Association of rs7688285 allelic variation coding for *GLRB* with fear reactivity and exposure-based therapy in patients with panic disorder and agoraphobia

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Abstract

The gene coding for glycine receptor β subunits (*GLRB*) has been found to be related to panic disorder and agoraphobia (PD/AG) and to be associated with altered insular BOLD activation during fear conditioning, as an intermediate phenotype of defensive system reactivity in healthy subjects. In a multicenter clinical trial on PD/AG patients we investigated in three sub-samples whether *GLRB* allelic variation (A/G; A-allele identified as «risk») in the single nucleotide polymorphism rs7688285 was associated with autonomic (behavioral avoidance test BAT; n=267 patients) and neural (differential fear conditioning; n=49 patients, n=38 controls) measures, and furthermore with responding towards exposure-based cognitive behavioral therapy (CBT, n=184 patients). An interaction of genotype with current PD/AG diagnosis (PD/AG vs. controls; fMRI data only) and their modification after CBT was tested as well. Exploratory fMRI results prior to CBT, revealed A-allele carriers irrespective of diagnostic status to show overall higher BOLD activation in the hippocampus, motor cortex (MC) and insula. Differential activation in the MC, anterior cingulate cortex (ACC) and insula was found in the interaction genotype X diagnosis. Differential activation in ACC and hippocampus was present in differential fear learning. ACC activation was modified after treatment, while no overall rs7688285 dependent effect on clinical outcomes was found. On the behavioral level, A-allele carriers showed pronounced fear reactivity prior to CBT which partially normalized afterwards. In sum, rs7688285 variation interacts in a complex manner with PD/AG on a functional systems level and might be involved in the development of PD/AG but not in their treatment.

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1. Introduction

Anxiety disorders constitute the most prevalent group of mental disorders and are a leading cause of disability with high individual and societal burden (Gustavsson et al., 2011; Wittchen et al., 2011). In order to optimize their treatment and foster preventive approaches, it is necessary to better understand the underlying pathogenetic mechanisms and the potential of cognitive behavioral therapy (CBT) to affect these (Teachman et al., 2012). Twin studies demonstrate a heritability of anxiety disorders with 30%–50% of the individual variability to be explained by genetic factors (Gordon JA 2004; Shimada-Sugimoto et al., 2015). When specific candidate genes are identified, their functional relevance on multiple levels of analysis according to the Research Domain Criteria (RDoC) approach (Insel et al., 2010; Kozak and Cuthbert, 2016) is a research priority. Recently, the field of "therapygenetics" was introduced, where the association of genetic variants with (non-pharmacological) therapy outcome is studied and which adds a further layer of analysis.

A recent genome-wide association study (GWAS) (Deckert et al., 2017) found evidence for an association between the gene encoding the glycine receptor β subunit (*GLRB,* which plays an important role in the regulation of postsynaptic inhibition in neurotransmission and is involved in defensive motor reflex circuits (Lynch, 2004) and categorical (panic disorder (PD)) as well as dimensional (agoraphobia (AG)) characteristics of fear/anxiety, increased startle responses and neural indicators of defensive responding (Deckert et al., 2017). Specifically the rs7688285 single nucleotide polymorphism (SNP) was associated with *GLRB* expression changes and phenotypically showed the most robust impact. On a neural level, *GLRB* and rs688285 were associated to increased insular activation during fear conditioning as an intermediate phenotype of defensive system reactivity which could be replicated in two further, independent healthy control samples

(Lueken et al., 2017).

Defensive responses consist of phylogenetically old and adaptive behavioral adjustments (e.g., startle, fight, flight and freezing) and according autonomic reactivity (e.g., increased heart rate during flight) that prepare an organism to defend itself or flee a potentially harmful situation. Higher-level cognitive systems can modulate the engagement of these responses resulting in more complex behaviors such as prospective avoidance of potentially threatening situations (Mobbs et al., 2015). Together, defense responses are associated with a diverse neurofunctional system consisting of the cortical forebrain (e.g., (pre-)motor, prefrontal and anterior cingulate cortex; ACC), the insula, limbic (e.g., amygdala, hippocampus) and midbrain structures (Carvalho et al., 2010; Fanselow 1994; Mobbs et al., 2009; Wendt et al., 2017). These behaviors are modulated by learning experiences, especially fear conditioning. Through this process, the organism learns to discriminate between signals predicting threat (conditioned stimulus (CS) that is followed by an unconditioned stimulus (US); CS+) and cues predicting safety (CS that is never paired with an US; CS-) and integrates it in a way that eventually the CS+ alone evokes defensive reactions (e.g., freezing) to cope with the upcoming threat (Fullana et al., 2016; Lonsdorf et al., 2017; Sehlmeyer et al., 2009; Wendt et al., 2017).

Based on the initial findings of *GLRB*, we aimed to further explore the role of rs7688285 allelic variation in context of current PD/AG diagnosis and its treatment on multiple response levels (symptom reports, autonomic responding and neural data). Due to the current lack of studies on the intermediate *GLRB*-phenotype in the patient population, exploratory analyses on existing samples are a starting point to pave the way for future research on systematic conducted larger samples. Thus, we aimed to provide initial insights on a translational level. In line with previous research (Deckert et al., 2017; Lueken et al., 2017), we expected a) A-allele carriers (previously identified as carriers of genetic «risk»; Deckert et al., 2017; Lueken et al., 2017) to show signs of

enhanced defensive autonomic responding in a behavioral test as well as altered fear processing on a neural level in a fear conditioning and extinction task. Furthermore, aiming to translate findings from healthy subjects to the clinical population, we hypothesized that b) genotype would interact with current diagnosis in terms of a more pronounced effect in patients than in healthy subjects. Finally, investigating the potential of CBT as first-line treatment to modify bio-behavioral «risk» signatures as observed prior to treatment, we explored whether c) genotype interacts with the response toward exposure-based CBT in which dysfunctional defensive reactivity is modified by corrective fear-inhibitory learning in PD/AG patients (therapygenetic effect).

2. Experimental procedures

Participants

Participants represent sub-samples (behavioral avoidance test (BAT): n=267 patients AA/AG n=91, GG n=176; CBT completer: n=184 patients, AA/AG n=61, GG n=122; fMRI pre: n=49 patients AA/AG n=13, GG n=36, n=38 healthy controls/HC, AA/AG n=17, GG n=22; fMRI post**:** n=39 patients: A/AG n=9, GG n=30; n=29 HC, A/AG n=13, GG n=16; Supplementary Figure SF1) from the randomized controlled multicenter trial «Mechanism of Action in CBT» (MAC) with a total number of 369 enrolled patients (Gloster et al., 2009; Gloster et al., 2011). This study was part of the national research network PANIC-NET funded by the German Federal Ministry of Education and Research. The study with all of its subprojects was approved by the respective local ethical committees; written informed consent of all participants was obtained. Detailed information about inclusion and exclusion criteria, clinical assessment, treatment procedure, and measures of quality control for fMRI data can be found elsewhere (Gloster et al., 2009; Gloster et al., 2011; Straube et al., 2014) and in the Supplementary Methods 1.1-1.3. Patients were free from psychotropic medication, not related, and fulfilled a diagnosis of PD/AG according to DSM-IV-TR criteria (American Psychiatric Association 2000) as diagnosed by the Composite International Diagnostic Interview (Wittchen HU, Garczynski E, Pfister H. 1997) (CIDI). Treatment consisted of 12 sessions of standardized exposure-based CBT (Supplementary Methods 1.2). The Structured Interview Guide for the Hamilton Anxiety Scale (SIGH-A) (Shear et al., 2001) served as the primary of five outcome measure with reductions in symptom severity of at least 50% defining a clinically meaningful response (Gloster et al., 2009; Gloster et al., 2011) (Supplementary Methods 1.4).

Genotype data of patients were previously included in the GWAS study (Deckert et al., 2017) as one of two independent validation samples for *GLRB* locus identification and the baseline (t1) fMRI sample of controls was included in Lueken et al. (2017) as one of two independent samples. The

present work provides new data in terms of genotype associated fMRI, BAT and clinical outcomes in patients focused on the multilevel longitudinal aspect by investigating the therapygenetic effects of *GLRB* and the potential of CBT to modify bio-behavioral «risk» signatures.

Genotyping

Genotyping of rs7688285 on blood samples was performed with Sequenom´s MassArray® system (Sequenom, San Diego, CA, USA), as recommended by the manufacturers and described previously (Deckert et al., 2017). While Deckert at al. (2017) identified four SNPs (rs7688285, rs78726293, rs191260602, rs17035816) associated with dimensional or dichotomous agoraphobic phenotypes, we chose to pick rs7688285 for our analyses for three major reasons: 1) rs7688285 had by far the strongest association with the phenotype at the categorial level (PD/AG patients vs. HC), 2) in our sample only rs7688285 had a distribution that allowed to differentiate reasonably between genetic subgroups (see Supplementary Methods 1.7), 3) we could avoid the problems of multiple testing, by focusing on the most promising candidate SNPs. The rs7688285 SNP is a marker for the *GRLB* gene located on chromosome 4q31-34 (Deckert et al., 2017; Kaabi et al., 2006). According to Deckert et al., (2017), the minor A-allele of rs7688285 is associated with increased risk for the dichotomous agoraphobia phenotype based on the Symptom Checklist-90 and goes along with increased mRNA expression of *GLRB* in human midbrain tissue post mortem. Carriers of at least one A-allele were thus defined as carriers of this genetic «risk» compared to GG-homozygotes.

Psychophysiological data acquisition and analysis during BAT

Patients were assessed in a standardized behavioral avoidance test (BAT) consisting of an exposure to a small, dark and closed test chamber prior to (t1) and after CBT (t2). The procedure is described in detail elsewhere (Hamm et al., 2016; Richter et al., 2012) and in the Supplementary

Methods 1.5. The duration of tolerated exposure was obtained as index of behavioral fear response with a) *passive avoidance:* patients completely avoiding to enter the chamber, b) *active avoidance:* patients who first entered the chamber but left it before completing the intended exposure duration of 10 minutes and c) *no avoidance*. After each phase (anticipation/exposure/recovery), the intensity of experienced anxiety and panic symptoms were assessed by paper and pencil immediately on a 10-point Likert scale ranging from 1 (not at all) to 10 (very strong). During the entire test, the electrocardiogram (ECG) was measured as described elsewhere (Reif et al., 2014) in patients showing active or no avoidance .

To test the effect of genotype, reported fear levels and heart rate we conducted a mixed-model of variance including genotype as between-subjects factor and BAT phase as within-subject factor. The presence of avoidance behavior (active vs. no avoidance) was included as between-subjects factor to control for the effects of active avoidance (Richter et al., 2012). To test the genotype depended effect of CBT on BAT reactivity we additionally included the within-subject factor time (t1 vs. t2).

fMRI data acquisition and analysis

A previously validated (Reinhardt et al., 2010) fear conditioning and extinction task was applied: during the acquisition phase (A), the US (white noise) and one of two previous neutral stimuli (colored sphere) were paired (reinforcement rate of 50%) to become the fear related conditioned stimulus (CS+) while the other stimulus was never paired and consequently acquired safety signal properties (CS-). In the extinction phase (E), both CS were presented again without the US. Preprocessing and first level analyses followed previous publications (Kircher et al., 2013; Lueken et al., 2017; Reif et al., 2014) (see also Supplementary Methods 1.5). On group level, only those trials in which no US was delivered during acquisition were analyzed to avoid an overlap with

neuronal activation directly related to the presentation of the US (Kircher et al., 2013).

A flexible factorial design including gender, age, years of education, center and BDI value as covariates of no interest was used to examine activation differences during presentation of CS+ and CS- separately for A (early, late) and E between A-allele carriers and GG-homozygotes in patients and controls. At t1, these contrasts of interest were calculated: (1) main effect of genotype to reveal the general influence of «risk» status on brain activation, (2) interaction between genotype and diagnosis to test for the diagnosis specific influence of genotype, (3) interaction between genotype and CS-type to test for the differential learning effects depending only on genetic «risk», (4) interaction between genotype, CS-type and diagnosis to investigate the effects of genotype on CS-processing in the presence of current psychopathology. Finally, using a separate flexible factorial analysis, including patients' and controls pre- and post-treatment data, we explored if neural signatures related to the A-allele genotype and diagnosis in patients were modified after CBT by testing in patients (1) the interaction between genotype and time (t1, t2) and (2) between genotype, time and CS-type (see also Supplementary Methods 1.6).

In accordance with previous analyses (Kircher et al., 2013; Lueken et al., 2017; Reif et al., 2014), a Monte Carlo simulation at threshold $p<0.005$ (uncorr.) and a minimum cluster size of 142 contiguous voxels was used to correct for multiple comparisons at $p<0.05$ for all contrasts of interest. Clusters were localized using the Anatomy Toolbox v1. Post hoc small volume corrections of the amygdala were performed using the masks of the Automated Anatomical Labeling (aal) implemented in SPM5. Beta values from significant peak voxels of these clusters were extracted for bar graph visualization.

3. Results

Sample characteristics

Genotype dependent sample characteristics for all subsamples are given in the Supplementary Table S1. Before treatment, patients with and without A-allele did not differ in demographic and clinical characteristics suggesting comparable severity of panic/agoraphobic and depressive symptoms. Genotype distribution did not deviate from the Hardy-Weinberg equilibrium. Among the fMRI sample, patients and controls only differed in education but not in their neuropsychological characteristics. Patients scored higher than controls in the Anxiety Sensitivity Index (ASI) and the Beck Depression Inventory II (BDI II).

Psychophysiological assessment during BAT

Detailed results are reported in Table S2a. In short, genotype was not associated with the frequency of active and passive avoidance behavior or mean duration of BAT exposure during pre-treatment assessment. In those patients entering the test room, A-allele carriers reported more pronounced fear during BAT exposure relative to phases of anticipation and recovery as compared to GG-homozygotes (Figure 1a) and irrespective of avoidance behavior. Increased fear reports went along with a higher heart rate acceleration from last minute of anticipation to first minute of BAT exposure in A-allele carriers relative to GG-homozygotes (Figure 1b), again in both active- and non-avoiders. This genotype associated modulation was specific for fear activation during exposure as overall heart rate did not differ between genotype groups across all BAT phases.

Please insert Figure 1 here

Neural correlates of fear conditioning/fMRI

Detailed results are reported in Table 1.

Main effects of GRLB. Across groups (PD/AG vs. controls) and stimulus types (CS+/CS-), an overall main effect of higher activation of the A-allele genotype was observed in the left hippocampus, bilateral motor cortex (MC) and bilateral insula (Figure 2a).

*Interaction effect genotype X diagnosis. A*cross both stimulus types, an overall interaction between genetic A-allele genotype and group (PD/AG vs. controls) was found in the bilateral anterior and middle cingulate cortex (ACC/MCC), left insula (located more anterior than the cluster identified in the main effect) and right MC. A-allele carrying controls showed higher activation, while A-allele carrying patients exhibited a reverse pattern (Figure 2b).

Interaction effect genotype X CS. Regarding the A-allele effects on differential conditioning, an interaction between stimulus type and genotype across groups during early acquisition was found in the left amygdala. Over both groups (PD/AG and controls), the A-allele genotype showed higher activation during CS- versus CS+ presentation (Figure 2c).

Interaction effect genotype X diagnosis X CS. An overall interaction effect between the A-allele, diagnosis and stimulus type was found in the left hippocampus. During *early acquisition,* diagnosis specific effects of the A-allele genotype depending on stimulus type were found in the right MC, midline ACC, MCC, and bilateral hippocampal regions. A-allele carrying versus GG-homozygote patients showed higher activation to CS+ than CS- in these regions, whereas controls showed the reverse pattern. During *late acquisition*, more activation for CS- > CS+ was found in the right amygdala of A-allele carrying patients, whereas controls showed the reverse pattern (Figure 2d).

During *full extinction* an interaction was found in the bilateral MC where A-allele carrying versus GG-homozygote patients showed higher activation to CS+ than CS-; controls showed the reverse pattern.

Please insert Table 1 here

Please insert Figure 2 here

Modification of GLRB related processes after CBT

Treatment response

As reported in detail elsewhere (Gloster et al., 2011), primary outcomes improved considerably during treatment. Dimensional and responder analyses revealed that symptom reduction did not differ depending on rs7688285 genotype (see supplementary materials: Results 2.1 and Table S3).

Psychophysiological assessment during BAT

Detailed results are reported in Table S2b. We found an overall decrease of avoidance behavior during BAT after treatment, as indicated by an increase of tolerated exposure duration from t1 to t2 in pre-treatment avoiders. However, this decrease did not differ between genotype groups. In those patients entering the test chamber during both assessments, mean heart rate was found to decrease from t1 to t2 in the GG-variation but not in the A-allele group (Figure 1c) over all BAT phases and irrespective of avoidance behavior. Reported fear decreased from t1 to t2 which did not differ between genotype groups.

Neural correlates of fear conditioning/fMRI

Detailed results are reported in Table 2. Analyses are focused on the patient group only to reduce complexity. To further test the group specificity of the clusters found in patients, we calculated ANOVAs based on extracted individual parameter estimates (using the VOI function of SMP5).

Interaction effect genotype x time. Analysis across pre- and post-treatment showed that activation related to the A-allele genotype was altered following CBT overall in the ACC, MCC and visual

processing areas. A-allele carriers vs. GG-homozygotes showed increased activation in the cingulate cortex after CBT (Figure 3).

Interaction effect genotype x time X CS. The interaction between genotype, time and stimulus type revealed higher activation in the motor cortex during acquisition post-treatment for A-allele carriers towards CS+ than CS-. Tthis effect seemed to be treatment unspecific, because it was found in controls as well.

Please insert Table 2 here

Please insert Figure 3 here

4. Discussion

This study investigated the role of rs7688285 allelic variation coding for *GLRB* expression and PD/AG pathophysiology in context of defensive responding. Across autonomic and neural levels, we explored how neural intermediate phenotypes of genetic variants are associated with the presence of current PD/AG diagnosis and the potential of exposure-based CBT to act upon these multilevel (patho-)physiological «risk» signatures.

The following main results were obtained: First, we found general genotype dependent effects in key brain regions related to fear conditioning and extinction as well as on the autonomic level of defensive responding. Second, and unlike initial expectations, the neurofunctional signatures associated with *GLRB* A-allele genotype and *GLRB* X stimulus type found in healthy subjects – mainly the anterior insula (Deckert et al., 2017; Lueken et al., 2017) – were not further amplified in patients with current diagnosis, as A-allele carrying patients showed several reverse patterns of neurofunctional activation that was more comparable to GG-homozygote controls. Third, the neural and autonomic signatures in patients were partly modified after CBT treatment. Noteworthy, results regarding treatment response revealed no pronounced differences as a function of rs7688285 genotype.

In line with previous findings from partly independent data-sets (Deckert et al., 2017; Lueken et al., 2017) (see participants section), we found increased insular activity over A-allele carrying patients and controls within a cluster in the mid insula. Further, increased activity in the hippocampus and MC was found to be associated with allelic variation. However, we found the A-allele presence to interact with the current presence of PD/AG diagnosis. Insular activation in an anterior cluster, hippocampus, motor cortex and cingulate activation was differentially associated with allelic variation and diagnostic status. This interaction revealed reverse patterns in patients vs. controls:

the neural signature in the patient A-allele group was more comparable to GG-homozygote controls. This leads to the yet preliminary assumption that intermediate phenotypes as identified in healthy subjects (Lueken et al., 2017) cannot simply be extrapolated to clinical groups in terms of a linear relation. Instead, the relationship between genotype and the presence of current clinical diagnosis might be more complex, possibly following an inverted u-shaped function (Cools and D'Esposito 2011; Vijayraghavan et al., 2007; Waal and Preston 2017).

Differential fear learning was also found to be associated with rs7688285 allelic variation and diagnosis. During early acquisition, A-allele carrying patients showed more activity toward the threat (CS+) than to the safety signal (CS-) in bilateral hippocampal regions, whereas controls showed the reverse pattern – as previously reported for the independent healthy sample 1 in Lueken et al. (2017). As early acquisition is crucial for establishing the association between CS+ and aversive stimuli (Kircher et al., 2013), and considering the hippocampus to be highly involved in memory formation (Rothschild et al., 2017), its higher activation toward CS+ could indicate higher priority of encoding threat. A similar activation pattern was also found in the motor cortex. As *GLRB* plays an important role in defensive motor reflex circuits (Lynch 2004), the motor cortex activation might indicate top-down involvement of voluntary motor control to react to potentially harmful stimuli reflecting intentional defensive behavior in response to an approaching threat. In contrast, on the level of amygdala reactivity, higher activation was associated with enhanced safety signal (CS-) processing during late acquisition in A-allele carrying patients but not in A-allele carrying controls. However, during early acquisition, A-allele carriers over both groups showed higher amygdala activation toward the CS-, in line with the independent healthy sample 1 in Lueken et al. (2017). Again, that supports the idea of potential non-linearity in phenotypes in the context of current diagnosis.

During BAT, A-allele carrying patients demonstrated elevated fear reactivity as reflected by more

pronounced fear ratings and autonomic arousal compared to GG-homozygotes. This genotype effect was limited to the exposure phase suggesting fear specific effects evoked by proximal threat (Hamm et al., 2016). In line with the previous observation of increased fear potentiated startle during BAT (Deckert et al., 2017), we found the *GLRB* A-allele genotype to be associated with heightened defensive reactivity possibly preparing for a behavioral fight/flight response. However, we did not find increased tendency for active or passive avoidance suggesting that an open display of this kind of behavioral fear response depends on additional factors (Helbig-Lang et al., 2014).

Concerning the expected influence of *GLRB* genotype on the response to CBT, treatment effects were not found to be affected by allelic variation on the level of reported symptom severity. On the neural level, however, reactivity in the cingulate cortex was partly modified after CBT: the general genotype associated effect in A-allele carriers was reversed, whereas the differential fear learning effect during early acquisition in the ACC and hippocampus was found to be unaltered after CBT. Furthermore, the previously reported activation reduction in the inferior frontal gyrus (Kircher et al., 2013) was not associated with genotype (Supplemental Results 2.1). Our findings of increased overall activation of the cingulate cortex, confirm this region to be a sensitive area in terms of activation changes from prior to post treatment in anxiety and depressive disorders (Dunlop et al., 2017; Lueken et al., 2016; Siegle et al., 2012). The cingulate cortex is crucial for fear expression, attention and motor control, functionally connected with the amygdala, anterior insula and hippocampus (Bush et al., 2000) and plays an important role in fear regulation (Etkin et al., 2011). Our pre-treatment data suggest that patients carrying the A-allele activate more of these neurofunctional resources when processing threat (CS+), while controls carrying the A-allele are more focused on safety signals (CS-). This also underlays the assumption of non-linearity in the interaction of phenotype and current psychopathology.

During post-treatment BAT, we observed unaltered heart rate responses in A-allele carrying

patients, while a decrease from the pre-treatment assessment was observed in the GG-homozygote group. Persistent autonomic arousal during the fear challenge suggests still strongly pronounced physiological fear reactivity (Hamm et al., 2016; Richter et al., 2012) after treatment. Hence, in A-allele carrying patients, CBT might be effective in reducing avoidance behavior, but fail to normalize exaggerated physiological responding during threat. Future research needs to test whether residual defensive reactivity might favor symptom relapse on the long run.

Several limitations must be considered. Since genetic variance cannot be manipulated in human samples, the relationship between investigated data and allelic variation in rs7688285 must be correlative. Additionally, diagnosis also represents an unrandomized factor. Therefore, it is possible that PD/AG diagnosis and altered fear reactivity are confounded by sharing causes. In this case, the descriptive potential of our results is nevertheless informative. Furthermore, our results have to be interpreted with caution and fMRI results must be classified as exploratory because of the small sample sizes in the genotype subgroups especially when conducting interaction analyses. We cannot exclude that our results could either represent false positive effects or that important differences might have been missed due to false negative findings. However, although this sample is likely underpowered, the clinical relevance of these exploratory significant results on the rs7688285 associated neural activation in PD/AG is high. Complex analyses are needed to provide at least preliminary information about group specific activation to better understand the role of genes in the complex environment of factors possibly influencing psychopathologies. Another limitation is possible selectivity of the patient sample. Since participation was based on voluntary consent information and the fMRI-setting itself can be afflicted with anxiety, we cannot exclude that some patients – especially with additional claustrophobia – deliberately avoided participation. This could have lead to a potentially not representative sample regarding the severity of anxiety or functional level. The more valuable however, is the data at hand of at least a small part of a

patient-group that is difficult to investigate in the fMRI. Our data benefit from coming from a large and controlled clinical trial which makes them valuable. Further longitudinal investigations are needed on how risk factors contribute to the development of PD/AG and how the brain is shaped by both genetic risk and environmental factors (e.g., life events/learning) (Kuhn et al., 2016). This is of particular relevance in clarifying the complex transition from (neural) endophenotypes to the development of current psychopathology and is feasible in ongoing clinical multicenter trials (Heinig et al., 2017).

To conclude, we consider our findings to support the hypothesis of the rs7688285 SNP coding for *GLRB* being associated with PD/AG. We provide preliminary evidence that it represents a risk factor for altered fear reactivity on a physiological and neurofunctional level and that it interacts with the presence of current PD/AG diagnosis in a rather complex way. A-allele carrying patients showed more pronounced autonomic fear reactivity during BAT and altered fear processing on a neural level. A modification of these (dysfunctional) signatures seems to be possible but not exhaustive. Noteworthy, we found no evidence that the genotype affects treatment success on clinical (symptom reduction) or behavioral (BAT avoidance) levels. This suggests that rs7688285 allelic variation might be involved in the development of anxiety disorders but not necessarily in their modification via CBT. Our results may help to expand the knowledge of rs7688285 function on intermediate phenotypes. So far, the A-allele is supposed to be the risk factor. Our data however suggest first evidence, that this «risk»-allele as identified in subclinical populations may reverse its mechanism of action in PD/A patients. Interactions between genetic «risk»-variants, normal and pathological forms of fear processing and its modification by psychological interventions seem to be complex and not necessarily linear. Thus – although preliminary – our findings can provide an important contribution to the yet young field of gene x environment interactions and intermediate phenotypes, especially to the newly investigated *GLRB* in the context

of anxiety disorders. Future systematic research on larger samples of the patient-population is needed to clarify if the rs7688285 A-allele becomes a resilience factor in patients with current PD/AG at least on a neural systems level.

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Figure legends

Figure 1. Main effect of *GLRB* genotype on BAT outcome measures in PD/AG patients. A: Main effect of *GLRB* genotype on reported fear (F(2,468)=3.33, p=.04; A: n=91, G: n=176) at t1. **B:** Heart rate increase from last minute of anticipation phase to first minute of exposure phase (F(1,205)=5.69, p=.02; A: n=68, G: n=141) at t1 **C:** decrease of heart rate from t1 to t2 during BAT exposure phase (F(1,125)=5.08, p<.05; A: 39, G: n=90). A: carriers of at least one A-allele, GG: GG-homozygotes; Δbpm: deviation of beats per minute. See also Supplementary Table ST2.

Figure 2. *GLRB* genotype associated BOLD activation in fear conditioning and extinction at baseline. A: carriers of at least one A-allele: PD/AG n=13, controls n=17, GG: GG-homozygotes: PD/AG n=36, controls n=22; PD/AG: diagnosis of panic disorder and agoraphobia: HC: healthy control subjects; CS+: conditioned stimulus that is followed by the unconditioned stimulus (US) with a reinforcement rate of 50% (only unpaired CS+ were included in the analyses; CS-: conditioned stimulus that is never followed by an US). **A:** Main effect of *GLRB* genotype during full course of fear conditioning (MNI coordinates x, y, z: 16, -18, 52, 155 voxels, t=3,35, p<.001; -44, 4, 2, 337 voxels, t=3.37, p<0.001; -20, -42, 8,275 voxels, t=3,76, p>0.001). **B:** Interaction between *GLRB* genotype and presence of diagnosis during full course of fear conditioning (MNI coordinates x, y, z: 52, 4, 42, 535 voxels, t=3,79, p<.001; -34, 16, 14, t=3.68, p<.001; -10, 22, 24, t=3.65, p<0.001). **C:** Interaction between *GRLB* genotype and stimulus type (CS+ = threat; CS- = safety signal) during early acquisition (MNI coordinates x, y, z: -26, 4, -18, 53 voxels, t=3.33, p=.0.006, small volume correction using Automated Anatomical Labeling (aal) masks, family-wise error correction at p<0.05). **D:**

Interaction between *GRLB* genotype, presence of PD/AG diagnosis and stimulus type during early and late acquisition (MNI coordinates x, y, z: 54, -24, 56, 911 voxels, t=3.75, p<0.001; 6, 10. 28, 3rd maximum of an 6006 voxels cluster, t=4.23, p=<0.001, -22. -14 -28, 199 voxels t=3.75, p<0.001; 34, 0 -28, 27 voxels, t=3.42, p=0.005, small volume correction using aal masks, family-wise error correction at p<0.05). Bar graphs illustrate the contrast estimates (arbitrary units (a.u.)) of activation. Error bars indicate the s.e.m. in all cases. Peak voxels of identified clusters based on the "Overlap between structure and function" of the Anatomy Toolbox v1.5 and the "Cluster Labeling" of the aal implemented in SPM5 are given. See also Table 1.

Figure 3. *GLRB* genotype associated BOLD activation in fear conditioning and extinction after CBT. A: carriers of at least one A-allele: PD/AG n=9, GG: GG-homozygotes n=30; CS+: conditioned stimulus that is followed by the unconditioned stimulus (US) with a reinforcement rate of 50% (only unpaired CS+ were included; CS-: conditioned stimulus that is never followed by an US. **A:** Overall interaction effect between *GLRB* genotype and time in patients (MNI coordinates x, y, z: 10, 28, 34, 2082 voxels, t=3.95, p<0.001). Bar graphs illustrate the contrast estimates (arbitrary units (a.u.)) of activation. Error bars indicate the s.e.m. in all cases. Peak voxels of identified clusters based on the "Overlap between structure and function" of the Anatomy Toolbox v1.5 and the "Cluster Labeling" of the Automated Anatomical Labeling implemented in SPM5 are given. See also Table 2.