INCREASED DNA METHYLATION OF THE PRODYNORPHIN GENE PROMOTER IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH BIPOLAR DISORDER

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BACKGROUND
We have previously shown selective changes in DNA methylation of brain-derived neurotrophic factor (BDNF) gene promoter of Bipolar Disorder (BD) II subjects, highlighting the importance of epigenetic factors in mediating the onset and/or susceptibility to BD [1]. Here we studied the epigenetic regulation of prodynorphin gene (PDYN), the precursor of the opioid peptide dynorphin, recently suggested as a downstream effector of BDNF regulation [2].

AIMS
I. to examine the DNA methylation patterns in the proximal promoter region of PDYN in peripheral blood mononuclear cells (PBMCs);
II. to investigate whether PDYN expression levels are correlated with DNA methylation level;
III. to determine whether these changes are associated with alterations in the expression of DNA methyltransferases (DNMTs).

RESULTS
We observed a significant (p<0.05) hypermethylation of the PDYN promoter in BD II patients (CT: 17.14 1.25%; BDI: 18.06 1.26%; BDII: 21.55 1.08 %).

PDYN gene expression resulted to be significantly decreased in BD II subjects (0.56 0.09; P<0.05) but not in BD I (0.86 0.16) patients compared with controls (1.25 0.19).

Higher levels of DNA methylation were observed in BD subjects on pharmacological treatment with antidepressants (21.01 1.23 %) compared with those exclusively on mood-stabilizing agents (16.89 1.48 %; p < 0.05).

DISCUSSION
The dynorphin system has been associated with the regulation of mood [3] and in this study we provide evidence of epigenetic modulation of PDYN gene expression in PBMCs of BDI and BDII patients.

In particular, we found a significant and selective PDYN gene expression down-regulation and, consistently, a hypermethylation of PDYN promoter in BDII patients.

We suggest that pharmacological treatments might play a crucial role in the difference observed between BDI and BDII. Indeed, mood stabilizers (particularly lithium and valproic acid), mostly used to treat BDII patients, were associated with reduced DNA methylation of the PDYN promoter compared with antidepressant drugs. Conversely, BDI subjects, most of whom received a combination of mood stabilizers and antidepressants, showed hypermethylation of PDYN promoter region.

In addition, the study of the mRNA expression of four DNMT isozymes revealed that DNMT 3b, an enzyme critically involved in de novo DNA methylation, was selectively upregulated in BD type II subjects, where consistently, PDYN promoter hypermethylation was observed.

POSSIBLE LIMITATIONS OF THE STUDY
It could be argued that alterations in PDYN gene expression in peripheral cells might not be reflective of the same mechanism in the brain. However, it is important to mention a recent study showing a high similarity of CpG methylation patterns at PDYN promoter among brain tissues and PBMCs [4].

Further comparisons across larger samples, including unipolar and drug-free patients, in different illness phases are warranted to confirm these results.

CONCLUSIONS
The present findings, provide evidence for PDYN as a possible peripheral biological marker useful for differential diagnosis and, mostly, for prediction of treatment response, pointing out the relevance of epigenetic mechanisms in the pathophysiology of major psychoses.

METHODS
We conducted this study on PBMCs, that are accessible cells with potential for biomarker discovery in psychiatric disorders and contain the full complement of epigenetic enzymes found in most tissues, including neurons and peripheral nucleated cells. Total DNA and RNA were isolated from 89 patients with BD on stable pharmacological treatment (BD I = 48, BD II = 41) and 42 healthy controls. To assess PDYN abundances and to quantify PDYN promoter DNA methylation, we used Real-Time RT-PCR and Real-Time Methylation Specific PCR, respectively.

REFERENCES

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