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Brief report

Further evidence for plasma progranulin as a biomarker in bipolar disorder

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ABSTRACT

Background: A recent study suggested that progranulin (encoded by the fronto-temporal dementia risk gene *GRN*) plasma levels are decreased in bipolar disorder (BD). Replication of this finding is however lacking.
Methods: Progranulin plasma levels of bipolar patients ($n=104$) and healthy controls ($n=80$) were measured by enzyme-linked immunosorbent assay (ELISA). Participants were also genotyped for three single nucleotide polymorphisms (SNPs) in the *GRN* gene (rs2879096, rs4792938 and rs5848), and the effect of genetic variation on progranulin levels was examined.
Results: Plasma progranulin levels were decreased in BD (ANCOVA, $p=0.001$). Furthermore, age was significantly and positively correlated with plasma progranulin (Pearson's correlation, $r=0.269$, $p<0.001$). Also, lithium treatment but no other medication had a significant effect on progranulin plasma levels (ANCOVA, $p=0.007$). Specifically in BD, the *GRN* SNP rs5848 was associated with progranulin plasma levels (Kruskal–Wallis test, $p<0.005$).
Limitations: Subgroup analysis regarding bipolar I vs. bipolar II subtype and polarity of the episode at sampling (manic vs. depressed vs. mixed vs. rapid cycling vs. euthymic) could only be performed with limited validity due to the relatively small sample size. The suitability of peripheral progranulin as a biomarker for BD is limited due to the overlap between patients and controls.
Conclusion: The findings strengthen the evidence for progranulin being involved in pathomechanisms of bipolar disorder, and suggest a genetic determinant of progranulin concentrations that is relevant specifically in bipolar patients.

1. Introduction

Coding mutations in the progranulin gene (*GRN*) are one of the confirmed variants in autosomal dominant fronto-temporal lobar degeneration (FTLD). However, common genetic polymorphisms in this gene on the other hand are associated with schizophrenia and bipolar affective disorder (BD) (Rademakers et al., 2008; Momeni et al., 2010; Galimberti et al., 2012). Progranulin (PGRN) is involved in several physiological and pathophysiological processes, in the periphery as well as in the central nervous system. There is evidence that progranulin functions as a neurotrophic factor and modulates neurite outgrowth, neuronal differentiation and neuronal survival (Van Damme et al., 2008; Gao et al., 2010). Therefore it is not surprising that increased progranulin expression can be found in neuroinflammatory and neurodegenerative processes, like Alzheimer's disease, Creutzfeld–Jakob disease and amyotrophic lateral sclerosis (Ahmed et al., 2007). An involvement of progranulin in peripheral inflammatory processes is also presumed because it was recently shown that it interacts with the TNF-α receptor and its expression is regulated by cytokines like Il-1 β (Tang et al., 2011; Okura et al., 2010). Furthermore PGRN seems to be involved in the pathophysiology of cancer and the value of progranulin as diagnostic and

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prognostic biomarker in different cancer types is an ongoing research subject (Demorrow, 2013). Interestingly, there is growing evidence for a role of inflammatory processes in at least subgroups of bipolar patients, as increased levels of pro-inflammatory cytokines were observed in blood and cerebrospinal fluid of BD patients (Langan and McDonald, 2009; Drexhage et al., 2010). Studies on the proteomic profile of bipolar patients also add evidence for an involvement of the inflammatory system in BD (Alsaif et al., 2012).

Diagnostic biomarkers for bipolar disorder are not available to date. Due to the heterogeneity of the disorder it cannot be expected to develop a general fluid biomarker, but rather a set of different biomarkers to identify subgroups of bipolar patients with also potential implications on different treatment strategies. In unipolar depressive patients it was shown recently that add-on treatment with COX-2 inhibitors enhances the effect of antidepressant treatment (Abbasi et al., 2012). And an even more intriguing study investigating the antidepressant properties of the TNF-alpha antagonist infliximab revealed that especially a subgroup of treatment-resistant depressive patients showing high baseline inflammatory markers improved when being treated with infliximab (Raison et al., 2013). This can be extended by hypotheses that there are subgroups in unipolar and also in bipolar patients which benefit more than others from adjunctive treatment with antiinflammatory agents, which might be due to increased levels of basal pro-inflammatory proteins (McNamara and Lotrich, 2012). In the present study we therefore attempted to replicate and extend previous findings (Galimberti et al., 2012) that BD patients had lower progranulin plasma levels compared to healthy controls to evaluate the potential of progranulin as a diagnostic biomarker for bipolar disorder.

2. Methods

2.1. Participants

Patients were recruited at the Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Germany. Controls were recruited mainly from hospital staff working at this Department. Plasma and DNA samples were collected in 104 BD patients, including 56 females and 48 males, mean age ± SD at sampling: 48.1 ± 12.56 years (20–76 years) and 80 controls, including 46 females and 34 males, mean age ± SD at sampling: 37.7 ± 13.93 years (20–71 years). In three of the participants, no DNA could be obtained. Among the BD patients were 64 bipolar type I and 40 bipolar type II. All patients received psychotropic medication. For demographic details of patients and controls see Supplementary Table 1. Diagnosis was established by administration of a semi-structured clinical interview for a WHO ICD-10 diagnosis, independently assessed by two trained psychiatrist (Fähndrich, 2007). Additionally, diagnosis was assessed by the operational criteria OPCRIT checklist for psychotic and affective illness (Opcrit 4 windows, 2009, MRC Social, Genetic & Developmental Psychiatric Center). In controls, presence of any psychiatric disorders or intake of psychotropic medication was excluded by applying the Mini-DIPS screening interview (Margraf, 1994). Exclusion criteria for patients and controls were severe medical conditions, severe neurological disorders, mental retardation, current carcinomas and infectious diseases. Exclusion criteria for controls were a history of psychiatric disorder or first-line relatives with psychiatric disorders. Only subjects who gave written informed consent were enrolled in the study, which compiled with the Declaration of Helsinki and was approved by the Ethics Committees of the University of Würzburg.

2.2. DNA isolation and GRN SNPs analysis

High-molecular weight DNA was isolated from EDTA blood by using a standard desalting method. DNA samples were aliquoted and stored at −20 °C until use. For the association study, three SNPs already shown to be associated with BD (Galimberti et al., 2012) were analyzed, rs2879096 and rs4792938 are located in the *GRN* sequence, whereas rs5848 is located in the 3'UTR and was shown to influence mRNA transcription through miR-659 regulation (Rademakers et al., 2008). SNPs were genotyped by using TaqMan methodology. Each Taqman 5'-nuclease assay employed 25 ng of genomic DNA as template. Assay-on-demand products, ABI assay IDs: C_15835934_10, C_32346749_10, and C_7452046_10 were used for rs2879096, rs4792938 and rs5848 genotyping, respectively.

2.3. Plasma collection and progranulin ELISA

Blood samples were collected from fasting subjects (12 h) at the same time (8.00) in the morning to avoid circadian variation. Plasma was collected from EDTA blood. Samples were centrifuged at 4 °C at 2300 rpm for 15 min and plasma was aliquoted in 500 µl in LoBind tubes and stored at −80 °C until use (Eppendorf, Hamburg, Germany). Progranulin expression levels in plasma samples were measured in duplicate using the Adipogen human Progranulin enzyme linked immunosorbent assay (ELISA) kit (Incheon, Korea). ELISA was performed in accordance to manufacturer's instructions and plasma samples were diluted 1:200. The sandwich ELISA detects only full-length progranulin but not progranulin cleavage products or fragments (Finch et al., 2009).

2.4. Statistical analysis

Data were analyzed using the software SPSS 20 (SPSS Inc., Chicago, IL). Covariates sex and age were tested for their independence from the progranulin levels with an ANOVA and led to the exclusion of age as covariate. Progranulin plasma levels in cases and controls were compared by the analysis of covariance (ANCOVA). Progranulin plasma levels in subgroups of bipolar patients (bipolar I vs. II; current episode) were also compared by analysis of covariance (ANCOVA). Effects of the psychopharmacological treatment on progranulin levels in the bipolar patients were analyzed by ANCOVA and Student's *t*-tests (antipsychotics vs. anticonvulsants vs. lithium vs. combination and add-on antidepressants). Furthermore, Pearsons's correlation comparing age and progranulin plasma levels was performed. As previous data demonstrated lower plasma levels in patients, one-sided testing was performed. A χ^2 test was used to test for differences in SNP and haplotype distribution between cases and controls. Haplotypes as well as linkage disequilibrium were analyzed by Haploview 4.2. The correlation between progranulin plasma levels and genotypes was tested by analysis of variance (ANOVA) and non-parametric tests (Kruskal–Wallis) when genotype subgroups were too small in number.

3. Results

3.1. Plasma progranulin levels

ELISA of blood plasma revealed that bipolar patients feature significantly lower progranulin levels (ANCOVA, $p=0.001$) compared to healthy controls. Patients had a mean progranulin concentration of 141.3 ng/ml (± 31.5 ng/ml SD, with a range between 77.1 ng/ml and 273.9 ng/ml) while controls had a mean concentration of 152.4 ng/ml (± 49.6 ng/ml, with a range between 45.3 ng/ml and 292.4 ng/ml). Sex had no influence on progranulin levels (ANOVA,