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Research report

The microtubule-associated molecular pathways may be genetically disrupted in patients with Bipolar Disorder. Insights from the molecular cascades

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ABSTRACT

Bipolar Disorder is a severe disease characterized by pathological mood swings from major depressive episodes to manic ones and vice versa. The biological underpinnings of Bipolar Disorder have yet to be defined. As a consequence, pharmacological treatments are suboptimal. In the present paper we test the hypothesis that the molecular pathways involved with the direct targets of lithium, hold significantly more genetic variations associated with BD. A molecular pathway approach finds its rationale in the polygenic nature of the disease. The pathways were tested in a sample of ~7000 patients and controls. Data are available from the public NIMH database. The definition of the pathways was conducted according to the National Cancer Institute (http://pid.nci.nih.gov/). As a result, 3 out of the 18 tested pathways related to lithium action resisted the permutation analysis and were found to be associated with BD. These pathways were related to Reelin, Integrins and Aurora. A pool of genes selected from the ones linked with the above pathways was further investigated in order to identify the fine molecular mechanics shared by our significant pathways and also their link with lithium mechanism of action. The data obtained point out to a possible involvement of microtubule-related mechanics.

1. Introduction

Bipolar Disorder (BD) is a mental disease characterized by pathological mood swings, both depressive and manic. It affects ~1% of the population world-wide and results in high economic and societal costs for the communities in which patients live (American Psychiatric Association, 2000). Pharmacological treatment is considered necessary for BD (American Psychiatric Association, 2002), even if the present treatments present some (major) flaws. First the efficacy of drugs is limited: up to one third of subjects, and more than half of patients diagnosed with BD may experience relapses during their lifetime despite treatment. Further, the drugs that are in use today (mood stabilizers, second-generation antipsychotics and antidepressants), though generally effective in the short term, do not dramatically change the natural history of the disorder (Angst and Sellaro, 2000). Finally, the appearance of severe side effects is a somewhat common experience. The absence of a detailed knowledge about the biological background of BD is one of the reasons for the lack of a highly efficient and/or specific drug treatment. Consistently, we are witnessing a shift of the scientific paradigm of the biological basis of BD from the monoamines to a more comprehensive picture of biological pathways involved with neurodevelopment, neurodegeneration and in general with the normal functioning of the brain (Berk et al., 2011). For decades the investigation of monoamines did not yield a solid breakthrough for the understanding of BD, and now that the perspective is changing, pressed by the findings form genome-wide investigations among others, we are still waiting for a consistent biological understanding of BD. Thus, it is still impossible to engineer a drug based on consistent biological evidence about the pathophysiology of BD. Genetics holds the potential to unravel the genes that harbor the variations that significantly segregate in patients versus healthy controls. This field has been extensively and completely reviewed recently (Sullivan et al., 2012). Things are really far to be elucidated though, and this field of research appears to be still in its infancy. Moreover, the brain proved to be complex to investigate and the polygenic nature of BD further hinders any potential biological understanding of the disorder. Finally, both rare and common variations may be involved in the

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Table 1
Sample under analysis.

Sample	Ancestry	Case (*n*)	Case ♂ (*n*)	Case ♀ (*n*)	Control (*n*)	Control ♂ (*n*)	Control ♀ (*n*)
Trinity College Dublin	Irish	150	72	78	797	236	561
University of Edinburgh	Scottish	282	121	161	275	141	134
Pritzker Neuropsychiatric Disorders Research Consortium	European–American	1130	426	704	718	366	352
Systematic Treatment Enhancement Program for Bipolar Disorder (STEP1)	European–American	922	418	504	645	336	309
Systematic Treatment Enhancement Program for Bipolar Disorder (STEP2)	European–American	659	281	378	191	79	112
Thematically Organized Psychosis (TOP) Study	Norwegian	203	83	120	349	176	173
University College London (UCL)	British	457	182	275	495	212	283
Grand total	/	**3803**	**1583**	**2220**	**3470**	**1546**	**1924**

pathophysiology of BD (Sullivan et al., 2012), therefore the investigation of very large samples is needed to tell apart the noise from the true signal, and to extrapolate the impact of rare variations. For such a reason, during the recent years some international consortiums joined forces to create the larger genetic samples ever seen for psychiatric diseases, BD included. Some of these datasets are available for international researchers to be investigated. In the present paper we studied ~7000 BD patients and controls from the public NIMH dataset (Table 1). We used a molecular pathway analysis to investigate the sample, a technique which proved to be more powerful compared to single SNPs analysis and which takes into account the polygenetic nature of the disorder in a more specific way compared to common genome-wide investigations (Holmans, 2010). For the identification of biological pathways possibly involved with BD we choose to use, as starting point, the molecular pathways involved with lithium, given its relevance for the treatment of BD. We examined several molecular networks related to these elements under the hypothesis that the pathways involved in the pharmacodynamics of lithium could explain, at least in part, the pathophysiology of the disorder.

A case-control mega-sample was used to test the hypothesis that the molecular networks derived from lithium's molecular targets are also involved in the genetic risk of BD. This process entails a cross-relation between the pharmacodynamics of a benchmark drug for the treatment of a disease, and its genetic background. This cross-relation was not thoroughly previously demonstrated in literature and the analysis process that is undertaken in the present work is therefore to be considered as exploratory.

2. Materials and methods

2.1. Datasets

Genetic and phenotypic data were available from the NIMH (https://www.nimhgenetics.org). Table 1 reports the characteristics of the samples under investigation. 3803 cases and 3470 controls were analyzed.

2.2. Quality control of single datasets

Quality control was performed on genotypes generated by various GWAS platforms, with quality control conducted separately using a common approach.

The SNPs successfully genotyped in each study and common to all platforms were pruned to remove high LD and lower frequency SNPs and were then used for relatedness testing in each sample and in the combined total sample.

Common quality control parameters were applied: (i) missing rate per SNP < 0.05 (before sample removal below), (ii) missing rate per individual < 0.02, (iii) missing rate per SNP < 0.02 (after sample removal above), (iv) missing rate per SNP difference in cases and controls < 0.02, (v) SNP frequency difference to HapMap < 0.15, and (vi) Hardy–Weinberg equilibrium (controls) $P > 1 \times 10^{-6}$.

Samples size varied between ~150 and ~1200 individuals. The number of SNPs per study after quality control varied between 250,000 and 680,000.

On average, the quality control processes excluded 15 individuals per study (with a range of 0–100 individuals) and 38,000 SNPs per study (with a range of 5000–160,000 SNPs).

The pool of eligible SNPs relatedness testing and population structure analysis, were further pruned to remove LD (leaving no pairs with $r2 > 0.05$) and lower frequency SNPs (minor allele frequency < 0.05).

2.3. Genetic quality control

Genetic quality control included relatedness testing and principal components analyses. Identical subjects (pi-hat > 0.9) were identified and one was included after random choice. Relatives (pi-hat > 0.2) were excluded as well, by choosing randomly one representative. Principal component estimation was performed with the non-related subset of individuals. The population structure and the study of origin were chosen as covariates along with gender. Cases were grouped in a single dataset and a relatedness test was conducted. Pairs (pi-hat > 0.9) and relatives (pi-hat > 0.2) were excluded and one randomly chosen representative was reintegrated in the sample groups. The genetic heterogeneity across samples was controlled at the level of the genetic quality control in the analysis' flow. The rationale under this choice was that the SNPs found to specifically segregate in one or another sample, were excluded from the analysis, was lower in the analysis' flow. Nevertheless, this cannot rule out the possibility that pooling heterogeneous samples may lead to spurious differences between patients and controls.

2.4. Flowchart of the analyses

As a first step of the work, the pathways under analysis were selected from the National Cancer Institute (http://pid.nci.nih.gov/) database according to their involvement with lithium-based therapy. Below are reported the pathways under investigation.

- Androgen receptors
- Aurora
- Calcineurin
- cdc42
- glucose metabolism
- Hedgehog
- Insulin
- Integrin
- LKB1
- LPA
- p53
- Presenilin
- Reelin
- Stem
- Trans
- Trk