Integration of Data Sources and Omics to Identify Susceptibility Variants for Bipolar Disorder

K Truvé[1], D Vizlin-Hodzic[2,5], TZ Parris[3], S Salmela[3], E Berger[4], H Ågren[5], K Funa[6]

1 Bioinformatics Core Facility, Sahlgrenska Academy at University of Gothenburg. 2 Institute of Neuroscience and Physiology, Department of Physiology, Sahlgrenska Academy at University of Gothenburg. 3 Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy at University of Gothenburg. 4 Proteomics Core Facility, Sahlgrenska Academy at University of Gothenburg. 5 Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at University of Gothenburg.

6 Sahlgrenska Cancer Center at the Sahlgrenska Academy, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

Aim

The focus of our current research is to identify variants and genes involved in development of bipolar disorder (BD). Ultimately, knowledge gained could be used to develop diagnostic tests and improve current treatments.

Background

Bipolar disorder (BD) is a severe chronic psychiatric disorder affecting >1% of the population worldwide [1]. The disease is characterized by recurrent episodes of mania and depression. About 15% of patients with bipolar disorder are expected to die from suicide [2]. Thus, early detection, diagnosis and initiation of correct treatment are critical.

RNA-seq

Our previous results from RNA-seq [3] showed the *NLRP2* gene to be the most differentially expressed gene (Fig 1).

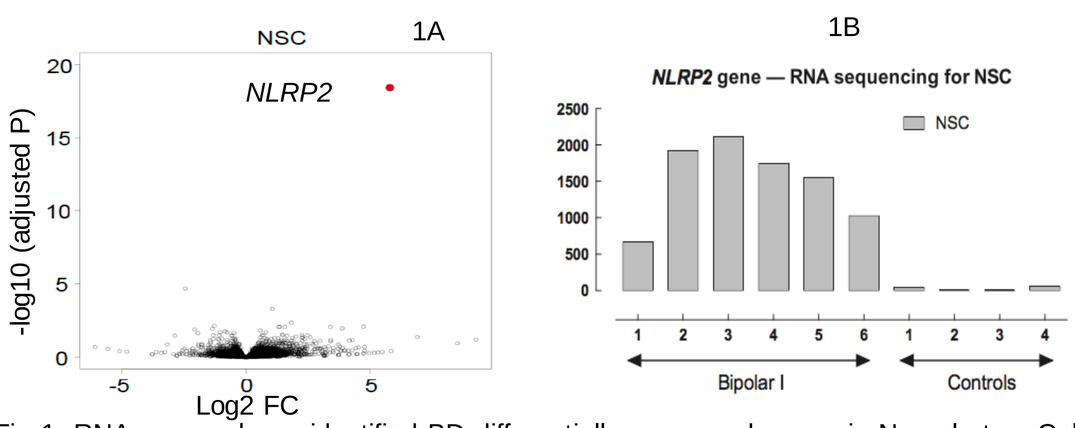


Fig 1. RNA-seq analyses identified BD differentially expressed genes in Neural stem Cells (NSC). 1A) Volcano plots of log10 adjusted P-values versus the log2 fold change between six BD patients and four controls. 1B) Altered *NLRP2* gene expression in six BD patients and four controls. Y-axis shows normalised gene-counts.

Study design

We performed Whole Genome Sequencing (WGS) and quantitative proteomics in the same sample as we previously performed RNA-seq. Variants were filtered as illustrated in Fig 3, and candidate variants were genotyped in a larger cohort. Variants in candidate genes were further investigated in a dbGAP data set (n=66) with WGS data from six large families affected with DB.

Results

Proteomics

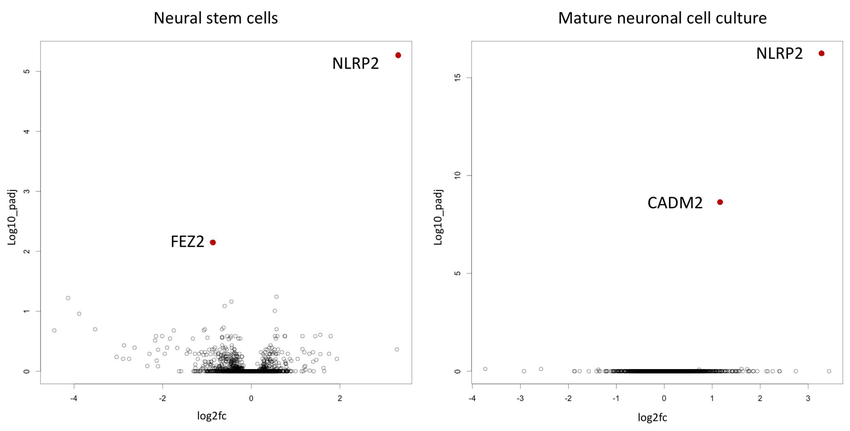


Fig 2. The volcano plot shows how the expression of proteins is distributed in the space of log2 fold change (log2fc) and adjusted p-values (log10_padj). Two proteins were statistically significant in neural stem cells (FEZ2, NLRP2) and two in matureneuronal cell culture (CADM2, NLRP2).

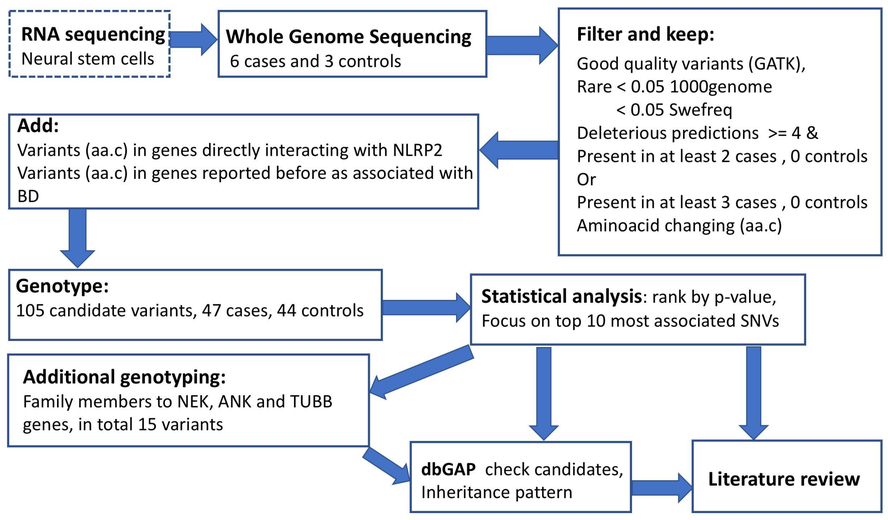
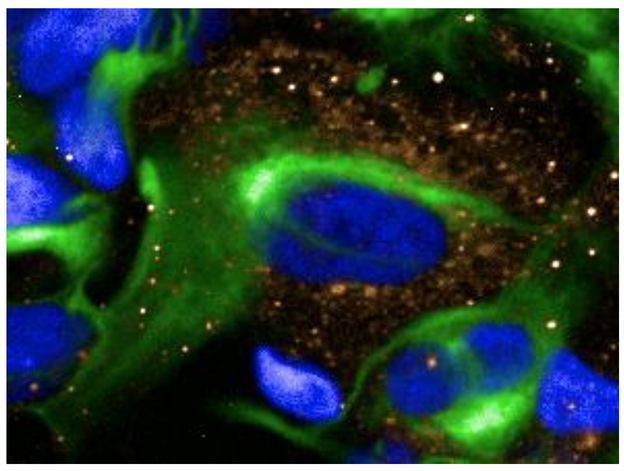


Fig 3. Overview of workflow showing some steps taken in the analysis, including different types of data resources and filtering of candidate variants.

WGS, Genotyping and DbGAP

In total, 120 candidate variants were selected after WGS to be genotyped in a larger cohort. Among the most associated variants, four genes were the most promising as being involved in disease development: *ANK3* that has been proposed in several studies, and the novel findings *NEK3, NEK7* and *TUBB1*. NEK3 and NEK7 have been shown to affect microtubule acetylation and microtubule dynamic instability [4,5]. Interestingly, ANK3 has also been shown to increase microtubule dynamics, suggesting dysfunctional microtubule as involved in BD development. Furthermore NEK7 has previously been shown to activate the NLRP3 (relative NLRP2) inflammasome [6]. A possibility of a similar activation of NLRP2 will be explored.

NLRP2 Protein Expression in NSC



It was confirmed with immunocytochemistry that the protein NLRP2 is expressed in NSC (Fig 4). We plan to investigate in more detail how the expression of NLRP2 can be influenced by certain genetic variants.

Fig 4. NLRP2 protein expression in NSC NESTIN / NLRP2 / DAPI

Methods

WGS was performed on HiSeqX. Tools used from mapping to candidate variants include, BWA, Samtools, GATK HaplotypeCaller and Annovar. Databases used for filtering were 1000genome and SweGen. Genotyping of candidates were performed using iPLEX Sequenom MassARRAY platform. The PLINK tool was used for Chi-square calculation. Proteins were identified, quantified and abundance among sample groups compared using Proteome Discoverer and DESeq2.

References

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Correspondence: Katarina Truvé, Bioinformatics Core Facility, Sahlgrenska Academy at University of Gothenburg, email: katarina.truve@gu.se

The Sahlgrenska Academy

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