samples showed elevated levels of IgG against BP180-NC16A (above the cut-off value >9 U/ml). Increased levels of autoantibodies against BP230 (above the cut-off value >9 U/ml) were found in 4.3% (3/70) in the FTLD group and 7.5% (3/40) in the control group. One FTLD patient, but none of the control subjects, had increased levels of both BP180 and BP230 autoantibodies. FTLD patients with the *C9orf72* repeat expansion had more often increased autoantibody levels for both BP180 (12.5%) and BP230 (8.3%) when compared to those not carrying the *C9orf72* repeat expansion (9.1% for BP180, 2.3% for BP230) and control subjects (4.9% for BP180, 7.5% for BP230). However, all of these observed differences showed only as trends, as none of them reached the statistical significance (Fisher`s exact test) (Table 2). Out of the seven FTLD patients with elevated levels of BP180 autoantibodies, two patients had a history of other autoimmune disease (one celiac disease and one colitis). Out of the three control subjects with elevated BP180 levels, two subjects had a history of other autoimmune disease (one sarcoidosis and one psoriasis). As for the subjects with elevated levels of BP230 autoantibodies, none of the FTLD patients but one control subject had a history of other autoimmune disease (psoriasis).

Investigation of the autoantibodies according to the FTLD clinical subtypes indicated that increased levels of BP180 autoantibodies were detected in 10.4% (5/48) of the bvFTD patients, 7.7% (1/13) of the nfvPPA patients, 0.0% (0/3) svPPA patients and 16.7% (1/6) of the FTLD-MND patients. The one FTLD-MND patient with elevated BP180 autoantibody levels presented with bvFTD as the FTLD phenotype. Increased BP230 autoantibody levels were detected in 4.2% (2/48) of the bvFTD patients, 7.7% (1/13) of the nfvPPA, and in 0.0% (0/3) of the svPPA and in 0.0% (0/6) FTLD-MND patients. There were no statistically significant differences between the groups (Fisher's exact test).

4. Discussion

To our knowledge, this is the first study investigating the relationship between clinical BP and FTLD and analyzing serum BP180 and BP230 autoantibody levels in FTLD patients. Several epidemiological studies have revealed that BP is associated with neurological diseases, such as dementia, cerebral stroke, motor neuron disease and multiple sclerosis. Association with some psychiatric disorders, such as schizophrenia and bipolar disorder, has also been described [12–16]. The combination of both neurological and psychiatric symptoms often observed in FTLD patients led us to hypothesize that FTLD could show comorbidity also with BP. Especially FTLD patients carrying the *C9orf72* repeat expansion have psychiatric symptoms prior the neurological diagnosis [28]. Interestingly, the only case with a clinical BP diagnosis in our FTLD cohort was a carrier of the *C9orf72* repeat expansion. BP was diagnosed four years after the first symptoms of FTLD at the age of 67 years. The age at onset of BP was about 10 years less compared to typical BP cohorts [29]. Also the phenotype of BP was a rare localized type.

We detected circulating BP180 autoantibodies in patients with FTLD slightly more often (10%) than a previous study with unspecified dementia patients (7%) [21]. In the general healthy population, increased levels of BP-autoantibodies are usually detected from 0-2% of participants [30]. In contrast, our recent work showed that BP180 autoantibodies were detected even more often in AD patients (17%) than in FTLD patients in this present study [23]. One possible reason underlying this difference may be the different age of the patients in these two cohorts. The average age of the FTLD patients in the present study was 65 years (61 years in the *C9orf72* expansion carriers group), being clearly lower compared to that of the AD patients (72 years) in the previous study [23]. Notably, the incidence of BP starts to significantly increase in people older than 70 years, reaching maximum in people aged above 90 years [31]. The disease activity correlates with serum BP180-NC16A autoantibody levels

[32]. Despite the presence of circulating autoantibodies against BP180, none of the AD patients had BP diagnosis or BP-like symptoms [23]. Our findings in FTLD patients are similar, although one patient in our FTLD group (a *C9orf72* repeat expansion carrier) had a clinical diagnosis of BP. Our findings regarding the BP230 analysis are in line with the previous studies: neurological patients have autoantibodies targeting BP230, but also similar percentage of control individuals show increased values [22,23,33]. Rationally, the autoantibodies targeting BP180 are likely more important than those against BP230 when considering the immunological association of BP and neurological diseases, since the autoantibodies against BP180, rather than BP230, actually play a more crucial role in the development of clinical BP [34]. Moreover, dystonin (in skin dystonin-e or BP230) is in fact expressed as a different isoform, dystonin-a, in the central nervous system [35], and the sequences targeted by commercial BP230-ELISA differ between these two isoforms (i.e. significant amount of epitopes targeted by BP230-ELISA are actually present only in the dystonin-e isoform) [36]. Interestingly, one recent study reported two patients with dementia (AD and dementia with Lewy bodies) that developed mild clinical BP in ages of 91 and 93 years old. These patients had autoantibodies against BP230, but not against BP180, suggesting that some milder inflammatory phenotypes of BP are associated only with BP230 autoantibodies, and that these autoantibodies could be associated with cross-reactive immune response against neural and cutaneous isoforms of dystonin [37].

We observed that increased autoantibody levels were most frequently detected in patients carrying the *C9orf72* repeat expansion, even though the difference between the *C9orf72* repeat expansion carriers and non-carriers was not statistically significant. Notably, serum sample of the one *C9orf72* repeat expansion carrier who had clinical BP diagnosis was not available for our BP-autoantibody analyses, which moderates the difference between the FTLD *C9orf72* repeat expansion carriers and non-carriers or controls. Either way, this finding is intriguing since recent studies have suggested that

the *C9orf72* repeat expansion is associated with immune system dysregulation. Murine models have demonstrated that the loss-of-function in the *C9orf72*, mimicking the haploinsufficiency in *C9orf72* repeat expansion carriers, causes severe systemic inflammation with increased levels of several pro-inflammatory cytokines and autoantibodies [8,9]. In addition to the findings regarding the interrelationship between the *C9orf72* and inflammation, other studies have suggested that FTLD in general could be associated with immune system dysfunction. A genome-wide association study by Ferrari et. al (2014) linked FTLD patients with the HLA locus 6p21.3, suggesting possibly altered immune system functions in FTLD. Also, increased concentrations of anti-AMPA GluA3 antibodies were detected in patients with FTLD [11]. Recently, we discovered that prevalence of cancer is significantly lower in FTLD patients compared to neurologically healthy controls and AD patients [38], and that the *C9orf72* repeat expansion may affect the prevalence of immunological diseases in FTLD [27]. Both of these findings may indicate immunological disturbances in the FTLD pathogenesis. Our present findings further suggest that subset of FTLD patients may have an increased risk for immunological dysfunction, by associating 10% of FTLD patients with increased levels of BP autoantibodies. In addition, our data implicate that the presence of the *C9orf72* repeat expansion may enhance this association.

A limitation of this study is the small cohort size, particularly from a statistical point of view. However, FTLD is a rare condition and our cohort is one of the largest FTLD cohorts worldwide. Our cohort is clinically and genetically well-characterized and it includes a high proportion of the *C9orf72* repeat expansion carriers and definite FTLD cases according to the latest criteria. By dividing patients with dementia into accurate diagnostic groups, as into the different clinical FTLD subgroups, this present study is able to provide more specific information than some previous studies that have compared patients with dementia in general to control subjects. We have already excluded in our previous studies *GRN*, *MAPT* and *CHMP2B* mutations, which represent some of the most

common FTLN-causing mutations in other populations, in the majority of the cases without the *C9orf72* expansion in our cohort [5–7]. These mutations have not been systematically excluded in all of the patients in our cohort, but based on our previous studies, they are extremely rare in Finland [5–7]. Another limitation in our setting is the slight female dominance in our control group, especially when compared to the whole FTLN group. However, knee osteoarthritis is more common in women [39] and we collected patients to the control group from consecutive patients scheduled for knee arthroplasty and this was likely outcome.

In conclusion, our data suggest that particularly FTLN patients carrying the *C9orf72* repeat expansion are possibly associated with increased levels of BP autoantibodies, suggesting potential comorbidity of BP in a subset of FTLN patients. However, the lack of statistical significance (possibly due to our limited cohort size) should be noted, and no explicit conclusions can be made. Future studies with larger cohorts should define whether the non-significant trend seen in this preliminary study is seen also in other FTLN cohorts, and more specifically in older FTLN patients. Further studies should also aim to specify whether BP autoantibodies have a role in the pathways causing neurodegeneration, or whether neurodegeneration or neuroinflammation themselves cause the development of these autoantibodies. Understanding the differences in BP180 autoantibodies between clinical BP patients and neurological patients, as well as between cutaneous and neural tissues is crucial when solving the association between these two diseases. Furthermore, in patient care, clinicians should pay attention to the skin of the patients with neurodegenerative and psychiatric diseases to detect possible BP symptoms.

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Disclosure statement

The authors have no conflict of interest to report.

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Table 1. Characteristics of the total FTL D cohort screened for comorbid diagnosis of BP.

<i>Characteristics</i>	Patients with FTL D² N=196	FTL D with C9orf72 repeat expansion² N=56	FTL D w/o C9orf72 repeat expansion² N=117
Age ¹ , mean \pm SD	67.9 \pm 8.0	64.3 \pm 8.2	69.3 \pm 7.5
Gender, % female	49.0%	50.0%	51.3%
bvFTD, n	132	40	76
nfvPPA, n	37 ³	6 ³	27
svPPA, n	8	1	6
FTL D-MND, n	19	9	8
Comorbid Bullous Pemphigoid, n	1/196 ³	1/56 ³	0/117

¹ = Age is calculated from the date of the last visit at the university hospital.

² = Total FTL D group (N=196) includes 23 patients without the analysis of the C9orf72 repeat expansion.

³ = Patient having both FTL D and Bullous Pemphigoid was a carrier of the C9orf72 repeat expansion and had nfvPPA as the FTL D clinical phenotype.

Table 2. Characteristics of patients and controls included in the BP analyzes and their BP180-NC16A and BP230 autoantibody values.

<i>Characteristics</i>	Patients with FTLD⁵ N=70	FTLD with <i>C9orf72</i> repeat expansion⁵ N=24	FTLD w/o <i>C9orf72</i> repeat expansion⁵ N=44	Controls⁶ N=61	P-value
Age ¹	64.9±8.7	61.1±9.1	67.1±8.0	60.2 ±12.6	
Gender, %female	51.4%	58.3%	47.7%	62.3%	
BP180 NC16A ² (U/ml)	3.52 (0.0-20.8)	3.85 (0.3-20.8)	3.29 (0.0-12.2)	3.06 (0.3-27.1)	
BP180 NC16A positive ³ % (n)	10% (7)	12.5% (3)	9.1% (4)	4.9% (3)	p=0.337 ⁴
BP230 ² (U/ml)	3.16 (0.1-38.9)	3.80 (0.2-38.9)	2.79 (0.1-19.5)	2.71 ⁵ (0-24.1) ⁶	
BP230 positive ³ % (n)	4.3% (3)	8.3% (2)	2.3% (1)	7.5%(3)⁶	p=0.666 ⁴

¹ = Age is calculated from the date of the blood sample, data is given as mean ± standard deviation.

² = Data given as mean (range).

³ = Representing participants reaching the BP180 NC16A or BP230 cutoff value (> 9 U/ml).

⁴ = In statistical analysis, no statistically significant differences were found: FTLD vs. Controls BP180- NC16A p=0.337 (Fisher's exact test), FTLD with the *C9orf72* repeat expansion vs. Controls BP180 p=0.344 (Fisher's exact test). FTLD vs. Controls BP230 p=0.666 (Fisher's exact test).

⁵ = Total FTLD group (N=70) includes two patients without the analysis of the *C9orf72* repeat expansion.

⁶ = BP230 autoantibody levels were analyzed from 40 control subjects.

Supplementary Figure 1. (Graphical Abstract)

