Title

Neurogranin as a potential synaptic marker in the cerebrospinal fluid of patients with a first episode psychosis

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Abstract (max 250 words, now 222)

The neurodevelopmental hypothesis is central in the conceptualization of schizophrenia, but ante-mortem human evidence is lacking. Neurogranin is a post-synaptic protein important for calmodulin-related mechanisms and long-term potentiation establishment. Measurement of neurogranin in cerebrospinal fluid (CSF) is thought to reflect synaptic degeneration in neurodegenerative disorders. We examined CSF neurogranin by Meso Scale Discovery immunoassay in 40 first episode psychosis (FEP) patients, of which 22/40 were antipsychotic-naïve and 20 healthy controls. Although not statistically significant, the CSF neurogranin level was lower in FEP patients (342 pg/mL ±207) compared to controls (427 pg/mL ±189, mean difference 85, 95% CI=-25 – 195). Utilizing multivariate analyses, the variation of CSF neurogranin in the FEP group and the group difference were coupled to the use of antipsychotic medication, which was associated with lower levels of CSF neurogranin, and smoking, being associated with higher levels. No relationships were seen between CSF neurogranin and symptom scores or cognitive measures. Our study indicates that levels of CSF neurogranin are not altered in FEP, which may contradict the neurodevelopmental and particularly the synaptic pruning hypothesis, but there are important caveats that are discussed. With the study design in mind, our study suggests that neurogranin may be physiologically altered with antipsychotic treatment, thus indicating a possible new second messenger of interest in dopamine receptor D2- and 5-hydroxytryptamine 2 A receptor-mediated signaling that is accessible for *in vivo* measurements in humans. Longitudinal clinical and experimental studies are needed to examine this further.

Keywords

schizophrenia, neurogranin, neurodevelopmental hypothesis, synaptic pruning, calmodulin

Introduction/Background

The neurodevelopmental hypothesis of schizophrenia (Murray and Lewis 1987, Weinberger 1987, Cannon 2015) postulates that the disease develops when maturational processes act upon pre- and perinatal insults, leading to an aberrant neuronal development. Due to their timing in ontogenesis, in combination with the peak age-related incidence of schizophrenia, two developmental mechanisms are of particular interest, that is myelination and synaptic pruning (Huttenlocher 1979, Feinberg 1982, Petanjek, Judas et al. 2011). In support of this, studies on post-mortem brain tissue have shown reductions in the content of synaptic proteins, indicating reduced numbers of synapses, in discrete brain regions in schizophrenic patients (REF). There is a large body of evidence supporting exaggerated or aberrant synaptic pruning in schizophrenia, from neuropathology, genetics, and animal models (Bakhshi and Chance 2015, Sekar, Bialas et al. 2016, Osimo, Beck et al. 2018). However, a core issue is the availability of clinical evidence in this regard, particularly in patients who have not been treated with antipsychotic medication. One possible approach is to quantify proteins of the synaptic machinery in cerebrospinal fluid (CSF) of patients with schizophrenia. Indeed, some studies have shown reductions in the synaptic proteins chromogranin A and B in CSF in schizophrenia (REF), suggesting de-arranged synaptic function. Neurogranin is a postsynaptic calmodulin-binding protein, important for Ca2+ signal transduction and establishment of

long-term potentiation (LTP) (Diez-Guerra 2010). In neurodegenerative disorders, particularly Alzheimer´s disease (AD), CSF neurogranin levels are increased in a way that is thought to reflect ongoing synaptic degeneration, since it corresponds with other biomarkers of synaptic damage and synaptic activity, such as hippocampal atrophy and cortical fluorodeoxyglucose (FDG) hypometabolism investigated with positron emission tomography (PET) (Portelius, Zetterberg et al. 2015, Tarawneh, D'Angelo et al. 2016, Selnes, Stav et al. 2017). Interestingly, in the context of schizophrenia, polymorphisms of the neurogranin gene *NRGN* are associated with an increased risk of disease (Stefansson, Ophoff et al. 2009), as well as phenotypical variations, both in cognitive performance and cerebral structure, in patients with schizophrenia (Ohi, Hashimoto et al. 2013, Thong, Qiu et al. 2013). Recently, neurogranin production was shown to be triggered by the kynurenine signaling pathway (Oliveros, Wininger et al. 2017), which is activated in schizophrenia (Erhardt, Blennow et al. 2001, Oliveros, Wininger et al. 2017).

The purpose of this study was to examine CSF neurogranin in a first episode psychosis (FEP) cohort of whom a considerable proportion were expected to either have or subsequently develop schizophrenia and to be devoid of antipsychotic medication, in order either to support or refute the hypothesis of aberrant synaptic pruning in schizophrenia. *Our hypothesis* was that we would detect an increase in CSF neurogranin levels in FEP patients as compared with controls, as a consequence of increased synaptic turnover, due to ongoing accelerated synaptic pruning.

Material and methods

Patients and healthy controls were recruited as part of the Karolinska Schizophrenia Project (KaSP), an ongoing longitudinal study of first episode psychosis (FEP) in Stockholm, Sweden. Subjects in this study were included from March 2011 through January 2014.

FEP patients

Patients with a FEP (n=40) were recruited at first contact with in- or outpatient clinics. Inclusion criteria were a diagnosis of either schizophrenia, schizophreniform psychosis, schizoaffective disorder, delusional disorder, psychosis not otherwise specified (NOS) or brief psychosis, according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Diagnosis was based on a structural interview using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I), or a consensus procedure using all medical information that was available at the time of inclusion. Exclusion criteria were drug or alcohol abuse (except for tobacco), autism spectrum disorder, neurologic illness or severe other illness. Patients with previous or ongoing antipsychotic treatment longer than 1 month were excluded from the study. Diagnosis was re-assessed for a subgroup of 15 patients at follow-up after 1.5-2 years.

Patients were rated using the Positive and Negative Symptoms Scale (PANSS), Global Assessment of Functioning (GAF), Clinical Global Impression (CGI), the Alcohol Use Disorders Identification Test (AUDIT-E) and Drug Use Disorders Identification Test (DUDIT-E). Cognitive performance was assessed using the MCCB (Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery) battery (Nuechterlein, Green et al. 2008) (see below). Lumbar puncture (see below), routine blood and urine analysis was performed. Absence of major brain abnormalities was determined based on a 3T magnetic resonance imaging (MRI) examination. Information about Duration of Untreated Psychosis (DUP) was given by the patient or the patient's relatives. For the majority of patients (n=34) all procedures were performed within a 10-day period from study inclusion, whereas in 6 patients these were completed in a period of 14 to 40 days.

Healthy controls

20 healthy controls (HC), as to be matched for sex and age, were recruited via advertisement. All healthy controls underwent medical and psychiatric examination (supported by the Mini International Neuropsychiatric Interview), routine blood analysis, lumbar puncture, 3T MRI and cognitive assessment with the MATRICS battery. Exclusion criteria were previous or current psychiatric or neurological illness, first-degree relative with psychotic illness and previous or current drug abuse or dependence. The latter was evaluated by a clinical interview and with Alcohol Use Disorders Identification Test (AUDIT-E) and Drug Use Disorders Identification Test (DUDIT-E). All healthy controls were free from medication.

Ethics

The study was approved by the Regional Ethics Committee in Stockholm and conformed to the tenets of the Declaration of Helsinki. All subjects included had given written consent after receiving written and oral information.

Cognitive assessment

The MCCB (Nuechterlein, Green et al. 2008) is a cognitive test battery purposely designed to capture cognitive domains relevant to schizophrenia. Seven cognitive domains are assessed, using 10 different tests. Previous studies showing a relationship between CSF neurogranin and cognitive function have done so either with a) “global” cognitive function, determined by the Mini Mental Status Examination (ref) or the Montreal Cognitive Assessment (Bereczki, Bogstedt et al. 2017) or b) episodic verbal memory recall (Casaletto, Elahi et al. 2017). As an approximate equivalent to the former we used the MCCB composite T score and of the latter immediate recall raw scores from the Hopkins Verbal Test-Revisited (HVLT-R), which is a part of the MCCB.

CSF collection and analysis

CSF was collected by lumbar puncture as previously described (Orhan, Fatouros-Bergman et al. 2018), centrifuged, divided into aliquots and frozen at -80° C within 1 h of sampling. All tubes used were of polypropylene. Amounts of lymphocytes, albumin, total IgG, IgG and IgM antibodies for *Borrelia burgdorferi* was determined at a clinical laboratory.

Electrochemiluminescence immunoassay for neurogranin

Measurement of CSF neurogranin was performed on the Meso Scale Discovery (MSD; Rockville, MD, USA) platform, as described in detail previously (Portelius, Zetterberg et al. 2015). The in-house generated monoclonal antibody Ng7, which binds to an epitope including amino acids 52–65 (Kvartsberg, Duits et al. 2015) was used as the capturing antibody. Summarily, QUICKPLEX 96-well plates were coated with Ng7 overnight at room temperature, and the remaining protein binding sites blocked with 5% MSD Blocker for 1h at room temperature. Full-length neurogranin calibrators (with concentrations ranging between 31.3–2000 pg/ml), blanks, and 50 µl of CSF samples were incubated in duplicate together with the detector antibody and polyclonal neurogranin anti-rabbit antibody (07-425; sMerck Millipore, USA, Upstate) overnight. Next, a MSD Sulfotag goat anti-rabbit antibody was incubated for 1 h at room temperature. MSD Read Buffer T with surfactant was used for immediately reading on the Mesoscale instrument Quickplex SQ 120 reader. All measurements were performed by board-certified laboratory technicians who were blinded to clinical data.

Statistical analysis

All analyses were performed in IBM SPSS Statistics Version 22. Shapiro-Wilks normality test was used for assessment of normality distribution. For between group comparisons of demographic and clinical characteristics t-test was used for continuous variables and chi-square test for dichotomous variables. Bivariate relationships between CSF neurogranin and demographical and clinical variables were, for continuous variables, explored either with Pearson or Spearman correlation methods, as appropriate. For dichotomous variables, effect sizes were expressed according to Cohen's d and group mean values were compared using t-test. Multiple linear regression models were controlled for assumptions about linear relationship, collinearity, and residual normality. Threshold for statistical significance was set at $p<0.05$.

Results

Demographics and clinical characteristics

Forty FEP patients were diagnosed at baseline with schizophrenia (n=11), schizophreniform disorder (n=14), schizoaffective syndrome (n=1), delusional disorder (n=3), brief psychotic disorder (n=1) and psychotic disorder not otherwise specified (n=9). One patient was diagnosed with affective psychosis at baseline, but did receive schizoaffective disorder diagnosis at follow up and thus retained. Out of the 40 patients at baseline 15 patients participated in re-assessment after 1,5 years. All patients with a diagnosis within the schizophrenia spectrum (schizophrenia, schizophreniform disorder, schizoaffective disorder) at baseline and who had completed 1.5 year follow up retained a diagnosis within the spectrum. All patients with schizophreniform disorder at baseline (n=6) received a schizophrenia diagnosis at follow up.

FEP patients and healthy controls did not significantly differ with regard to height, weight, body mass index (BMI) and education, but differed in their mean age (FEP 29 ±7, HC 25 ±7, p= 0.028) (Table 1). As expected, the groups differed in the proportion of individuals using nicotine (smokers, smokeless tobacco), although these differences were statistically not significant.

Psychotropic medication

At the time of the CSF sampling, 22 of the FEP patients were naïve to antipsychotics, whereas 18 were treated with antipsychotics, with a mean ± SD treatment duration of17.8 ±12.6 days. Of the patients 15/40 were treated with a benzodiazepine and 5/40 with an antidepressant (Table 1). Twelve of the patients were naïve to all psychotropic medications. In descending order of frequency, patients were treated with olanzapine, risperidone, quetiapine, aripiprazole, and haloperidol, with olanzapine being administered to 61% of the patients. Of those treated with antipsychotics 83% were on monotherapy. Antipsychotic treatment longer than one month was an exclusion criterion, however, after inclusion it became apparent that one patient notwithstanding had been treated for 57 days, and this subject was retained. Using the minimum effective dose method as by Leucht et al 2014 (Leucht, Samara et al. 2014), the mean haloperidol equivalent dose was 8.5 mg.

Relationships between CSF neurogranin, demographic and clinical variables

Relationships between CSF neurogranin and demographic and clinical variables for FEP patients and HC reported in Table 1 are found in Supplementary Table 1 and Supplementary Table 2. None of the relationships were statistically significant. The relationships between CSF neurogranin and possible confounders in the FEP group was explored in a multivariate analysis, with 4 covariates chosen on criterion of descending effect sizes (smoking,

antipsychotic treatment, smokeless tobacco use), and age. Age was included since age has shown the most consistent influence on CSF neurogranin in previous studies (De Vos, Jacobs et al. 2015, Kester, Teunissen et al. 2015, Abner, Jicha et al. 2016, De Vos, Struyfs et al. 2016, Mattsson, Insel et al. 2016, Casaletto, Elahi et al. 2017). This yielded a significant model (ANOVA), p=0.027, adjusted R^2 = 0.178, with smoke (ß=0.317, p=0.040), and antipsychotic treatment (ß=-0.323, p=0.040) emerging as statistically significant. Substituting smokeless tobacco use for BMI did not change these results. Substituting antipsychotic treatment for haloperidol equivalents increased p-values from 0.040 to 0.052 for both smoking and haloperidol equivalents.

In the FEP patient group multivariate analysis was used to examine the relationship between CSF neurogranin and PANSS positive, negative, and GAF function scores. Based on the previous analysis, antipsychotic treatment and smoking were used as covariates, together with DUP. None of these models were statistically significant. The same strategy was applied for HTLV-R and MCCB composite T scores, with the latter only showing a statistical significant model, with DUP and smoking as significant predictors. Adding education and age still yielded to a significant model (ANOVA p= 0.014) with DUP (ß = -0.558, p= 0.001) and smoking (ß = -0.365, p=0.034) as statistically significant.

CSF neurogranin levels between groups

FEP patients had a lower but statistically non-significant mean neurogranin level (342 pg/mL ±207) compared to HC (427 pg/mL ±189) (mean difference 85, 95% CI=-25 – 195, p=0.127, Cohen´s d=0.429) (Table 1, Figure 1). Selecting only the FEP patients who had a schizophrenia spectrum disorder diagnosis either at baseline or at 1.5 year follow up (n=27)

and comparing their mean neurogranin level (328 pg/mL ±191) to HC (427 pg/mL ±189) showed a slightly larger mean decrease (99, p=0.084).

As our main analysis, multivariate regression was performed to investigate the relationship between FEP/HC group membership and neurogranin, using age, antipsychotic medication, and smoking as covariates. This yielded a significant model (ANOVA p=0.010) with adjusted R^2=0.155, with no significant effect of FEP/HC status on CSF neurogranin (ß = 0.042, p=0.766) but significant effects for antipsychotic treatment (ß = -0.327, p=0.020) and smoking (ß = 0.281, p=0.025). An independent samples t-test between levels of CSF neurogranin in the HC (427 pg/mL, SD = 189) and FEP patients with antipsychotic treatment only (302 pg/mL, SD = 183). This showed that there was a statistically significant difference (Cohen´s d = 0,672, p = 0,043, two-tailed).

Discussion

Contrary to our hypothesis, we did not find any significant difference in CSF neurogranin between FEP patients as compared with controls. The moderate reduction present on a group level was associated with the presence of antipsychotic medication.

The neurodevelopmental hypothesis of schizophrenia, more specifically the hypothesis of aberrant synaptic pruning, provided the rationale for this study. Synaptic pruning is the neurodevelopmental process during early adulthood in which the excess of synapses produced during childhood and puberty are reduced (Huttenlocher 1979, Feinberg 1982, Petanjek, Judas et al. 2011). A large body of evidence supports this hypothesis. Reduction in cortical neuropil (the neuropil volume is dominated by synapses) is possibly the most solid neuropathological finding in SZ (Bakhshi and Chance 2015), and reduction of the synaptodendritc system has

been demonstrated repeatedly in post mortem samples. More specifically, this includes density of spines (Glausier and Lewis 2013), diverse constituents of the presynaptic machinery (Osimo, Beck et al. 2018), and, importantly, also neurogranin (Broadbelt, Ramprasaud et al. 2006). Further, genetic findings implicate genes such as complement factor 4 (C4), involved in synaptic pruning (Sekar, Bialas et al. 2016) and neurexin 1 (NRXN1), involved in synaptic vesicle release (Levinson, Shi et al. 2012). Furthermore, animal models based on disruption of these genes do show reductions in synaptic density and function (Missler 2003, Missler, Zhang et al. 2003, Sekar, Bialas et al. 2016).

However, evidence of structural aberrations of the synaptic system in the living patient with schizophrenia are much rarer, particularly in drug-naïve patients. Given the possible effect of antipsychotics (see below) the latter aspect appears critical. Landén and coworkers found a decrease in the synaptic vesicle protein chromogranin A (CgA) and CgB, in CSF of both drug-naïve and medicated patients (Landen, Grenfeldt et al. 1999), but these findings were the opposite in the only other drug-naïve cohort where CSF-CgA (Miller, Kirchmair et al. 1996) has been analyzed. Synaptosomal-associated protein 25 (SNAP-25), a presynaptic SNARE complex protein, has been examined in the CSF of patients with chronic schizophrenia, with an increase seen both off and on antipsychotic treatment (Thompson, Kelley et al. 2003). Neuronal cell adhesion molecule (NCAM/CD56), a membrane protein important for synaptic plasticity, is reduced in the CSF of antipsychotic-naïve patients (Vawter, Hemperly et al. 1998). To our knowledge there has been no further confirmatory studies in antipsychotic free subjects, while chronic medicated or chronic but temporarily not medicated patients have shown either increased or unchanged levels of synaptic proteins (van Kammen, Poltorak et al. 1998, Hidese, Hattori et al. 2017). Comprehensively, some studies indicate reduced levels of synaptic system proteins, but results are either unreplicated or contradictory.

In the current study, we only included FEP patients who were naïve or only briefly exposed to antipsychotics, and thus a cohort we regard as well suited for the purpose of studying synaptic dynamics in schizophrenia. An obvious explanation for the findings of the current study would be that there is no exaggerated synaptic pruning in schizophrenia, and that the associations between the neurogranin gene and risk of schizophrenia (Stefansson, Ophoff et al. 2009), or cognition and brain structure in schizophrenia (Ohi, Hashimoto et al. 2013, Thong, Qiu et al. 2013), are not reflected in neuronal levels of the protein. In addition to the caveat that our study may be underpowered to detect minor differences that escape statistical significance, there are however important methodological aspects that will be discussed subsequently.

CSF neurogranin as "synaptic marker"

The CSF concentration of neurogranin likely represents the collective neuronal contribution at one given time point, mainly from the neocortex (Represa, Deloulme et al. 1990). Neither the physiological process of neuronal maturation (Huttenlocher and Dabholkar 1997) nor the pathology in schizophrenia (Bora et al. 2011) is likely to be uniformly distributed across the neocortex. As a consequence, it is possible that anatomically restricted alterations in synaptic dynamics are not reflected in the CSF. If exaggerated synaptic pruning is a core etiopathological process in schizophrenia, it should be present no later than the FEP stage. Yet, a putative secondary increase (or decrease) in CSF neurogranin may have occurred considerably earlier than at the FEP stage. Clearly, methods with spatial information and studies in prodromal stages with longitudinal follow up are needed to overcome these problems.

Based on the wealth of data from the neurodegenerative field, particularly in Alzheimer's disease (AD), CSF neurogranin appears to be well suited as a synaptic biomarker. CSF neurogranin shows a robust separation between AD patients and controls (De Vos, Jacobs et al. 2015, Portelius, Zetterberg et al. 2015, Tarawneh, D'Angelo et al. 2016), also in prodromal stages of AD (Kester, Teunissen et al. 2015, Tarawneh, D'Angelo et al. 2016). Importantly, CSF neurogranin correlates with rate of increase in cerebral atrophy, including that of the hippocampus, (Portelius, Zetterberg et al. 2015, Tarawneh, D'Angelo et al. 2016), rate of decrease in cortical metabolism as measured by FDG-PET (Portelius, Zetterberg et al. 2015, Selnes, Stav et al. 2017) and core AD biomarkers such as tau and p-tau (Portelius, Zetterberg et al. 2015, Tarawneh, D'Angelo et al. 2016). Notably, though, it is always the case of an increase of CSF neurogranin levels in AD, while the pathological process in AD most definitely includes a synaptic loss. The current explanation is that CSF neurogranin reflects both cumulative synapse density *and* intensity of the synaptic loss/turnover (Mattsson, Insel et al. 2016, Tarawneh, D'Angelo et al. 2016). If these processes are balanced, no net effect on CSF neurogranin levels would be detected, which is a fully plausible situation in a FEP patient with schizophrenia. To complicate matters further, neurogranin levels measured in neuronal derived exosomes in blood in AD patients are not increased, but reduced (Goetzl, Kapogiannis et al. 2016, Winston, Goetzl et al. 2016). Also, CSF neurogranin is *reduced* in both Parkinson´s disease (Selnes, Stav et al. 2017) and frontotemporal dementia (Janelidze, Hertze et al. 2016, Lista, Toschi et al. 2017), and in the latter is paralleled by reduced neuronal derived exosomal neurogranin in blood (Goetzl, Kapogiannis et al. 2016). Thus there may be concomitant phenomena that limit how well CSF neurogranin reflects synapse densities and/or turnover (Chang, Schumacher et al. 1997).

Relationship with antipsychotics

In our study the use of antipsychotic medication is associated with lower CSF neurogranin levels, although the cross sectional study design unables any conclusions about causal relationships. A critical issue is if there is a confounding by indication, i.e. a true reduction in CSF neurogranin in FEP patients that is most apparent in the more severely ill patients in which start of antipsychotic medication could not wait. However, demographical and clinical parameters (Supplementary Table 3) show no major differences between medicated and unmedicated patients, arguing against this explanation. Still, the very short antipsychotic treatment period could already at this stage have lowered PANSS scores. To our knowledge no study has previously reported CSF neurogranin in relationship to antipsychotic medication.

If our finding is indeed a true effect of antipsychotic treatment, several interpretations are possible. First, that a low dose of antipsychotics over a very limited period of time leads to a reduced synapse density. In humans, a single dose haloperidol (5 mg/70 kg bodyweight) has shown a reversible reduction in striatal volume detected by MRI, an effect attributed to plasticity mechanisms such as alterations in dendritic arborization or synapse dynamics (Tost, Braus et al. 2010). Chronically antipsychotic treated monkeys and mice (Lidow, Song et al. 2001, Ibi, de la Fuente Revenga et al. 2017), show reduced spine density, but this can be detected also after a single haloperidol dose (Engmann, Giralt et al. 2016). However, in both the former studies, despite these structural changes, densities of synapses *per se* were unchanged, indicating that this is not the most plausible explanation in our study.

A second interpretation is that the lower levels of CSF neurogranin associated with antipsychotic medication reflects a pharmacological effect on second messenger systems. Neurogranin is a critical part of the Ca2+ mediated signaling pathway (Diez-Guerra 2010). Briefly, neurogranin binds calmodulin (CaM), making it strategically available in the synapse

(Zhong and Gerges 2012), thus regulating CaM activity in response to Ca2+ influx and CaM:s subsequent effects and establishment of LTP (Zhong, Cherry et al. 2009). Overexpressing neurogranin in animals leads to increased LTP, enhances extinction learning (Zhong, Brown et al. 2015), while knock out animals show reduced LTP and deficiencies in learning (Pak, Huang et al. 2000, Huang, Huang et al. 2004, Zhong, Cherry et al. 2009). It is well known that antipsychotics disrupt LTP in animal models (Price et al 2014), while it is not clear exactly how this effect is mediated (Beaulieu and Gainetdinov 2011). Downstream processes do partly converge on the CaM system, and thus the relationship between neurogranin and antipsychotics in our study could be an effect of dopamine receptor D2 blockade on such pathways. Our findings could thus indicate a new mechanism for dopamine receptor D2 mediated effects on Ca2+ signaling, and, regardless, could provide an in vivo CSF biomarker for second messenger systems in antipsychotic treatment. As can bee seen in Table 1, our study has data for other classes of compounds (antidepressants and anxiolytics - particularly benzodiazepines). However, effects are too small for meaningful multivariate statistical analyses in this cohort.

Relationship with smoking

In our study, smoking appears to have the opposite relationship on CSF neurogranin than antipsychotic medication i.e. be associated with higher levels. Tobacco smoke contains a wealth of pharmacologically active compounds, including nicotine. Nicotine asserts its effect trough nicotinic acetylcholine receptors (nAChRs), mainly localized presynaptically were nAChRs stimulates the release of neurotransmitters, including dopamine, and induces LTP (Role and Berg 1996). A possible effect on the postsynaptically located neurogranin is most likely a secondary effect of such a neurotransmitter. Our finding highlights a second messenger of interest for nicotine transmission, and also proposes a novel crossroad for the

clinically important interaction between dopaminergic blocking compounds and nicotine (Kumari and Postma 2005).

Conclusion and future directions

Our conclusion is that CSF levels of neurogranin appear not to be altered in patients with a FEP, but instead there is a reduction associated with antipsychotic use. The latter finding potentially provides a novel view into antipsychotic related second messenger systems that can be monitored in vivo, but clearly longitudinal and experimental studies are needed to test this hypothesis. The lack of significant alterations of CSF neurogranin can be explained by limitations of the underlying hypothesis, general limitation of CSF biomarkers, or the relatively small sample size (40 patients with different psychiatric diagnoses). For future studies on the synaptic system in schizophrenia and other disorders we suggest that a) presynaptic proteins may be preferable, b) methods with spatial resolution (Finnema, Nabulsi et al. 2016) can overcome some of the limitations of CSF based methods and c) pharmacological treatment, particularly antipsychotic use, and smoking appear to be important cofactors.

Conflicts of interest

KB has served as a consultant or at advisory boards for Alzheon, CogRx, Biogen, Novartis, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to the work presented in this study. HZ has served at scientific advisory boards for Eli Lilly, Roche Diagnostics, Wave, Samumed and CogRx, has received travel support from Teva and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. The authors declare no conflict of interest

Acknowledgments/funding

We express our deep gratitude toward the patients, their relatives and

the healthy volunteers for their participation. KB is supported by the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences. HZ is a Wallenberg Academy Fellow supported by grants from the European Research Council and the Swedish Research Council. We thank the KaSP personnel for their help with recruitment of subjects, and the staff at the neurochemistry laboratory in Mölndal for their expertise.

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Figure 1. CSF neurogranin in patients and controls.

Values are pg/ml, bars represent mean. FEP: First Episode Psychosis, HC=Healthy Control

Table 1. Demographic and clinical characteristics of the study population

Variable	**FEP (n=40)**	**HC (n=20)**	**p-value**
Age, years	29 ±7	25 ±4	0.028
Female sex	15, 38%	9, 45%	NS

Height, cm	175 ±9	175 ±9	NS
Weight, kg	71 ±13	69 ±11	NS
BMI, kg/m^2	23 ±4	22 ±3	NS
Education, years	14 ±3	15 ±2	NS
Smokers	38%	00%	NS
Smokeless tobacco users	718%	2.10%	NS
Tobacco users	1025%	2.10%	NS
Antipsychotic treatment, n	1845%	-	-
Duration of antipsychotic treatment, days	17.8 ±12.6	-	-
Benzodiazepines treatment, n	15, 38%	-	-
Antidepressant treatment, n	5, 13%	-	-
DUP, months (median, min-max)	4 (0-48)	-	-
PANSS-positive	20 ±6	-	-
PANSS-negative	16 ±8	-	-
PANSS-general	38 ±12	-	-
PANSS-total	73 ±22	-	-
GAF function	42 ±11	-	-
GAF symptom	32 ±7	-	-
CSF neurogranin, pg/ml	342 ±207	427 ±189	NS

Numerical values are mean ± SD, n, or percentage as appropriate, unless otherwise stated.

DUP: duration of untreated psychosis, FEP: First Episode Psychosis, GAF: Global Assessment of Functioning Scale, HC: Healthy Control, PANSS: Positive And Negative Syndrome Scale for Schizophrenia, NS: Not statistically significant.

Supplementary Table 1. Relationship between CSF neurogranin and continuous demographic and clinical variables

FEP=First Episode Psychosis, DUP=Duration of Untreated Psychosis, HC=Healthy Controls, PANSS=Positive And Negative Syndrome Scale for Schizophrenia. R is Pearson or Spearman correlation coefficient, as appropriate.

Supplementary Table 2. Relationship between CSF neurogranin and dichotomous demographic and clinical variables

FEP=First Episode Psychosis, HC=Healthy Controls. CSF neurogranin values are mean +/- SD, expressed in pg/ml.

Supplementary Table 3. Demographical and clinical variables in antipsychotic treated and untreated patients.

AP= antipsychotics, DUP=Duration of Untreated Psychosis, FEP=First Episode Psychosis GAF= Global Assessment of function, PANSS=Positive And Negative Syndrome Scale. Values are mean +/- SD unless stated. NS=Not statistically significant.

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