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DISSERTATION

Potential Biomarkers of Schizophrenia - Three Genetic Studies

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Content

Abstract

The role of genetics in psychiatric disorders is well established, and yet, the complexity of the genetic basis for most of these disorders creates major challenges in this research area. Therefore, investigating biological markers has been an important approach in psychiatric genetic research for several decades. This dissertation presents three genetic studies dealing with possible biomarkers in different domains of human psychophysiology. The first study explored the impairment of early visual processing and its link to cholinergic system. Single nucleotide Polymorphism (SNP) rs904952 of cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) on the performance on Backward Masking Task was found to be of significant influence (p=0.021). A second study investigates the role of dystrobrevin binding protein 1 (DTNBP1) in the regulation of cognitive processes, finding a significant influence of DTNBP1 (rs909706) on attention (p=0.030). In the third study, the effect of inetr-alpha-trypsin inhibitor heavy chain 3 (ITIH3) (rs2535629) on treatment response in schizophrenic patients was considered. Results suggest that the improvement of negative symptoms under clozapine treatment might be regulated by ITIH3 (rs2535629). These results once again underline the importance of the cholinergic system in the pathogenesis of schizophrenia, as well as the important role of DTNBP1 in regulation of cognitive processes. Furthermore, it adds evidence to the growing body of knowledge that individualized treatment for psychotic disorders might be the logical next step. Taken together, the results of these studies outline the real complexity of psychiatric disorders and stimulate the need for further research.

Abstract

Die Rolle der Genetik in der Entstehung psychiatrischer Erkrankungen ist seit langem bekannt. Die Komplexität der genetischen Grundlage der meisten psychiatrischen Erkrankungen erschwert jedoch die Forschung. Deswegen hat die Identifikation von so genannten Endophenotypen die genetische Psychiatrieforschung der letzten Jahrzehnte geprägt. Die vorliegende Dissertation stellt eine Zusammenfassung von drei genetischen Studien dar, die

möglichen Biomarker verschiedener Domänen der menschlichen Psychophysiologie untersucht haben. Ein Zusammenhang der frühen visuellen Verarbeitung mit dem cholinergem System wurde untersucht. Es wurde ein signifikanter Einfluss von cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) (rs904952) auf frühe visuelle Verarbeitung gefunden (p=0.021). In der zweiten Studie wurde die mögliche Rolle von dystrobrevin binding protein 1 (DTNBP1) in der Regulierung von kognitiven Prozessen untersucht und ein signifikanter Einfluss von DTNBP1 auf Aufmerksamkeit gefunden (p=0.030). In der dritten Studie wurde die Rolle von inetr-alpha-trypsin inhibitor heavy chain 3 (ITIH3) (rs2535629) hinsichtlich der Therapieantwort auf antipsychotische Medikation bei Schizophreniepatienten untersucht. Es zeigte sich ein statistisch signifikanter Einfluss auf die Besserung von Negativsymptomen unter der Behandlung mit Clozapin. Die Ergebnisse dieser Studien zeigen die Bedeutung des cholinergen Systems in der Pathogenese der Schizophrenie und die Rolle von DTNBP1 in der Regulation kognitive Funktionen. Außerdem leisten sie einen Beitrag zu der Erforschung individualisierter Behandlung psychiatrischer Krankheiten. Insgesamt unterstreichen diese Ergebnisse die Komplexität der genetischen Architektur psychiatrischer Störungen und stimulieren weitere Forschung.

Introduction

The role of genetics in the development of psychiatric diseases has been extensively studied in recent decades. The heritability of spectrum disorders is considered to be 41-86% (Kendler, 1983; Sullivan et al., 2003). Psychotic disorders are polygenic diseases that cannot be explained in the classical Mendelian way. The complexity of the phenotype and the involvement of many different genes complicate the research. Therefore, the need for biological markers with circumscribed phenotypic expression and simple genetic outline was evident. The concept of endophenotypes was adopted for schizophrenia in 1967 (Gottesman and Shields, 1967). Gottesman and Gould (2003) define

endophenotypes as markers that are: 1. associated with the disease in the population, 2. are heritable,3. are state-independent (manifest in every state of disease), 4. Cosegregate within families affected by the disease, and 5. occur in non-affected family members at a higher rate than in a general population (Gottesman and Gould, 2003).

In current genetic studies, we investigated the potential endophenotypes from different domains and in the case of the third study, a biological marker in a broader sense. We further search for their links to known candidate genes.

The nicotine system has been long associated with schizophrenia. A number of studies suggest the correlation of certain genetic variants with the diagnosis of schizophrenia (Leonard et al., 2002; Stassen et al., 2000; Stephens et al., 2009). The cholinergic nicotine system is strongly associated with the gating system (Brinkmeyer et al., 2011; Chen et al., 2011; Millar et al., 2011), and is one of the well-studied endophenotypes of schizophrenia (Dawson et al., 2000; Perry et al., 2001). Acetylcholine is specifically linked to visual processing. Its modulatory function has been outlined in different reviews and studies (Groleau et al., 2015; Kang et al., 2014; Logemann et al., 2014). Impairment in visual processing among schizophrenia patients has been shown in multiple studies using Visual Backward Masking (VBM)(Jahshan et al., 2014; Perez et al., 2012). VBM with a shine-through masking paradigm revealed significantly impaired visual processing not only in schizophrenia patients, but also in their first-degree relatives compared to healthy controls. The impairment in relatives was present at a rate between patients and healthy controls (Chkonia et al., 2010) underlining the strong genetic component of the deficit. In order to further investigate the role of the cholinergic system in the regulation of early visual processing, in our first study we examined the association of cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) gene polymorphisms with the performance in VBM shine-through paradigm.

Cognitive impairment in psychiatric patients has shown high heritability (Greenwood et al., 2007). The unaffected first-degree family members of

schizophrenic patients show poorer performance on cognitive tasks compared to control subjects (Faraone et al., 2000; Hilti et al., 2010), herewith showing a strong genetic component. Therefore, cognitive functions are considered a potential endophenotypes for schizophrenia.

Dystrobrevin binding protein 1 (DTNBP1) is one of the most well-studied and promising candidate genes of schizophrenia. The association of DTNBP1 with schizophrenia has been shown in multiple studies (Funke et al., 2004; Li et al., 2005; Pae et al., 2009; Rethelyi et al., 2010; Riley et al., 2009; Schwab et al., 2003). There is a body of evidence suggesting an association of DTNBP1 with cognitive functions in healthy individuals (Markov et al., 2010; Wolf et al., 2011) and psychotic patients (Baek et al., 2012; Varela-Gomez et al., 2015). Memory appears the most frequently reported cognitive domain to be associated with DTNBP1 (Alfimova et al., 2010; Donohoe et al., 2007; Hashimoto et al., 2009). Nevertheless. the variety of different tasks used across all studies makes the interpretation of findings difficult. In the second study presented in this dissertation, we tested the association of six Single Nucleotide Polymorphisms (SNPs) in DTNBP1 with four cognitive domains which were constructed based on the outcome of multiple cognitive tests.

Genome Wide Associations Studies (GWAS) were recently installed in genetic research where a genome-wide set of polymorphisms is compared across large samples comprising thousands of subjects. Psychiatric disorders, as mentioned above, are polygenic diseases where multiple genes with small effect size play a role. Therefore, GWAS is particularly important in psychiatric genetic research; it is able to detect the polymorphisms with small effect sizes, which would be "invisible" in studies with smaller sample sizes. One of the genes seeming highly significant in a recent large scale GWAS study of five psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013) is inter-alpha-trypsin inhibitor heavy chain 3 (ITIH3). The association with schizophrenia was confirmed in a case-control study by Sasayama et al., 2014 (Sasayama et al., 2014). The significant association of ITIH3 and the nearby chromosomal region with suicidal behavior in schizophrenic and bipolar patients (Finseth et al., 2014)

suggests an important role of this region in the pathogenesis of psychotic disorders.

Treatment response is a major challenge in psychiatric disorders and can be viewed as a biomarker of psychiatric disorders. Previous research suggests a link to the genetic background of treatment response. Polymorphisms in AHI1, CACNA1C, NRXN1, COMT and DRD2 showed significant influence on treatment response when using different antipsychotic medication (Chen et al., 2015; Kang et al., 2015; Lett et al., 2011; Porcelli et al., 2015a; Porcelli et al., 2015b). In the third study, we examined whether ITIH3 plays a role in a treatment response to antipsychotic medication.

The aim of the presented studies is to contribute to a better understanding of the complex genetic architecture of psychotic disorders. We examined potential biomarkers in different neurocognitive domains such as early visual processing and cognitive functioning. We also investigated whether the genetic polymorphisms can potentially modulate treatment response in psychiatric disorders

Methods

Participants and procedures

Study 1: Two samples were investigated. The first consisted of 224 schizophrenic patients recruited at the Department of Psychiatry and Psychotherapy, Charité, Berlin, Germany and 224 matched healthy controls recruited in general population. The second consisted of 50 schizophrenic patients recruited at Asatiani Psychiatric Institute, Tbilisi, Georgia, their 57 first-degree relatives and 51healthy controls recruited in the general population. In the German sample, we tested the association of CHRNA7 polymorphisms with the diagnosis of schizophrenia. In the Georgian sample, the association with the diagnosis as well as the association with the performance on VBM was examined.

In both patient collectives, substance abuse and comorbid psychiatric or organic diseases were exclusion criteria. For relatives in the Georgian sample and healthy controls in both samples any axis one or two diagnosis were exclusion

criteria. All participants gave informed consent and both studies were approved by the ethical committees of the corresponding institutions.

All participants of the Georgian sample were tested on visual acuity and underwent the testing on visual backward masking with the shine-through paradigm. In the shine-through masking paradigm, the subjects are presented vernier stimulus, two vertical bars which are slightly offset either to the left or to the right. After vernier, the masking stimulus follows, which is a 25 aligned vernier stimuli without offset. After the presentation of both stimuli, the subject should indicate the direction of the offset of vernier stimulus. As an outcome, variable Stimulus Onset Asynchrony (SOA) was measured.

Study 2: A sample of 91 schizophrenic patients recruited at the Department of Psychiatry and Psychotherapy, Charité, Berlin, Germany was examined. Patients with substance abuse and comorbid organic diseases were excluded from the study. All participants gave informed consent. The study was approved by the ethical committee of the Charité. All participants were tested with an extensive test battery for cognitive functions.

Study 3: Four different samples designated as A (N=89), B (N=88), C (=45) and D (N=34), constituted a total of 256 individuals with schizophrenia or schizoaffective disorder were recruited in Germany and USA at different sites. Sample A received various antipsychotics and received follow up for 6 weeks. Sample B received clozapine and received follow up for six months. Sample C received clozapine, risperidone, olanzapine or haloperidol for 14 weeks in a double-blind trial. Sample D received clozapine for 6 months. The treatment response was assessed with the Brief Psychiatric Rating Scale (BPRS). All participants gave informed consent. The study was approved by the local ethical committees of each corresponding recruitment site.

Genetic analyses

The genetic analyses for **studies 1** and **2** were performed in the Genetics Section, Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Berlin, Germany. Approximately 30ml blood samples of each individual were used. DNA extraction was made using standard high-salt method (Lahiri and Nurnberger, 1991). For **Study 1,** five SNPs of CHRNA7 (rs3826029, rs2337506, rs982574, rs904952, rs2337980) and for **Study 2** six SNPs of DTNBP1 (rs909706, rs1018381, rs2619522, rs760761, rs2619528, rs1011313) were genotyped.

Primers were designed and the relevant DNA regions were amplified by polymerase chain reaction (PCR). The PCR products were cut by allele-specific restriction enzymes and visualized after gel electrophoresis.

For **Study 3,** blood samples of each subject were collected. DNA was extracted using the standard high-salt method (Lahiri and Nurnberger, 1991). One SNP (rs2535629) of ITIH3 was genotyped using TaqMan Assay (Applied Biosystems Inc., Foster City, CA).

Statistical analysis

Study 1: The statistical analysis was conducted using SPSS v. 16.0.

For the analysis of categorical data, like allelic variants and sample groups, the Chi-square test was used. For quantitative, normally distributed data such as demographic parameters, *F* statistics was used. For quantitative non-normally distributed data, like SOA Kruskal-Wallis and Mann-Whitney tests were applied.

Study 2: The statistical analysis was conducted using SPSS v. 17.0. For preselection of the initial 33 outcome variables, the two-tailed Pearson's correlation was used. Eleven selected variables were then included in factorial analysis. Varimax rotation with an Eigenvalue cut-off set to 1.0 was used. Four cognitive domains were derived. Then, a multivariate analysis of variance (MANOVA) with genotype as the between-group factor was used to measure the association between Polymorphisms in DTNBP1 and these cognitive domains.

Study 3: The statistical analysis was conducted using SPSS v. 22.0. For continuous variables, Analysis of Covariance (ANCOVA) was used.

For categorical variables, Pearson's $\chi 2$ or Fisher's exact test were applied. There were significant differences in minor allele frequencies among patients of European or African-American descent; therefore all association tests were performed separately among ethnicities.

Results

Study 1: We found a significant association of the rs904952 with the diagnosis of schizophrenia in the German sample ($X^2(1)=6.37$; p =0.009) with T being a risk allele for schizophrenia. The result remained significant after the Bonferroni correction. As found in previous research (Chkonia et al., 2010), there were significant differences between patients, relatives and controls in SOA, with relatives presenting between patients and controls (H[2]=68.3, $p < 0.0001$). In the patient group, the rs904952 significantly affected the SOA. Carriers of T/T genotype performed significantly worse compared to carriers of C/T and C/C genotypes (U = 5, p = 0.021). None of the other SNPs were significantly associated with SOA in any group.

Study 2: We identified two cognitive domains, which were predicted by a genetic polymorphism in DTNBP1. The carriers of A/A genotype of SNP rs909706 showed poorer performance on set-shifting (G/G vs. A/A $\beta = -0.31$, p = 0.004; and G/A vs. A/A $\beta = -0.12$, p = 0.243) and sustained attention (G/G vs. A/A $\beta = -0.24$, p = 0.018 and G/A vs. A/A $\beta = 0.18$, p = 0.076) tasks compared to A/G and G/G carriers. After a correction for multiple testing, the association with attention remained significant ($F(2, 88) = 5.39$, p = 0.006, p corr = 0.030) whereas the set-shifting became a non-significant trend ($F(2, 88) = 4.69$, p = 0.012, p corr =0.060). None of the other SNPs and none of the tested haplotypes were significantly associated with cognitive functions.

Study 3: A significant influence of a genotype on treatment response was found. Carriers of A/A genotype on rs2535629 showed greater improvement of negative symptoms under clozapine treatment than carriers of A/G and G/G

genotypes among European ancestry. ($F_{1,87}=8.8$, $p=0.004$, $p_{corr}=0.032$).

Discussion

In the presented three studies, we investigated potential biomarkers of psychotic disorders, early visual processing, cognitive functioning and treatment response.

The various results of the Study 1 are interesting. We replicated the results of previous studies (Leonard et al., 2002; Stassen et al., 2000; Stephens et al., 2009) and once again showed the association of CHRNA7 with a diagnosis of schizophrenia in the German sample. In the Georgian sample, we observed the same trend, however the association was insignificant, presumably because of the small sample size. Chkonia (Chkonia et al., 2010) reported impaired early visual processing in psychotic patients and their relatives. In the Georgian sample in our study, we found the same result. Patients showed the worst performance on a visual backward masking task followed by relatives and healthy controls, thereby outlining the strong genetic component of the phenomenon. Finally, we found a significant association of the same rs904952 with early visual processing. The carriers of T/T genotype performed worst on VBM Task compared to carriers of C/T and C/C genotypes. However, this result was seen only in the patients group.

Although the present study is limited by its small sample size, it does provide additional evidence to the existing body of research regarding the role of cholinergic nicotine system and the impairment of early visual processing in psychotic disorders. Although replication is needed, our results may provide insight into underlying genetic mechanisms of early visual processing, thereby stimulating a need for further research.

In Study 2, we found a significant association between sustained attention and a polymorphism in DTNBP1. Set-shifting showed an insignificant trend after correcting for multiple testing. Unlike most reports from similar studies, we found no significant association of memory with DTNBP1. This may be due to the type of memory investigated in our study compared to previous research. The verbal memory examined in our study was reported only by Alfimova et al. (2010) and was measured in healthy individuals. Again, the small sample size is

another limitation of our study.

The exact mechanisms with which DTNBP1 regulates cognitive functions must be explored; however, it has been linked with the glutamatergic system. Talbot et al. (2004) (Talbot et al., 2004) found decreased DTNBP1 protein in hippocampal glutamatergic neurons of schizophrenic patients. Glutamate itself is an important neurotransmitter involved in the regulation of a wide range of cognitive functions (Bustillo et al., 2011; Klamer et al., 2011). Another line of evidence links DTNBP1 with visual processing, schizophrenia patients carrying risk haplotype of DTNBP1 showed poorer early visual processing compared to non-risk haplotype carriers in the study of Donohoe et al., (2008)(Donohoe et al., 2008). This last finding provides a link with the results of the Study 1. It's possible that in the regulation of early visual processing not only cholinergic system plays a role, as Study 1 showed, but the glutamatergic system and the DTNBP1 as part of it, could be involved as well.

In Study 3, we found an impact of ITIH3 on treatment response in schizophrenic patients. SNP rs2535629 was significantly associated with an improvement of negative symptoms under clozapine treatment. The effect was seen in the European ancestry of samples B and D. Positive psychotic symptoms respond well on antipsychotic medication whereas negative symptoms are difficult to treat and are mostly a long lasting burden for patients (Tsapakis et al., 2015). Therefore, it is particularly important to increase the understanding of the underlying biological and genetic mechanisms of negative symptoms. Our results provide additional insight in the possible role of ITIH3 in psychiatric disorders, in particular with regard to treatment response to clozapine. Furthermore, it suggests some practical implications in the treatment of psychotic disorders, like an early switch to clozapine in subjects with particular ITIH3 genotype.

Conclusion

Overall, the results of these three studies outline: 1. A strong genetic background of early visual processing and its link to the cholinergic system; 2. The possible

regulatory function of DTNBP1 and thereby the glutamatergic system on attention in psychotic patients; 3. A potential regulatory function of ITIH3 on treatment response of negative symptoms in psychotic patients. Schizophrenia patients are often known to be heavy smokers (Dalack et al., 1998; de Leon et al., 1995; de Leon and Diaz, 2005; Lasser et al., 2000)(nicotine affects cholinergic system) and nicotine is proven to improve attention in schizophrenia patients (Hahn et al., 2013; Morisano et al., 2013). Hence, based on the combined results of Studies 1 and 2, together with previous research, it can be concluded that both the cholineric and glutamatergic systems are involved in the regulation of attention and early visual processing. The regulatory role of these two neurotransmitter systems on the same two phenotypic traits suggests a new hypothesis, that there may be an association between attention and early visual processing, thereby provoking a call for new research. For example, the possible role of impaired early visual processing in the negative symptoms of schizophrenic patients is specifically interesting in its possible link to attention deficiency. Furthermore, it might be interesting to investigate the possible role of ITIH3 in the regulation of the cognitive processes in schizophrenia patients.

Research of the past few decades clearly shows the importance of genetics in the pathogenesis of psychiatric disorders. Further genetic research could reshape the current classification systems of psychiatric disorders like ICD-10 and DSM-V, which are based on phenotypic expression, shifting it to a more pathophysiologically-based classification. Also, there is considerable genetic potential in the treatment of psychiatric disorders. Individualization of treatment based on specific genetic profiles could spare patients unnecessary drug trials resulting in unfavorable side effects and delaying effective treatment. Additionally, treatment costs could be significantly reduced.

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Eidesstattliche Versicherung

„Ich, George Bakanidze, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: "Potential Biomarkers of Schizophrenia - 3 genetic studies" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Datum 11.09.2017 Unterschrift

<u>Anteilserklärung an den erfolgten Publikationen</u>

George Bakanidze hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1:

Bakanidze, G., Roinishvili, M., Chkonia, E., Kitzrow, W., Richter, S., Neumann, K., Herzog, M.H., Brand, A., Puls, I., 2013. Association of the Nicotinic Receptor alpha7 Subunit Gene (CHRNA7) with Schizophrenia and Visual Backward Masking. Frontiers in psychiatry 4, 133.

Planung der Studie, Genotypisierung, Statistische Analyse,
Manuskriptentwurf, Fertigstellung der Publikation

Publikation 2:
Bakanidze, G., Brandl, E.J., Hutzler, C., Aurass, F., Onken, S., Rapp, M.A., Puls, I., 2016. Association of Dystrobrevin-Binding Protein 1 Polymorphisms with Sustained Attention and Set-Shifting in Schizophrenia Patients. Neuropsychobiology 74(1), 41-47

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Publikation 3:
Brandl, E.J., Lett, T.A., Chowdhury, N.I., Tiwari, A.K., Bakanidze, G., Meltzer, H.Y., Potkin, S.G., Lieberman, J.A., Kennedy, J.L., Muller, D.J., 2016. The role of the ITIH3 rs2535629 variant in antipsychotic response. Schizophrenia research 176(2-3), 131-135.

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Curriculum Vitae

Aus datenschutzrechtlichen Gründen wird mein Lebenslauf in der elektronischen Version der Dissertation nicht veröffentlicht.

Komplette Publikationsliste

1. Bakanidze, G., Roinishvili, M., Chkonia, E., Kitzrow, W., Richter, S., Neumann, K., Herzog, M.H., Brand, A., Puls, I., 2013. Association of the Nicotinic Receptor alpha7 Subunit Gene (CHRNA7) with Schizophrenia and Visual Backward Masking. Frontiers in psychiatry 4, 133.

2. Bakanidze, G., Brandl, E.J., Hutzler, C., Aurass, F., Onken, S., Rapp, M.A., Puls, I., 2016. Association of Dystrobrevin-Binding Protein 1 Polymorphisms with Sustained Attention and Set-Shifting in Schizophrenia Patients. Neuropsychobiology 74(1), 41-47.

3. Brandl, E.J., Lett, T.A., Chowdhury, N.I., Tiwari, A.K., Bakanidze, G., Meltzer, H.Y., Potkin, S.G., Lieberman, J.A., Kennedy, J.L., Muller, D.J., 2016. The role of the ITIH3 rs2535629 variant in antipsychotic response. Schizophrenia research 176(2-3), 131-135.

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