CONVERGENT ANALYSIS OF GENOME-WIDE GENOTYPING AND TRANSCRIPTOMIC DATA SUGGESTS ASSOCIATION OF ZINC FINGER GENES WITH LITHIUM RESPONSE IN BIPOLAR DISORDER

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Lithium is the mainstay treatment in bipolar disorder (BD) for its effectiveness in the acute phases of illness and in prevention of recurrences. Response to lithium has a strong genetic background, but findings from pharmacogenetic studies have only marginally succeeded in identifying lithium response genes. Moreover, lithium's mechanism of action is complex, and while it modulates the expression and function of a hundreds of molecular players, most of these effects have been shown to be unspecific and not relevant for its clinical efficacy. It is therefore of crucial importance to conduct studies aimed at identifying lithium-modulated genes that are most likely responsible for predisposing patients to respond to the treatment. To this regard, approaches exploiting data from different omic platforms can constitute powerful tools to identify genes and pathways involved in lithium's response with a potentially higher translational value.

In the present study we applied an integrated analytical approach using genome-wide expression and genome-wide genotyping data from BD patients characterized for lithium response aiming at identifying, through convergent findings, lithium-responsive genes that may serve as biomarkers of its clinical efficacy.

We tested the effect of in vitro treatment with lithium chloride 1mM for one week on the transcriptome of lymphoblastoid cell lines (LCL) from 10 full responders (FR) and 10 non-responders (NR) patients and identified genes significantly influenced by the treatment. Genes were tested for differential expression after in vitro lithium treatment in both FR and NR using the paired t-test implemented in limma in R (v. 3.3.3). We focused on genes altered by lithium exclusively in FR, as these genes could be involved in modulating clinical efficacy of this drug. Findings from this approach were integrated with findings from a gene-based analysis performed with MAGMA (de Leeuw et al., 2015) using genome-wide genotyping from an extended sample of 205 BD patients characterized for lithium response (Alda et al., 2002; Manchia et al., 2013).

The expression of 29 genes was significantly changed by lithium in FR but not in NR. Two of these genes, zinc finger protein 429 (ZNF429; $p = 0.0003$) and zinc finger protein 493 (ZNF493; $p = 0.0005$), were respectively the fourth and the fifth most significant genes in the gene-based analysis. Validation with quantitative real-time PCR confirmed the under-expression of ZNF493 in FR after lithium treatment [fold change (FC) = 0.71; $p = 0.036$)], while ZNF429 showed a trend for downregulation (FC = 0.82, $p = 0.06$).

Using convergent analyses of data from genome wide genotyping and gene expression studies, we identified two zinc finger protein genes as lithium-responsive targets that may be involved in modulating lithium efficacy in BD. These genes codify for zinc finger proteins, a large family of

functional domains involved in several functions comprising transcriptional activation, regulation of apoptosis and protein folding. To our knowledge, this is the first evidence supporting the involvement of zinc finger proteins in lithium mechanism of action and response.

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