Schizophrenia Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

A network of synaptic genes associated with schizophrenia and bipolar disorder

Diego A. Forero [a,b,c,d,e,1], Liesbet Herteleer [c,d,1], Sonia De Zutter [a,b], Karl-Fredrik Norrback [f,g], Lars-Göran Nilsson [h], Rolf Adolfsson [f,g], Patrick Callaerts [c,d,*], Jurgen Del-Favero [a,b,**]

[a] Applied Molecular Genomics Unit, Department of Molecular Genetics, VIB, Belgium
[b] University of Antwerp, Antwerp, Belgium
[c] Laboratory of Behavioral and Developmental Genetics, VIB, Belgium
[d] Catholic University of Leuven, Leuven, Belgium
[e] Laboratory of NeuroPsychiatric Genetics, School of Medicine, Universidad Antonio Nariño, Bogotá, Colombia
[f] Department of Clinical Sciences, Division of Psychiatry, University of Umeå, Umeå, Sweden
[g] Sunderby Hospital, Sweden
[h] Department of Psychology, Stockholm University, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 8 May 2015
Received in revised form 4 February 2016
Accepted 5 February 2016
Available online xxxx

Keywords:
Genomics
Synaptic genes
Psychiatric genetics
Neural plasticity

ABSTRACT

Identification of novel candidate genes for schizophrenia (SZ) and bipolar disorder (BP), two psychiatric disorders with large epidemiological impacts, is a key research area in neurosciences and psychiatric genetics. Previous evidence from genome-wide studies suggests an important role for genes involved in synaptic plasticity in the risk for SZ and BP. We used a convergent genomics approach, combining different lines of biological evidence, to identify genes involved in the cAMP/PKA/CREB functional pathway that could be novel candidates for BP and SZ: *CREB1*, *CREM*, *GRIN2C*, *NPY2R*, *NF1*, *PPP3CB* and *PRKAR1A*. These 7 genes were analyzed in a HapMap based association study comprising 48 common SNPs in 486 SZ, 351 BP patients and 514 control individuals recruited from an isolated population in Northern Sweden. Genetic analysis showed significant allelic associations of SNPs in *PRKAR1A* with SZ and of *PPP3CB* and *PRKAR1A* with BP. Our results highlight the feasibility and the importance of convergent genomic data analysis for the identification of candidate genes and our data provide support for the role of common inherited variants in synaptic genes and their involvement in the etiology of BP and SZ.

1. Introduction

Schizophrenia (SZ) and bipolar (BP) disorder are severe psychiatric disorders, affecting approximately 1% of the population worldwide. They are complex disorders affected by genetic and environmental factors (Craddock and Sklar, 2013; Owen, 2012). Despite their high heritability, the identification of genetic risk factors and biological pathways for BP and SZ has proven difficult and the pathological mechanisms that lead to disease remain to be established (Alaerts and Del-Favero, 2009; Allen et al., 2008; Craddock and Sklar, 2013; Owen, 2012; Seifuddin et al., 2012). However, even though associations for individual genes have been uncovered, the results from these studies are not conclusive and reveal the genetic heterogeneity and complexity of BP and SZ (Allen et al., 2008; Craddock and Sklar, 2013; Owen, 2012). This observation is further reinforced by the recent results of the SNP based genome-wide association studies for BP and SZ (Chen et al., 2013; Ripke et al., 2013). Recent studies have highlighted that schizophrenia and bipolar disorder and several other psychiatric disorders share risk loci implicating common biological pathways in the disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013; Cross-Disorder Group of the Psychiatric Genomics et al., 2013; Network and Pathway Analysis Subgroup of Psychiatric Genomics, 2015).

Previous evidence from genome-wide studies suggests an important role for genes involved in synaptic plasticity in the risk for SZ and BP (Hall et al., 2014). Specifically, results from recent SNP and CNV chip experiments and exome sequencing studies show multiple alterations in genes involved in signaling pathways at the postsynaptic density, such as the N-methyl-D-aspartate receptor signaling complex and the cAMP/PKA/CREB functional pathway (Hall et al., 2014).

As part of an integrated effort towards identifying disease genes for psychiatric disorders, and given the apparent significance of focusing on biological pathways (Cross-Disorder Group of the Psychiatric Genomics, 2013; Cross-Disorder Group of the Psychiatric Genomics et al., 2013; Network and Pathway Analysis Subgroup of Psychiatric Genomics, 2015), we used a convergent genomics approach (Ogden et al., 2004;

* Correspondence to: P. Callaerts, Laboratory of Behavioral and Developmental Genetics, VIB, Belgium.
** Correspondence to: J. Del-Favero, Applied Molecular Genomics Unit, Department of Molecular Genetics, VIB, Belgium.
E-mail addresses: Patrick.Callaerts@cme.vib-kuleuven.be (P. Callaerts), jurgen.delfavero@molgen.vib-ua.be (J. Del-Favero).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.schres.2016.02.012
0920-9964/

Le-Niculescu et al., 2009; Ayalew et al., 2012) for the identification of novel candidate genes for BP and SZ, focused on tagging SNPs located in genes that are involved in the cAMP/PKA/CREB functional pathway. In total, 7 novel genes were analyzed in detail using a HapMap based SNP association study, comprising 351 BP patients, 486 SZ patients and 514 control individuals, all from Northern Swedish origin.

2. Materials and methods

2.1. In silico analysis of functional candidate genes

UniHI and *KEGG* servers (Chaurasia et al., 2007; Kanehisa et al., 2008) were used for the retrieval and identification of protein–protein interaction networks and signaling pathways that are related to the cAMP/PKA/CREB pathway (Collins et al., 2006; Grant, 2012; Kandel, 2001).

The *GNF SymAtlas* (Su et al., 2004) was used to obtain expression data of the functional candidate genes in brain tissues. The *Stanley Brain database* (Higgs et al., 2006) was queried to retrieve differential expression data of BP and SZ postmortem human brains. The data from two previous linkage meta-analyses for BP and SZ were used to identify genes in genome-wide linked regions (Lewis et al., 2003; Segurado et al., 2003).

2.2. Subjects

All participants are Caucasians and originate from a geographically isolated population living in Northern Sweden. In total 351 BP patients, 486 SZ patients and 514 healthy controls were included in this study. The mean ages at examination were 56.3 and 58.5 years and male percentages were 47.2 and 65.3% for BP and SZ patients, respectively. Patients were evaluated by experienced psychiatrists and diagnosis was made according to DSM-IV criteria. Healthy controls were selected randomly from a longitudinal population-based study (the Betula project) and screened for history of psychotic events; their mean age at examination and M/F ratio were similar to those of the patients. The control group was recruited from the same geographical region of Northern Sweden as the patients and there was no evidence of population stratification in these samples (using the structure program for the analysis of several unlinked microsatellites). More information about these cohorts and the advantages of this isolated population for gene mapping can be found in previous publications (Alaerts et al., 2009; Strazisar et al., 2014). All subjects signed an informed consent and the project was approved by the institutional ethics committees of the Universities of Umeå and Antwerp.

2.3. Selection and genotyping of SNPs

In order to cover as much as possible of the genetic variation of the selected genes we used the CEU genotype data from the *HapMap* database (Consortium, 2005). htSNPs were chosen as predicted by Haploview (confidence interval minima for strong LD; upper: 0.9 and lower: 0.65; upper confidence interval maximum for strong recombination: 0.9; fraction of strong LD in informative comparisons must be at least: 0.9; exclude markers with a MAF < 0.01). Only haplotypes with an estimated overall frequency of 5% or greater were considered in the selection analyses. Tagging SNPs not covered with the htSNPs selection were added using the r2 option of *Tagger* (using a cut-off point of r2 = 0.8 and minor allele frequencies > 0.01) SNPs located in regions with a high repeat content were excluded. The genomic location of the 48 tagging SNPs analyzed in the present work is presented in Table S1.

Genomic DNA was extracted from peripheral blood using standard methods. The genotyping of all SNPs was performed using the MassARRAY iPLEX Gold technology (Sequenom Inc., San Diego, California), following the protocol provided by Sequenom (http://www.sequenom.com). The PCR and extension primers were designed using Assay Design 3.0 (Sequenom Inc). Analysis and scoring were performed using Typer 3.3 (Sequenom Inc). All genotypes were manually checked by two independent researchers and internal controls showed a good consistency of genotype results.

2.4. Statistical analysis

The *PLINK and UNPHASED* programs (Dudbridge, 2008; Purcell et al., 2007) were used for Hardy–Weinberg equilibrium (HWE) analysis, patient-control comparisons of single SNPs (using a χ^2 test) and haplotype analysis (using 1000 permutations). Gene-wide haplotype determination and patient-control comparisons were carried out using the *Haploview* and *UNPHASED* programs (using 1.000 permutations) (Barrett et al., 2005; Dudbridge, 2008). Empirical p values for single SNPs were derived by permutation using *PLINK and UNPHASED* programs (Dudbridge, 2008; Purcell et al., 2007). Identification of gene–gene interactions was performed using the *MDR* program (Hahn et al., 2003). Visualizations of haplotype block structures and linkage disequilibrium (LD) patterns were carried out using the *Haploview* program (Barrett et al., 2005).

3. Results

A literature search and database analysis (Chaurasia et al., 2007; Kanehisa et al., 2008; Kuhn et al., 2009) were performed to identify genes known to be key factors in the cAMP/PKA/CREB pathway (Fig. 1) (Kandel, 2001). Those genes were further selected for brain expression and being located in genome-wide significant linkage regions as well as postmortem expression changes in BP and SZ brains (Table 1). The 7 selected genes are: *CREB1* (cAMP responsive element binding protein 1), *CREM* (cAMP responsive element modulator), *GRIN2C* (glutamate receptor, ionotropic, N-methyl D-aspartate, 2C), *NF1* (neurofibromatosis 1), *NPY2R* (neuropeptide Y receptor Y2), *PPP3CB* (protein phosphatase 3, catalytic subunit, beta isoform) and *PRKAR1A* (protein kinase, cAMP-dependent, regulatory, type I, alpha) (Table 1).

To analyze the role of these novel candidate genes as susceptibility factors for BP and SZ in humans, we performed a detailed HapMap based SNPs association analysis in a well-characterized sample of BP and SZ samples recruited from Northern Sweden. In total we genotyped 48 SNPs in these 7 genes in a sample comprised of 351 BP patients, 486 SZ patients and 514 controls. All SNPs were in HWE and the MAFs and structures of haplotype blocks and LD profiles were similar to those found in the HapMap CEU samples (data not shown) (Consortium, 2005).

Allelic association analysis showed significant results for the BP sample with a SNP in *PPP3CB* (rs12775630; p = 0008) and *PRKAR1A* (rs4968898; p = 0009). Also for the SZ sample *PRKAR1A* was associated with 2 SNPs (rs8066131; p = 0004 and rs8076465; p = 0,03) (Fig. 2; Tables S2 and S3). HapMap based haplotype block analysis as well as sliding window haplotype analysis revealed significant associations with *NF1* (p = 0,01), *PPP3CB* (p = 0005) *and PRKAR1A* (p = 0,03) in the total BP and SZ samples (Fig. 3; Table S4).

Stratification of the association samples by gender, age at onset and bipolar I/bipolar II status showed several more significant associations, some of them showed stronger associations in specific subtypes for SNPs that were also significant in the total sample: rs4968898 in *PRKAR1A* had p = 0009 for the total BP sample and p = 0002 for BP type I patients only. Other SNPs in *PPP3C*B or in the other candidate genes appeared as significant only in specific phenotypic subgroups (rs3793663/*PPP3CB* with BP type II; rs12507396/*NPY2R* with SZ in female patients and rs9893241/*GRIN2C* for BP patients with an earlier onset) (Fig. 4 and Table S2).

Gene–gene interaction analysis on this set of candidate genes showed that a combination of SNPs in *PRKAR1A* and *PPP3CB* had a significant association with BP: rs3785906 in *PRKAR1A* combined with rs12571454 in *PPP3CB*(p = 0.0001, Fig. 5).

Please cite this article as: Forero, D.A., et al., A network of synaptic genes associated with schizophrenia and bipolar disorder, Schizophr. Res. (2016), http://dx.doi.org/10.1016/j.schres.2016.02.012