Introduction

Mutations in the progranulin gene (GRN) are the most frequent cause of autosomic dominant frontotemporal lobar degeneration (FTLD). Age at disease onset, as well as clinical phenotypes associated with such mutations are extremely wide, even in the same family [1] and include, besides the classical FTLD syndromes behavioural variant of frontotemporal dementia (bvFTD), progressive non fluent aphasia and semantic dementia [2–4], additional presentations such as corticobasal syndrome and progressive supranuclear palsy (PSP). In 2009, Velakoulis et al. [5] presented a post mortem study of young patients, diagnosed ante mortem with psychiatric illnesses including bipolar disorder (BPD) and schizophrenia, and demonstrated the presence of protein deposits [tau or TAR DNA Binding Protein (TDP)43] typical of FTLD brains. Moreover, genetic analysis in one case revealed a GRN mutation. Additional evidence of a clinical overlap between psychiatric disorders and genetically determined FTLD comes from the recent description of a patient with heterossexual pedophilia [6] who was a carrier of a GRN mutation and developed bvFTD over time, and from a second article reporting two clinically different, apparently sporadic FTLD cases sharing the previously described Thr272fs GRN mutation, who had a premorbid BPD history [7]. Basing on the assumption that FTD and schizophrenia might have a common aetiology in some families in which both syndromes coexist, Schoeder et al. [8] analyzed the morbidity risk for schizophrenia in first-degree relatives of 100 FTLD probands and compared it with first-degree relatives of 100 Alzheimer’s disease (AD) relatives, showing that the morbidity risk for schizophrenia was significantly higher in relatives of FTLD probands than in relatives of AD probands. Notably, in one family, a mutation in GRN was found [8]. A major contribution to achieve a correct diagnosis independent of the phenotypic presentation is the demonstration that progranulin plasma levels are extremely low in GRN mutation carriers [1,9–11].

Besides autosomic dominantly inherited GRN mutations, a contribution of GRN genetic variability has been previously shown in sporadic FTLD as well [12], even though another study did not confirm these data [13]. A further association analysis demonstrated that a single nucleotide polymorphism (SNP) in the GRN promoter influences the risk for FTLD [14]. Besides the susceptibility effect, GRN polymorphisms likely influence gene expression. In this regard, Fenoglio et al. [15] demonstrated that rs5848 TT genotype is associated with decreased GRN expression levels in brains and peripheral blood mononuclear cells (PBMC) from patients with AD. GRN is localized in a region of
chromosome 17q21 previously shown to be associated with BPD [16,17] and schizophrenia [18].

Given these premises, we carried out a GRN association study in patients with BPD and schizophrenia compared with controls. In addition, we measured progranulin plasma levels and correlated them with genetic data.

Results

Genetic variation within GRN was analyzed in a German population of 508 patients with schizophrenia and BPD as compared with 574 matched controls (Table 1). Both control and case populations were in HWE for all SNPs studied. Considering each SNP alone, a significantly decreased allelic frequency of the minor versus the wild-type allele was observed for rs2879096 (23.2 versus 34.2%, \( P < 0.001 \), OR:0.63, 95%CI:0.49–0.80, Table 2), rs4792938 (30.7 versus 39.7%, \( P = 0.005 \), OR:0.70, 95%CI:0.55–0.89, Table 2) and rs5848 (30.3 versus 36.8%, \( P = 0.007 \), OR:0.71, 95%CI:0.56–0.91, Table 2). For all three polymorphisms, there seemed to be an additive effect in that the effect was stronger when carrying two polymorphic alleles (see details in Table 2). Stratifying patients according to the diagnosis, a significant association could still be seen in both schizophrenia and BPD (Table 2). Regarding haplotype analysis, none of the selected SNPs were in strong LD (\( D' \) ranging from 0.1 to 0.9, data not shown). Accordingly, none of upper CI values met the criteria for haplotype analysis according to the method used [19].

Progranulin plasma levels were measured in 26 German BPD patients. As one of them had levels below the reference range [9], GRN was sequenced, but no causal mutations were identified. Although exceeding the lower cut-off level, mean progranulin levels in patients were lower than previously published data obtained in Italian controls [20]. We thus evaluated progranulin levels in an independent cohort of Italian volunteers (n = 29) and compared them with German cases (Table 3), again showing a significant difference in means levels ±SEM (180.81 ± 18.39 ng/ml in controls versus 89.69 ± 3.97 ng/ml in patients, \( P < 0.001 \), Figure 1). Similar levels were found also in an Italian population of 61 patients with BPD (116.14 ± 5.90 ng/ml versus controls, \( P < 0.001 \), Figure 1). Progranulin levels were not correlated with age either in patients (\( P = 0.07 \), \( P = 0.568 \) in the Italian population, \( P = 0.17 \), \( P = 0.662 \) in German patients, data not shown) or controls (\( P = 0.11 \), \( P = 0.578 \), data not shown). Stratification according to each of the SNPs studied, no significant differences in progranulin plasma levels were shown (data not shown).

Discussion

To our best knowledge, this is the first evidence that GRN variability decreases the risk to develop BPD and schizophrenia. In addition, progranulin plasma levels are significantly decreased in patients as compared with controls. Despite both the SNPs and progranulin levels were associated with the target phenotypes, no association between such SNPs and progranulin levels were observed. This could be due to a number of reasons, including the possible regulation of translation by microRNA, the interaction of additional variants not included in this study, or the effect of medications taken at time of plasma sampling. Unfortunately, at time of DNA sampling, no matched plasma samples were taken from German controls. Therefore, these preliminary results need a further confirmation in a larger and ethnically matched population.

Progranulin and the various granulin peptides derived by elastase cleavage are implicated in a range of biological functions, including development, wound repair, inflammation and tumorigenesis [21]. Whereas progranulin has anti-inflammatory properties, granulins display pro-inflammatory actions [22]. Our observation that progranulin levels are low in plasma from patients with schizophrenia and BPD could imply that the balance between progranulin and granulins is altered in favour of granulins, that increase the degree of inflammation. A number of findings suggest a role for inflammatory factors in schizophrenia and BPD pathogenesis. Suvisaari et al., [23] analyzed inflammatory markers in psychotic disorders and their association with metabolic comorbidity, antipsychotic medication, smoking, alcohol use, physical condition, and mood, showing that mononuclear phagocyte system was mostly related to metabolic comorbidity and antipsychotic medication use, whereas T-cell activation had a more direct relationship with both psychotic disorders and depressive symptoms. In addition, Interleukin (IL)-6 serum levels were significantly increased in patients with schizophrenia as compared with controls, whereas IL-10 concentration was increased in both patients with schizophrenia and BPD [24].

To date, a number of Genome Wide Association Study (GWAS) have been performed in patients with either schizophrenia or BPD [25]. Among these, an association with 17q21, the locus containing GRN, has been shown in Latino populations with schizophrenia [18] and in patients with BPD [26]. Notably, this locus has been implicated in several other neurological and psychiatric pathologies of the central nervous system, including FTLD (see [27] for review), PSP [28], and autism [29,30], supporting the hypothesis of common pathogenic mechanisms among these diseases. Regarding the occurrence of FTLD and schizophrenia, in support of the hypothesis that these diseases share a common aetiology, Schoder et al. [8] demonstrated that the morbid risk for schizophrenia is significantly higher in relatives of FTLD probands than in relatives of Alzheimer’s disease probands. In three mixed families, a causal GRN mutation was found, even in family members diagnosed with schizophrenia [8]. In addition, TDP43 pathology was found in patients whose first clinical presentation was consistent with schizophrenia or BPD [5]. Regarding our population, we did not have the opportunity to

### Table 1. Characteristics of German individuals included in the association study.

|                | CON     | CASES   | Schizophrenia | BPD     |
|----------------|---------|---------|---------------|---------|
| n              | 574     | 508     | 271           | 237     |
| Gender (M:F:unknown) | 230:341:3 | 229:273:6 | 146:120:5     | 83:153:1 |
| Mean age, yrs ±SEM (range) | 27.4±0.39 (18–68) | 28.30±0.47 (9–72)* | 27.13±0.59 (9–72)* | 30.14±0.76 (14–63)* |

*age at disease onset.

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follow up patients and their families to ascertain the development of dementia.

In our study, the association observed in the whole population was maintained after stratifying in patients with BPD and schizophrenia, suggesting common pathogenic pathways. In line with this hypothesis, two large GWAS in BPD and schizophrenia patients, respectively, lead to the identification of the same susceptibility genes [31,32]. Nevertheless, a replication analysis should be carried out to confirm data described here.

Regarding progranulin levels, we acknowledge that the majority of patients were treated at time of sampling, thus we can’t exclude an influence of therapies on progranulin levels.

In conclusion, we demonstrated that GRN variability contributes to schizophrenia and BPD development and that progranulin

| Table 2. Allelic and genotype frequencies given as % (n) in German cases compared with age-, gender- and ethnicity matched controls. |
|---|---|---|---|
| SNP | n* | Genotype % (n) | Allele % (n) |
| rs2879096 | Controls | 574 | 46.7 (268) | 38.2 (219) | 15.1 (87) | 65.8 (755) | 34.2 (393) |
| | BPD cases | 237 | 59.5 (141) | 37.1 (88) | 3.4 (8)* | 78.1 (370) | 21.9 (104)** |
| | SZ cases | 271 | 57.6 (156) | 36.3 (99) | 5.9 (16)** | 75.8 (411) | 24.2 (131) |
| | All cases | 508 | 58.3 (296) | 37.0 (188) | 4.7 (24)**** | 76.8 (780) | 23.2 (236)**** |
| rs3785817 | Controls | 574 | 51.4 (295) | 39.7 (228) | 8.9 (51) | 71.3 (818) | 28.7 (330) |
| | BPD cases | 237 | 59.1 (140) | 35.0 (83) | 5.9 (14) | 76.6 (363) | 23.4 (111) |
| | SZ cases | 271 | 55.7 (151) | 38.0 (103) | 6.3 (17) | 74.7 (405) | 25.3 (137) |
| | All cases | 508 | 57.1 (290) | 36.8 (187) | 6.1 (31) | 75.3 (767) | 24.5 (249) |
| rs4792938 | Controls | 574 | 38.5 (221) | 43.6 (250) | 17.9 (103) | 70.5 (692) | 28.7 (456) |
| | BPD cases | 237 | 48.5 (115) | 43.9 (104) | 7.6 (18)** | 70.5 (334) | 29.5 (140)** |
| | SZ cases | 271 | 46.5 (126) | 43.9 (119) | 9.6 (26)**** | 68.5 (371) | 31.5 (171) |
| | All cases | 508 | 47.2 (240) | 44.1 (224) | 8.7 (44)**** | 69.3 (704) | 30.7 (312)**** |
| rs9897526 | Controls | 574 | 76.3 (438) | 22.3 (128) | 1.4 (8) | 87.5 (1004) | 12.5 (144) |
| | BPD cases | 237 | 82.7 (196) | 16.0 (38) | 1.3 (3) | 91.1 (432) | 8.9 (42) |
| | SZ cases | 271 | 76.7 (208) | 21.8 (59) | 1.5 (4) | 87.6 (405) | 12.4 (67) |
| | All cases | 508 | 79.5 (404) | 19.1 (97) | 1.4 (7) | 89.1 (767) | 10.9 (111) |
| rs5848 | Controls | 574 | 39.9 (229) | 46.5 (267) | 13.6 (78) | 63.2 (725) | 36.8 (217) |
| | BPD cases | 237 | 48.5 (115) | 43.9 (103) | 7.6 (18)*** | 70.5 (334) | 29.5 (140)** |
| | SZ cases | 271 | 48.7 (132) | 43.9 (119) | 7.4 (20)**** | 70.7 (373) | 29.3 (159)** |
| | All cases | 508 | 48.2 (245) | 42.9 (218) | 8.9 (45)** | 69.7 (708) | 30.3 (308)** |

*P<0.001, OR: 0.20, 95% CI: 0.09–0.14.
**P<0.001, OR: 0.60, 95% CI: 0.44–0.81.
***P=0.0002, OR: 0.35, 95% CI: 0.20–0.61.
****P<0.001, OR: 0.28, 95% CI: 0.17–0.44.
*****P<0.001, OR: 0.63, 95% CI: 0.49–0.80.
P=0.0003, OR: 0.37, 95% CI: 0.22–0.63.
P=0.003, OR: 0.66, 95% CI: 0.49–0.90.
P=0.002, OR: 0.48, 95% CI: 0.31–0.77.
P=0.001, OR: 0.43, 95% CI: 0.30–0.63.
P=0.015, OR: 0.70, 95% CI: 0.55–0.93.
P=0.01, OR: 0.51, 95% CI: 0.30–0.85.
P<0.0001, OR: 0.33, 95% CI: 0.19–0.56.
P=0.019, OR: 0.62, 95% CI: 0.42–0.91.
P=0.007, OR: 0.71, 95% CI: 0.56–0.91.
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| Table 3. Characteristics of Italian (I) and German (G) subjects included in plasma level evaluation. |
|---|---|---|---|
| CON | BPD | BPD |
| | I | I | G |
| n | 29 | 61 | 26 |
| Gender (M:F) | 10:19 | 25:36 | 9:17 |
| Mean age at sampling, yrs±SEM (range) | 67.89±1.83 (50–83) | 52.35±1.63 (20–64) | 42.23±2.78 (23–72) |
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plasma levels are lower in patients with BPD than in controls, although this finding need a replication in a larger cohort.

Materials and Methods

Subjects
Five hundreds and eight patients with psychiatric disorders, including 229 males, 273 females, and 6 gender unknown, mean age±SEM at sampling: 48.99±0.65 years (range 22–85), mean age±SEM at disease onset: 28.30±047 years (range: 9–72) were recruited at the Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Germany. Two hundreds seventy one patients, including 146 males, 120 females, and 5 gender unknown, mean age±SEM at sampling: 47.00±0.94 years (range: 22–85), mean age±SEM at disease onset: 27.13±0.59 years (range: 9–72) were diagnosed with schizophrenia [33], whereas 237 patients including 83 males, 153 females and 1 gender unknown, mean age±SEM at sampling: 51.96±1.00 years (range: 25–82), mean age±SEM at disease onset: 30.14±0.76 years (range: 14–63) were diagnosed with BPD (type 1; n = 103, type 2: n = 78, not defined: n = 56) [34].

The control group consisted of 574 German volunteers matched for ethnic background and age (230 males, 273 females, 3 gender unknown, mean age±SEM: 27.4±0.39 years, range 18–68) and was recruited at the Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Germany. The age of controls did not significantly differ from that of patients’ age at disease onset (P>0.05). The control sample was composed of blood donors, staff members, and volunteers all originating from the Lower Franconia region. The sample was not systematically screened for psychiatric disorders; however, all subjects were free of medication, and the study was explained to them, so that the likelihood of severe psychiatric disorders in the control sample was low. Only subjects who gave written informed consent were enrolled in the study, which complied with the Declaration of Helsinki and was approved by the Ethics Committees of the University of Würzburg.

Plasma samples were collected in 26 German BPD patients, including 9 males and 17 females, mean age±SEM at sampling: 42.23±2.78 years (range 23–72). Additional plasma samples were collected from 61 Italian patients with BPD, including 25 males and 36 females, mean age±SEM at sampling: 32.35±1.63 years (range: 20–64) and 29 controls (10 males and 19 females), mean age±SEM at sampling: 67.89±1.83 years (range: 50–83) at the University of Milan, Policlinico Hospital, Milan, Italy. Psychiatric diagnoses were performed through the administration of a semi-structured clinical interview for a DSM-IV-TR Axis I disorders (SCID-I/P) [35] by trained psychiatrists.

This study has been approved by the Institutional Review board of the Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico. Details of patients and controls are shown in Table 1.

DNA isolation and GRN SNPs analysis
High-molecular weight DNA was isolated from EDTA blood by using a standard de-salting method. DNA samples were aliquoted and stored at −20°C until use. For the association analysis, four optimal tagging SNPs covering the GRN gene were sequenced (details in [14]). In addition, rs5848, located in the 3’UTR and previously shown to influence mRNA transcription through miR-659 regulation [12], was included in the analysis as well.

Tagging SNPs were analyzed by using TaqMan methodology. Each Taqman 5’-nucleotide assay employed 25 ng of genomic DNA as template. Assay-on-demand products, ABI assay IDs: C_15035934_10, C_27482034_10, C_32346749_10, C_25402484_10, C_7452046_10 were used for rs2679096, rs3785017, rs4792938, rs9897526 and rs5848 genotyping respectively. Details of the protocol used are given elsewhere [14].

GRN mutation scanning
The entire open reading frame including the noncoding exon 0 and exon-intron boundaries of exons 1–12 of the GRN gene was sequenced using specific primers, as previously described [36] in one patient with progranulin plasma levels under the normality threshold [9].

Statistical analysis
Allelic and genotypic frequencies were obtained by direct counting. Chi square test was used to test for Hardy Weinberg Equilibrium (HWE, http://www.husdyr.kvl.dk/hmm/kc/popgen/genetik/applets/kitest.htm). Chi² was used for differences in SNP distribution between cases and controls. Bonferroni’s correction was applied. The Odds Ratio (OR) was calculated along with its 95% Confidence Interval (CI). Haploview 3.2 software was used to test for LD and for differences in haplotype distribution between cases and controls. Statistical significance was estimated empirically using the bootstrap function in Haploview. Bootstrap P-values are calculated using 10000 bootstrap samples. Calculation of D’ is based on block definition by Gabriel et al. [19]. Progranulin levels were compared by using the Kurskall-Wallis one way analysis of variance with Dunn’s method for multiple comparisons.

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Author Contributions
Conceived and designed the experiments: DG CF. Performed the experiments: CV FC MS CC ER. Analyzed the data: DG CF. Contributed reagents/materials/analysis tools: BDO SKS JW MN JV CL DGO JK CP LG NB ACA. Wrote the paper: DG AR ES.
References

1. Pietroboni AM, Fumagalli GG, Ghezzi I, Fenoglio C, Cortini F, et al. (2011) Phenotypic heterogeneity of the GRN Asp22fs mutation in a large Italian kindred. J Alzheimers Dis 24(2): 253–259.

2. Neary D, Snowden JS, Gustafsson L, Passant U, Stuss D, et al. (1998) Frontotemporal lobar degeneration. A consensus on clinical diagnostic criteria. Neurology 51: 1546–1554.

3. McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, et al. (2001) Clinical and pathological diagnosis of frontotemporal dementia. Arch Neurol 58: 1003–1009.

4. Graft-Radford NR, Woodduff BK. (2007) Frontotemporal dementia. Seminaries in Neurology 27: 48–57.

5. Velmans D, Walterfang M, Mocellin R, Pantelis C, McLean C. (2009) Frontotemporal dementia presenting as schizophrenia-like psychosis in young people: clinicopathological series and review of cases. Br J Psychiatry 194: 298–305.

6. Rainero I, Rubino E, Negro E, Gallone S, Galimberti D, et al. (2011) Heterosexual Pedophilia in a Frontotemporal Dementia Patient with a Mutation in the Progranulin Gene. Biol Psychiatry 70(9): e83–e84.

7. Cerami C, Marcone A, Galimberti D, Villa C, Scarpini E, et al. (2011) From TDP-43-positive frontotemporal dementia. Human Mol Genet 17(23): 3631–3642.

8. Escamilla M, Hare E, Dassori AM, Peralta JM, Ontiveros A, et al. (2009) A genome-wide search for risk alleles in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. Rev Bras Psiquiatr 33(3): 268–274.

9. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, et al. (2002) The structure of haplotype blocks in the human genome. Science 296(5576): 2225–2229.

10. De Riz M, Galimberti D, Fenoglio C, Piccio LM, Scalabrini D, et al. (2010) Cerebrospinal fluid progranulin levels in patients with different multiple sclerosis subtypes. Neurosci Lett 2010 469(2): 234–236.

11. Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, et al. (2002) Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. Cell 111: 867–878.

12. Ahmed Z, Mackenzie IR, Hutton ML, Dickson DW (2007) Progranulin in frontotemporal lobar degeneration and neuroinflammation. J Neuroinflammation 4: 7.

13. Williams JJ, Craddock N, Russo G, Hamshere ML, Moskvina V, et al. (2011) Most genome-wide significant susceptibility loci for schizophrenia and bipolar disorder reported to date cross-traditional diagnostic boundaries. Hum Mol Genet 20(2): 387–391.

14. Ewold H, Wikman FP, Tерел BM, Buttersen ON, Tоррала MS, et al. (2005) Agromine-wide search for risk genes using homozygosity mapping and microarrays withI,494 single-nucleotide polymorphisms in 22 eastern Cuban families with bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 156B(4): 462–471.

15. Schirmer ER, Schulze K, Wildfogel T, Obel E, Paulsson J, et al. (2011) Evidence for sex-specific risk alleles in autism spectrum disorder. Am J Hum Genet 71: 1117–1123.

16. Goose BF, Hutton M (2008) Refining frontotemporal dementia with parkinsonism linked to chromosome 17. Arch Neurol 65: 460–461.

17. Williams HJ, Craddock N, Russo G, Hamshere ML, Moskvina V, et al. (2011) Localization of dishinibition-dementia-parkinsonism-amyotrophy complex to 17q21-22. Am J Hum Genet 55: 1139–1165.

18. Stone JL, Merriam B, Cantor RM, Yoon AI, Gilliam TC, et al. (2004) Identification of sex-specific risk alleles in autism spectrum disorder. Am J Hum Genet 75: 1117–1123.

19. Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011) Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near OX24. Nat Genet 45(10): 977–983.

20. The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011) Genome-wide association study identifies five new schizophrenia loci. Nat Genet 43(10): 967–976.

21. Reif A, Herterich S, Strobel A, Eldis AC, Saur D, et al. (2006) A neuronal nicotinic acetylcholine (NOS2) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 11(3): 286–300.

22. Weber H, Kittel-Schneider S, Gessner A, Domschke K, Neuner M, et al. (2011) Evidence for sex-specific risk alleles in bipolar disorder reported to date cross-traditional diagnostic boundaries. Hum Mol Genet 20(2): 387–391.