Symptom severity in schizophrenia patients with NPAS3, dysbindin-1 and/or TRIOBP protein pathology in their blood serum: a PANSS-based follow up study

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SYMPTOM SEVERITY IN SCHIZOPHRENIA PATIENTS WITH NPAS3, DYSBINDIN-1 AND/OR TRIOBP PROTEIN PATHOLOGY IN THEIR BLOOD SERUM: A PANSS-BASED FOLLOW UP STUDY

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Abstract

Background: *It has been proposed that aggregation of specific proteins in the brain may be a pathological element in schizophrenia and other chronic disorders. Multiple such aggregating proteins have now been implicated through post mortem investigation, including NPAS3 (Neuronal PAS domain protein 3), dysbindin 1 (encoded by the DTNBP1, Dystrobrevin Binding Protein 1, gene) and TRIOBP (Trio-Binding Protein, multiple isoforms). While the presence of protein aggregates in the brain is interesting in terms of understanding pathology, it is impractical as a biomarker. These proteins were therefore investigated recently in blood serum of schizophrenia patients and controls, showing patients to have higher levels of NPAS3 in their serum generally. TRIOBP-1 and dysbindin-1 were also found in an insoluble state, implying aggregation, but did not clearly corresponding to disease state.*

Subject and methods: *We revisit 47 of the originally recruited 50 patients with schizophrenia, all of whom are Croatian and aged between 18 and 72. We assessed their symptom specificity and severity using PANSS (the Positive and Negative Symptoms Scale), comparing those with NPAS3, insoluble dysbindin-1 and/or insoluble TRIOBP-1 in their blood serum to those lacking any such protein dysregulation.*

Results: *The frequency of each individual potential protein pathology among these patients was too low for meaningful statistical analysis, however the 11 patients that displayed one or more of these pathologies (NPAS3, dysbindin-1, TRIOBP-1 and/or TRIOBP-5/6) showed a subtle but significant increase in total PANSS scores compared to the 36 patients displaying none of the pathologies (p = 0.031), seemingly driven principally by increased scores on the general psychopathology scale.*

Conclusion: While the numbers of patients involved do not allow firm conclusions to be drawn at this time, this provides the first indication that disturbed proteostasis in blood serum, of proteins that aggregate in the brains of schizophrenia patients, may correlate with the severity of schizophrenia symptoms.

Keywords: *Blood serum; PANSS; Protein aggregation; Proteinopathy; Schizophrenia*

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INTRODUCTION

Schizophrenia is a serious and chronic mental illness, whose biological background is unclear, in part because of its genetic complexity (Trubetskoy et al. 2022). There are numerous hypotheses regarding the aetiology of schizophrenia, however none can fully explain all of its phenotypic features, or clearly define biomarkers for the conditions (Rubeša et al. 2011, Rubeša et al. 2018, Ružić Baršić et al 2020). Due to this lack of reliable biomarkers, diagnosis must still be performed through the use of a standardised interview. For this reason, we and others have been pursuing an alternative and complementary approach to investigating the biology of schizophrenia and other chronic mental illnesses, based on identifying protein aggregates, similar to those found in neurodegenerative disorders (Bradshaw & Korth 2019). This approach has led to five proteins

being implicated as aggregating in the brains of patients with schizophrenia: DISC1, dysbindin-1, CRMP1, TRIOBP and NPAS3 (Leliveld et al. 2008, Ottis et al. 2011, Bader et al. 2012b, Bradshaw et al. 2014, Nucifora et al. 2016). Furthermore, it appears that at least a subgroup of schizophrenia patients show general proteostasis imbalances in their brains, with decreases in proteasomal activity (Scott & Meador-Woodruff 2020), increases in ubiquitinated protein (Bousman et al. 2019), deficits in various components of the proteasome-ubiquitination system (Luza et al. 2020) and increases in insoluble protein (Nucifora et al. 2019), which together implies a reduced ability to degrade misfolded proteins and prevent aggregate formation. While potentially of high relevance for schizophrenia pathology, the presence of such proteins in the brain is of limited use for diagnosis, although, curiously, increased protein ubiquitarian is also present in the blood of schizophrenia patients

(Bousman et al. 2019), implying disrupted proteostasis to also occur in other, more easily analysed, tissues.

For this reason, a pilot study was recently conducted involving blood from 50 patients with schizophrenia, 50 patients with major depressive disorder and 50 matched control individuals (Samardžija et al. 2021). Blood serum samples from these participants were collected, and the insoluble protein fraction, which would be expected to contain any aggregated proteins, was purified. Both samples of the whole serum and this isolated insoluble protein fraction were then Western blotted, to check for the presence of proteins previously implicated as aggregating in the brains of patients with major mental illnesses. This revealed two proteins to be present in the insoluble fraction, and therefore likely to be aggregating. Insoluble dysbindin-1, previously found in brains samples of patients with schizophrenia, bipolar disorder and major depressive disorder (Ottis et al. 2011), was found in two patients with schizophrenia and no control individuals (Samardžija et al. 2021). TRIOBP meanwhile, which was found to aggregate in brains of schizophrenia and major depressive disorder patients (Bradshaw et al. 2014, Zaharija et al. 2022), was present in a larger number of samples, but did not show clear diagnostic specificity (Samardžija et al. 2021). The *TRIOBP* gene is complex and encodes a variety of different protein species (Zaharija et al. 2020), however species seen in the serum seem to correspond to the approximately 70 kDa TRIOBP-1 isoform in some patients, and the longer (200+ kDa) TRIOBP-5 or 6 in others (Samardžija et al. 2021). Finally, NPAS3, which was previously implicated as aggregating in a family with schizophrenia and a rare mutation (Nucifora et al. 2016) was not seen in the insoluble fraction, but was present in whole serum (Samardžija et al. 2021), which was unexpected given that it is a nuclear expressed protein mostly linked to the brain (Brunskill et al. 1999, Brunskill et al. 2005). Nevertheless, levels of NPAS3 in serum were higher in schizophrenia patients than control individuals, showing that while NPAS3 aggregation is not clearly visible in blood serum of patients, its proteostasis here is nevertheless disrupted (Samardžija et al. 2021). It therefore appears that TRIOBP and dysbindin-1 aggregates (TRIOBP and dysbindin-1 pathology) exist in the blood serum of some schizophrenia patients, and the same may be true of NPAS3, which at a minimum is dysregulated in a subset of patients.

At the time, as a result of the COVID-19 pandemic, it was not possible to return to patients to get further information regarding their diagnoses or symptoms.

In this paper, therefore, we revisit these schizophrenia patients with proteostasis imbalances of dysbindin-1, TRIOBP and/or NPAS3 in their blood serum, and use the Positive and Negative Symptom Scale (PANSS, Kay et al. 1987) in order to determine whether any of these proteostasis disruptions may be associated with particular symptoms of schizophrenia.

SUBJECTS AND METHODS

Previously, 50 patients with schizophrenia were recruited from the Psychiatry Clinic of the Clinical Hospital Centre Rijeka, each of whom donated a blood sample (Samardžija et al. 2021) and was diagnosed by ICD-10 and/or DSM-V criteria. All participants were Croatian and aged between 18 and 72. Of these, 47 individuals were available and willing to undertake a PANSS assessment. Each had the methodology explained to them and were only included in the study if they gave informed consent. In the original study (Samardžija et al. 2021), all patients were included if they had an appropriate diagnosis and gave informed consent, while control individuals were invited from blood donors based on demographic matching, only after giving informed consent, and were excluded if they had any medical record of neurological or psychiatric illness. Each PANSS assessment (Kay et al. 1987) was carried out by a trained psychiatrist, who was unaware of the protein pathology status of each patient. Data was also collected on age, sex, length of illness and current medication. Total contact time with each participant was between 60 and 100 minutes.

The patient cohort consisted of 31 males and 16 females, with a mean age of 47.09 years (standard deviation: 11.19 years). Average age upon receiving first treatment for schizophrenia was 29.43 years (standard deviation: 9.20 years). 17 of the patients had at least one first or second degree relative with a history of mental illness.

Statistical analyses were performed in JASP (JASP Team 2020) and SPSS (IBM). Comparison of PANSS symptoms between two protein status groups was performed by Student's t-test, after using the Shapiro-Wilk test for normality and Levene's test for equality of variance. Where variance was not equal, Welch's t-test was additionally performed. Comparisons of numbers of patients displaying a characteristic were performed using the Fisher Exact Test. Unless otherwise stated, values in the text and tables are given as the mean ± standard error of the mean.

RESULTS

Of the 47 patients available for PANSS analysis, two (both male) were previously determined to have insoluble dysbindin-1 in their blood serum, two (both male) had insoluble TRIOBP-1 and three (one female, two male) had insoluble TRIOBP-5/6. Seven individuals (two female, five male) had clearly visible, soluble NPAS3 in their blood serum. Two individuals displayed multiple of these potential protein pathologies, both males in their 50s: each displayed both NPAS3 and insoluble TRIOBP-1, while one additionally displayed insoluble dysbindin-1. All had a diagnosis of paranoid schizophrenia (ICD-10 F20.0), except for one with NPAS3 in their serum, whose diagnosis was residual schizophrenia (ICD-10 F20.5).

In total, therefore, 11 of the schizophrenia patients displayed some form of disrupted proteostasis in the blood, and we asked whether this group showed any difference in PANSS symptoms to the remaining 36 schizophrenia patients available for the study. This group with apparent protein pathology in their blood indeed showed significantly higher total PANSS scores (114.73 ± 5.51 compared to 103.39 ± 2.23, table 1, figure 1A). This increase in symptom severity seems to be driven principally by increases in general symptoms (table 1, figure 1B),

although no individual symptom scale reached statistical significance (table 1, figure 1C,D). The low number of patients expressing each individual protein in their blood prevent statistical analysis of each putative proteinopathy individually, however in figure 1 each protein is indicated with different shading for information. For example, the individual displaying NPAS3, dysbindin-1 and TRIOBP-1 had among the highest scores for negative and general psychopathology symptoms (39 and 67 respectively).

There was no significant difference between the two groups (with apparent protein pathology or without) in terms of their age when the serum sample was given or age at first treatment, nor did the groups differ significantly in terms of sex or whether they have first- or second-degree relatives with a history of mental illness (table 2). There were similarly no significant differences in the pharmacotherapies taken (table 3), however low samples numbers mean that pharmaceutical interaction with the protein pathology cannot be discounted. Notably, patients displaying a potential protein pathology may be less likely to use clozapine. Amongst patients receiving clozapine, there was no difference in daily dose between the two groups (233.3 ± 66.7 mg for those with apparent protein pathology, 215 ± 36.2 mg for those without).

Figure 1. PANSS scores for patients with or without protein pathologies detected in their blood serum. Total PANSS scores are (A) and on the positive (B), negative (C) and general psycho-pathology scales (D), amongst schizophrenia patients with one or more of the protein pathologies (NPAS3 in blood serum, insoluble dysbindin-1 in blood serum, insoluble TRIOBP-1 in blood serum and/or insoluble TRIOBP-5/6 in blood serum), compared to schizophrenia patients with none of the protein pathologies detectable. This data is also shown in table 1. Among the individuals with protein pathology, the specific protein(s) seen are indicated with shading. P-values represent two-tailed Student's t-tests except for the negative symptoms scale (*) in which two-tailed Welch's test is used, as Levene's test showed variances not to be equal.
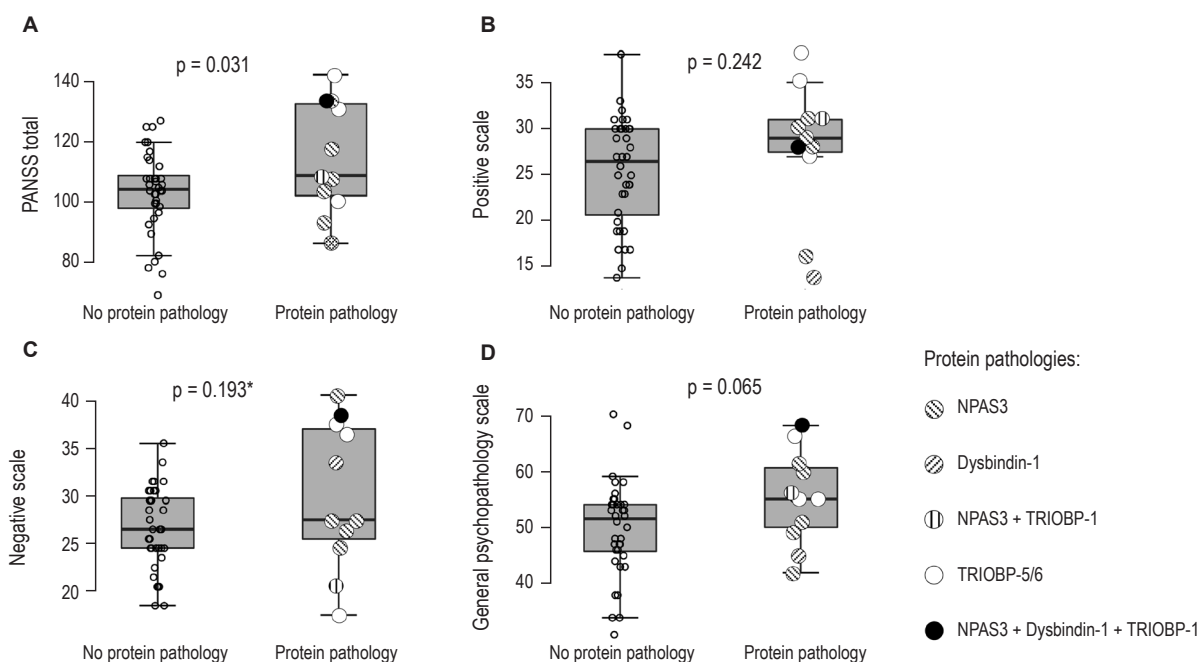
Table 1. Average symptom scores on PANSS. PANSS scores are shown both in total and on individual scales, for schizophrenia patients who displayed any of the protein pathologies (NPAS3 in serum, insoluble dysbindin-1, TRIOBP-1 and/or TRIOBP-5/6 in serum, n = 11) with patients that did not (n = 36). Scores are given with standard error of the mean. Student's t-test, Shapiro-Wilk normality test Levene's equality of variances test p-values are shown. Data is also shown visually in figure 1.

	Total PANSS	Positive	Negative	General psychopathology
Protein pathology	114.73 (± 5.51)	27.91 (± 2.15)	30.55 (± 2.32)	56.27 (± 2.47)
No protein pathology	103.39 (± 2.23)	25.42 (± 0.95)	27.19 (± 0.70)	50.78 (± 1.40)
Student's (p)	**0.031**	0.242	0.069	0.065
Shapiro-Wilk (p)	0.149	0.192	0.369	0.250
Levene's (p)	0.078	0.951	**0.001**	0.900

Table 2. Descriptions and demographic data regarding the patient groups. Mean age of patients at first treatment for schizophrenia and at time of giving the sample are given, along with standard errors of the mean and Student's two-tailed t-test. The sex of the patients is shown as number of females in the group, and the number of patients with family history of mental illness (at least one first or second degree relative) is shown, along with Fisher Exact Test statistics.

	Age when serum sample given	Age at first treatment	Sex	Family history
Protein pathology	47.45 (± 1.86)	28.92 (± 1.55)	3 / 11 female (27.3%)	5 / 11 (45.4%)
No protein pathology	46.97 (± 3.35)	31.09 (± 2.60)	13 / 36 female (36.1%)	12 / 36 (33.3%)
Student's (p)	0.902	0.499	-	-
Fisher	-	-	0.725	0.493

Table 3. Pharmaceuticals prescribed to patients in this study, separated into those who did or did not display a potential pathology, along with Fisher Exact Test statistics. In order to preserve patient anonymity, treatments are only included in this table if taken by at least 5 of the 47 patients.

	Aripiprazole	Clozapine	Haloperidol	Olanzapine	Paliperidone	Quetiapine
Protein pathology	3 / 11 (27.3%)	3 / 11 (27.3%)	1 / 11 (9.1%)	3 / 11 (27.3%)	5 / 11 (45.5%)	2 / 11 (18.2%)
No protein pathology	14 / 36 (38.9%)	20 / 36 (55.6%)	4 / 36 (11.1%)	9 / 36 (25.0%)	18 / 36 (50.0%)	3 / 36 (8.3%)
Fisher	0.722	0.168	1.000	1.000	1.000	0.578

DISCUSSION

The study of protein aggregates, and wider disruptions in proteostasis, is an emerging area of study in schizophrenia and other chronic mental illnesses (Bradshaw & Korth 2019), with several proteins seemingly forming insoluble protein aggregates in the brains of subsets of patients (Leliveld et al. 2008, Ottis et al. 2011, Bader et al. 2012b, Bradshaw et al. 2014, Nucifora et al. 2016).

While the presence of aggregated proteins in the brain is not a practical target for diagnosis of living patients, it is plausible that such aggregates, or biological correlates of them, may be present in tissues more easily accessible for diagnosis. Notably, similar enrichment of ubiquitinated proteins in the brains and erythrocytes of treatment-resistant schizophrenia patients, compared to controls, suggests similar defects in misfolded protein clearance in both tissues (Bousman et al. 2019). Specific transcriptional changes have also been associated with aggregation of the DISC1 protein, and are consistent between patients and a transgenic rat model (Trossbach et al. 2016, Trossbach et al. 2019). The same rat model also

shows DISC1 aggregation to affect the activity of peptidase enzymes known to be similarly altered in the blood of schizophrenia patients (Dal Mas et al. 2019, Nani et al. 2020a).

Where protein aggregates, or aberrant protein expression more generally, is seen in the blood of patients with major mental illness, this is unlikely to represent protein originating from the brain itself. While there is evidence that at least two aggregating proteins involved in mental illness, DISC1 and dysbindin-1, can pass between cells *in vitro* and in animal brains (Ottis et al. 2011, Bader et al. 2012a, Zhu et al. 2015, Zhu et al. 2017), the blood-brain barrier makes this transfer into the general blood circulation unlikely. Instead, it is more likely that general deficiencies in protein clearance of subgroups of patients with schizophrenia (Scott & Meador-Woodruff 2020, Bousman et al. 2019, Nucifora et al. 2019) manifest throughout the body, leading to correlation between protein pathology in the brain and elsewhere. Based on analogy with protein aggregation disorders like amyotrophic lateral sclerosis or frontotemporal lobe dementia, it is also plausible that many instances of protein aggregation in mental illness arise from familial or *de novo* mutations in genes encoding the aggregating proteins, in which case again pathology could occur throughout the body. In both scenarios, however, protein pathology would be expected to be more damaging in neurons than in most other cell types of the body, given the limited ability of neurons to clear large quantities of misfolded protein (Lim & Yue 2015, Kundra et al. 2020).

Previous work in brains and in blood serum implies that aberrant protein misfolding is likely to exist only in subsets of patients, and that while these proteins may overlap, each protein aggregates in its own subgroup of patients (Leliveld et al. 2008, Ottis et al. 2011, Bader et al. 2012b, Nucifora et al. 2019, Samardžija et al. 2021, Zaharija et al. 2022). It is therefore plausible that different protein aggregates reflect different biological subtypes of schizophrenia, and may reflect different symptomologies or treatment outcomes. While the number of patients with each individual apparent protein pathology here is too low to determine this at this time, the fact that patients with protein pathologies generally (in this case NPAS3, dysbindin-1 and/or TRIOBP) have higher symptoms supports this theory, and the fact that this was visible in blood serum, provides hope that this may ultimately have value in diagnosis and/or personalised treatment, likely in combination with other biomarkers.

This data also correlates well with previous experiments investigating levels of ubiquitinated protein in patients with schizophrenia and controls (Bousman et al. 2019). Elevated protein ubiquitination is indicative of increased protein misfolding and/or the inability of the proteasome to clear such misfolded proteins, both of which would increase the risk of protein aggregates forming in the cell. They saw increased ubiquitination in both the orbifrontal cortex and erythrocytes, suggesting that protein pathology in the blood could be reflective of that in the brain. Furthermore, in the orbifrontal cortex, this effect was higher in treatment resistance patients, suggesting that proteinopathy may highlight clinically relevant sub-groups of schizophrenia patients. Similarly, it fits with other protein models of schizophrenia, such as the use of NDEL1 enzyme activity as a biomarker in blood (Gadelha et al. 2013), and for which it is known that mouse models of schizophrenia display the same phenotype in both plasma and post mortem brain tissue (Nani et al. 2020a, Nani et al. 2020b).

Based on analogy with neurodegenerative disorders, it would be expected that protein aggregates in the brain of schizophrenia patients would increase with advancing age, however, it is not clear whether the same would apply to protein pathology in the blood, which is by nature a much more transient tissue. In this study, there was no indication that protein pathology was more common in older patients, or those who were first diagnosed and treated a longer time ago. It should be noted, however, that the average age of participants in this study is younger than in most publications that looked at protein pathology in the brain, as such studies have always been, to date, post-mortem analyses. There was also no indication in this study that protein pathology was associated with family history of mental illness, however the low number of patients means that this must be treated with caution. It is certainly possible that protein aggregation in schizophrenia arises, at least in part, due to inherited or *de novo* mutations that affect the folding of specific proteins. Similarly, while no interaction between pharmacotherapy and protein pathology was seen in this study, it may be detectable with increased patient numbers. It would be particularly interesting to follow up the (non-significant) trend towards patients with these pathologies being less likely to use clozapine, given the nature of clozapine as a "last line of defence", as this could potentially suggest a link between protein pathology in the blood and antipsychotic response.

CONCLUSION

This initial study suggests that the presence in the blood of specific proteins, which aggregate in the brains of schizophrenia patients, may be related to symptom severity in schizophrenia, and that further investigation in

larger groups of patients is warranted. It should be made clear that this is a limited study, with a low number of patients displaying each of the putative protein pathologies in their blood, compared to the proportion of schizophrenia patients previously seen to display protein aggregates of specific proteins in their brains (Leliveld et al. 2008, Ottis et al. 2011, Bader et al. 2012b, Nucifora et al. 2019, Samardžija et al. 2021, Zaharija et al. 2022). Similarly, we are unable, at this time, to formally rule out possible interactions between the protein pathology and antipsychotics or other treatment.

Comments for reviewers
None

Ethical Considerations
Does this study include human subjects? YES

Authors confirmed the compliance with all relevant ethical regulations.

Conflict of interest
No conflict of interest.

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