

ABSTRACT

A disrupted circadian clock has been linked to the risk of several neuropsychiatric disorders. Likewise circadian rhythms have been suggested as mediators of the mechanism of action of lithium in bipolar disorder. Nonetheless the relationship between the ‘clock genes’ that regulate circadian rhythms and lithium treatment response is not completely understood. To our knowledge there has not been a systematic pathway / enrichment analysis of clock genes in the context of psychiatric traits, in general, and of clinical response to lithium, in particular. The objective of this study was to perform formal gene-set enrichment analyses for circadian clock genes, using publicly available GWAS summary statistics from several psychiatric disorders, as well as the results from The International Consortium on Lithium Genetics (ConLiGen) GWAS (Hou et al., 2016a). Gene set enrichment analyses were carried out using both MAGMA and INRICH. These analyses reported a significant enrichment of gene-sets closely related to the ‘core clock’ machinery in the dichotomous lithium response phenotype. The specificity of these results suggests that the participation of circadian rhythms is especially relevant in the modulation of lithium response rather than in the overall risk of mental illness.

INTRODUCTION

The link between a disruption of the circadian rhythms and mental disease has been consistently observed and reported over the last two centuries. Disturbances in the timing and architecture of the sleep/wake cycle, partially regulated by the circadian clock, have been exhaustively described as very common comorbidities in different psychiatric disorders (Wulff et al., 2010). Besides sleep disturbances, alterations in the daily rhythms of endocrine function, appetite or activity have also been observed in a large proportion of psychiatric patients.

Likewise circadian rhythms have been suggested as mediators of the mechanism of action of lithium in bipolar disorder (Geoffroy et al., 2017). Nonetheless the relationship between the ‘clock genes’ that regulate circadian rhythms and lithium treatment response is not completely understood. To our knowledge there has not been a systematic pathway / enrichment analysis of clock genes in the context of psychiatric traits, in general, and of clinical response to lithium, in particular. The objective of this study was to perform formal gene set enrichment analyses for circadian clock genes, using publicly available GWAS summary statistics from several psychiatric disorders, as well as the results from The International Consortium on Lithium Genetics (ConLiGen) GWAS (Hou et al., 2016a).

METHODS

Phenotypes

GWAS summary statistics from schizophrenia, bipolar disorder, major depressive disorder, ADHD, Autism Spectrum Disorders, PTSD, chronotype and continuous/dichotomous lithium response were used as reference.

Gene-set enrichment analyses

Two methods were used in our analyses, MAGMA (<http://ctglab.nl/software/magma>) and INRICH (<http://atgu.mgh.harvard.edu/inrich/downloads>). These methods involve the use of competitive tests to evaluate the enrichment of a specific set of genes.

Gene-based analyses

MAGMA software was also used to carry out genome-wide gene association studies (GWAS) based on the results of the ConLiGen (lithium response) GWAS.

Generation of circadian gene-sets

Based on previous literature and other available databases, curated gene sets related to circadian control were generated. These sets fell into three categories: clock modulator genes, core clock genes and genes following a circadian pattern of expression.

REFERENCES

Geoffroy et al. (2017) World J Biol Psychiatry 28:1-4.
 Hou et al. (2016a) The Lancet 387:1085-1093.
 Hou et al., (2016b) Human Molecular Genetics 25:3383-3394.
 Wulff et al. (2010) Nat Rev Neurosc 11:589-599.

RESULTS

MAGMA enrichment analyses

Gene set enrichment analyses using MAGMA reported a significant enrichment of a set of 19 genes that constitute the ‘consensus core clock’ in the dichotomous lithium response phenotype (Figure 1. Competitive P-value=0.0005; corrected P-value=0.0336). None of the circadian gene sets showed a significant enrichment in any of the other psychiatric traits.

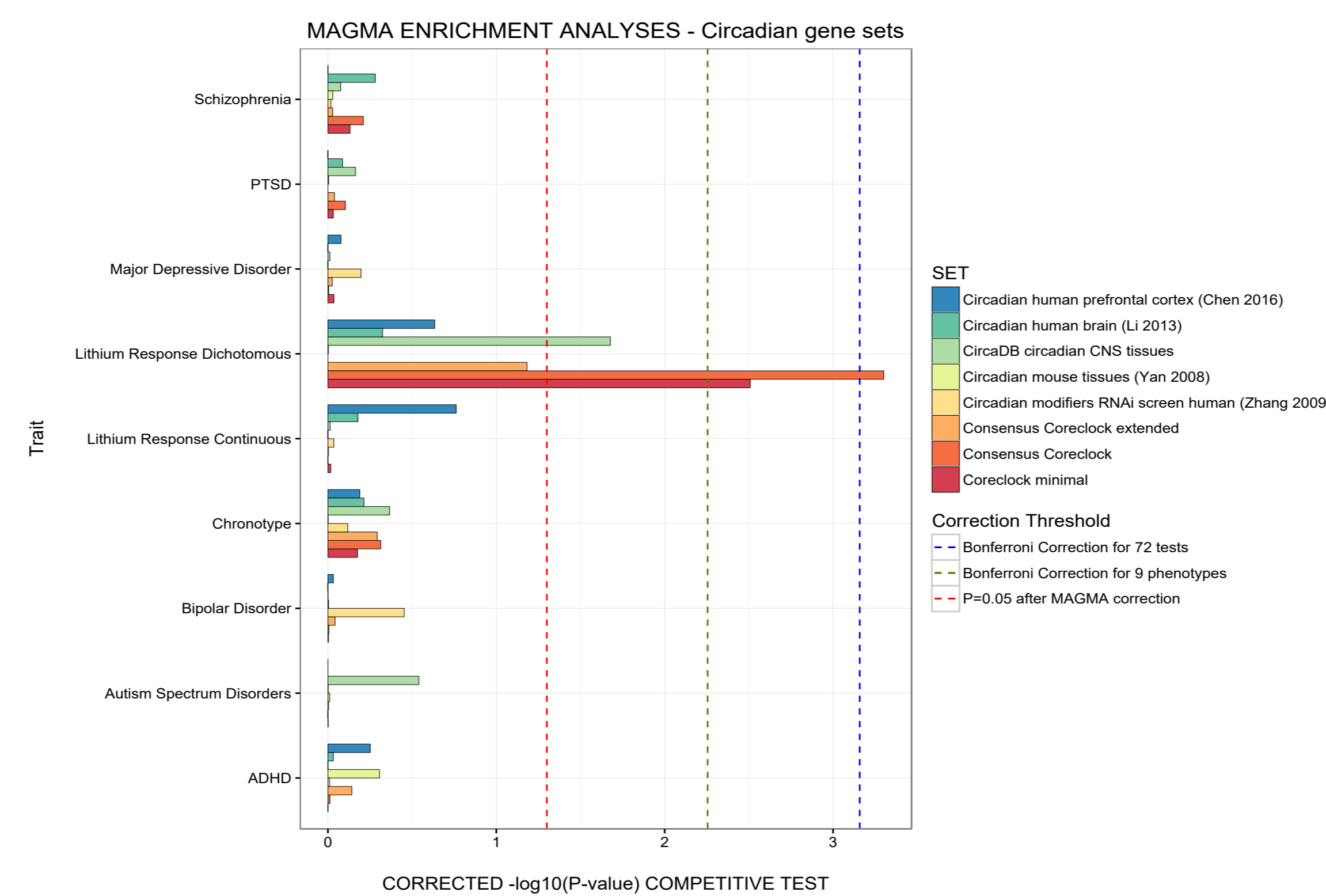


Figure 1. Results of MAGMA enrichment analyses. Eight different gene-sets related to circadian clock system were tested in nine phenotypes. X-axis: $-\log_{10}(P\text{-value})$; Y-axis: traits analyzed in this study. Dashed vertical lines: correction thresholds.

INRICH enrichment analyses

Gene set enrichment analyses using INRICH were carried out using different clumping SNP P-value significance thresholds (0.00001, 0.0001, 0.001, 0.01). The ‘extended core clock’ gene-set at a clumping P-value of 0.001 was found to be associated with dichotomous lithium response (Figure 2. Competitive P-value=0.004; corrected P-value=0.041). Some of the circadian gene sets also showed a significant enrichment in chronotype and schizophrenia but not bipolar disorder (Hou et al., 2016b).

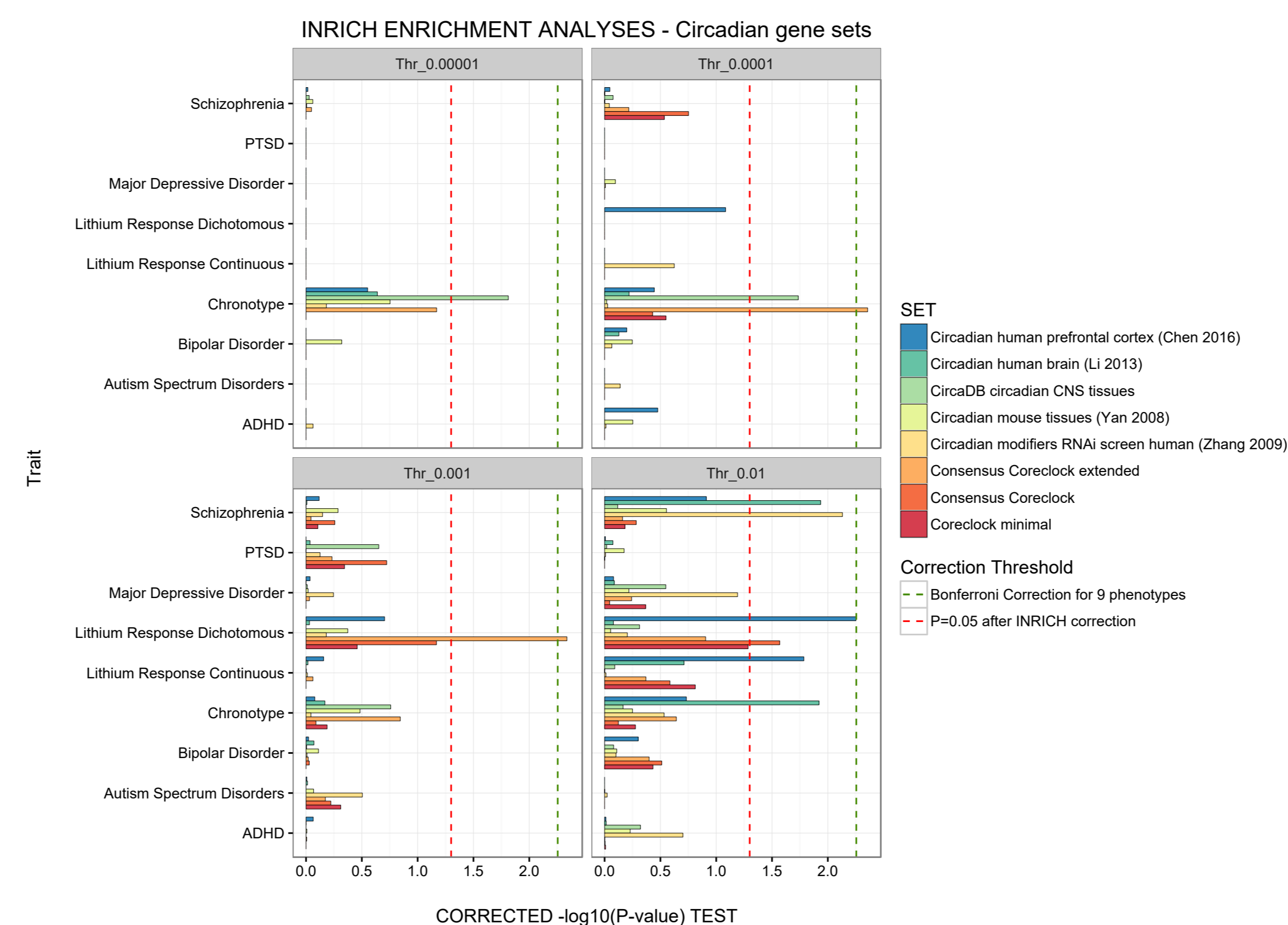


Figure 2. Results of INRICH enrichment analyses. Eight different gene-sets related to circadian clock system were tested in nine phenotypes. X-axis: $-\log_{10}(P\text{-value})$; Y-axis: traits analyzed in this study. Dashed vertical lines: correction thresholds.

GWAS based on lithium response phenotypes

Gene-based analyses were carried out based on the two available phenotypes related to lithium response in bipolar disorder: longitudinal (Figure 3) and dichotomous (Figure 4) response. Although some of the genes achieved suggestive P-values, none of them survived correction for the number of genes analyzed (N=17,910 genes).

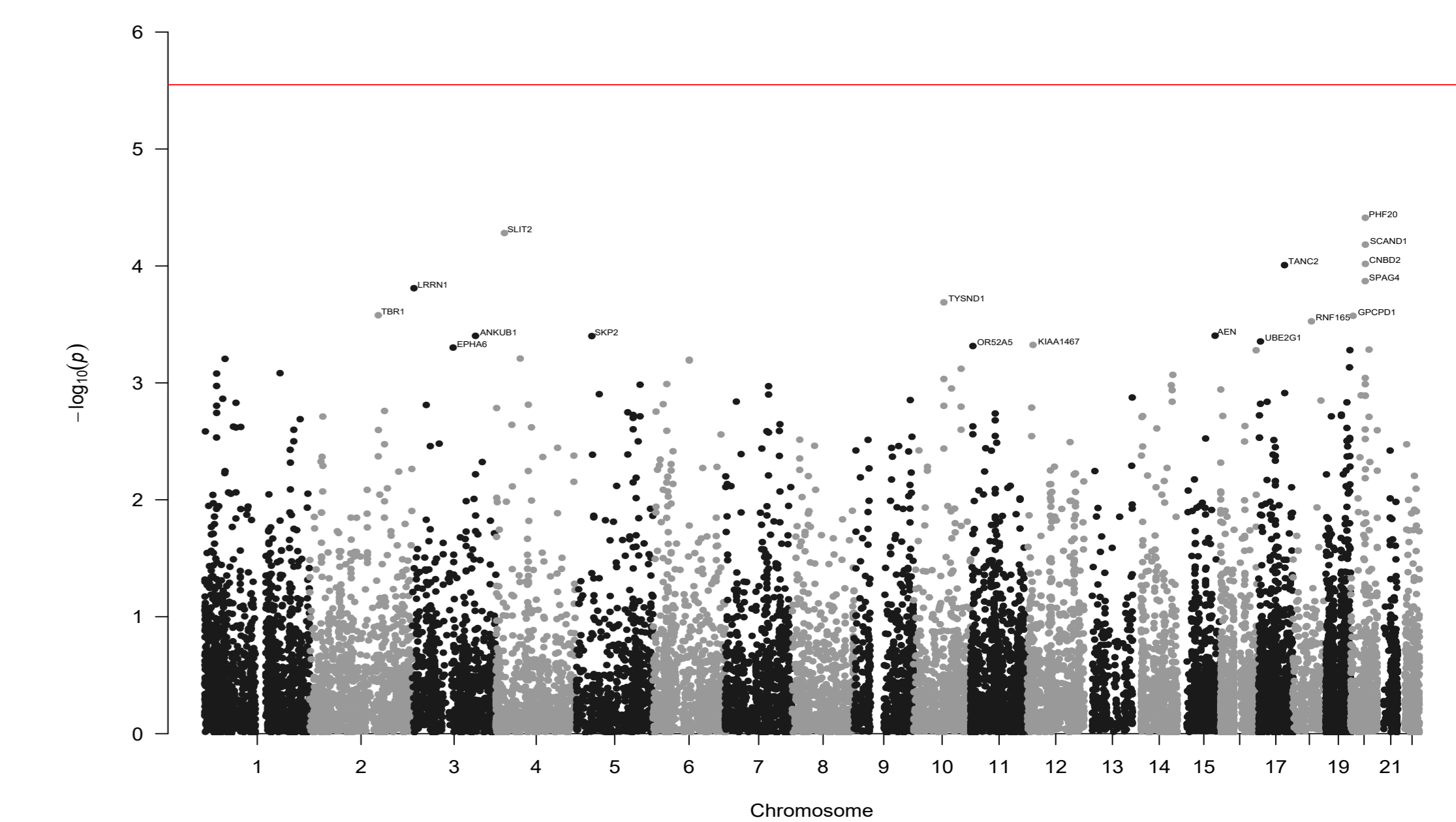


Figure 3. Manhattan plot of the GWAS of continuous response to lithium in bipolar disorder. Y-axis: $-\log_{10}(P\text{-value})$. Red horizontal line: multiple testing correction threshold.

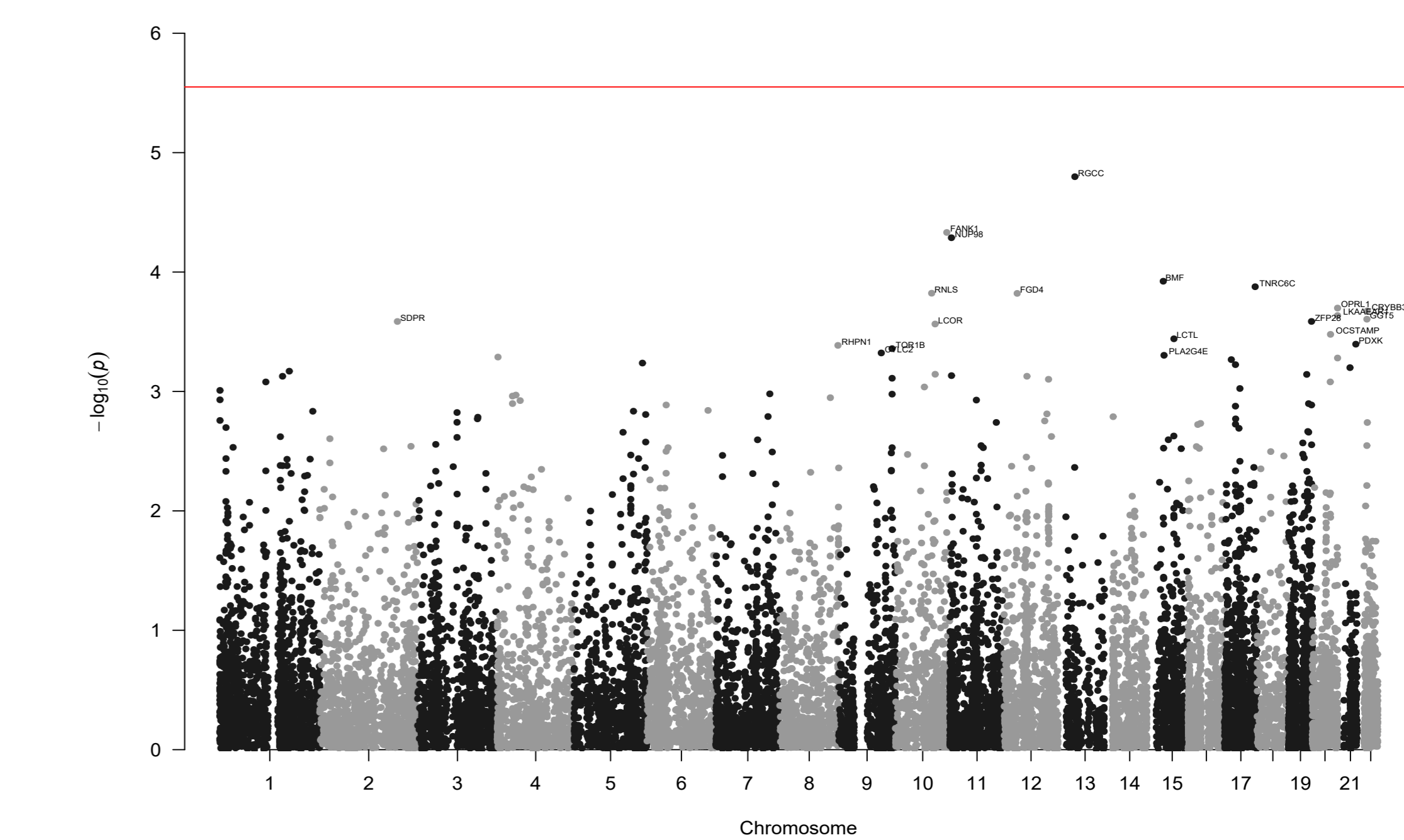


Figure 4. Manhattan plot of the GWAS of dichotomous response to lithium in bipolar disorder. Y-axis: $-\log_{10}(P\text{-value})$. Red horizontal line: multiple testing correction threshold.

DISCUSSION

Our results suggest the involvement of those genes that constitute the core clock machinery in the determination of the clinical response to lithium in bipolar disorder patients. The specificity of these results suggests that the participation of circadian rhythms is especially relevant in the modulation of lithium response rather than in the overall risk of mental illness. A better understanding of potential links between circadian mechanisms, genetic risk factors, and the lithium treatment response may open new avenues into the clinical management of bipolar disorder.

GRANTS

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