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ASPECTS OF CYCLON AND BDNF GENE EXPRESSION IN SCHIZOPHRENIA PATIENTS

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ABSTRACT:

The pathogenesis of the schizophrenic illness is still not fully elucidated. Many studies have been conducted revealing different aspects but may be the studies of greatest significance are studying the genetic aspects of expression of trophic factors and enzymes associated with nervous system development and plasticity. In this relation we aimed at measuring the Cyclon and BDNF genes expression in blood of patients suffering from schizophrenia and to test for correlation between them. Our result did not reveal correlation in spite of their connection with the disease.

Key words: schizophrenia, *ccdc86*, *Cyclon*, BDNF

The pathogenesis of the schizophrenic illness is still not fully elucidated. In this relation many studies have been performed examining the function of different systems in the organism. One of these directions is associated with the activity of the immune system and its effects on neural plasticity, glial activation and eventually the associated functional disorders of CNS. In this regard several studies for genetic associations with schizophrenia a linkage is found with components of the IL-3 signaling pathway – the gene coding for IL-3 (3) and the genes coding for the two subunits of the IL-3 receptor (4,11)

In 2008 Akemi Hoshino и Hodaka Fujii(10) identified the gene *CCDC86* to be directly induced by IL-3 in a IL-3 dependent pro-B cell line (14) and named it *Cyclon* (cytokine induced protein with coiled-coil domain)(10). In a consequent study the gene was identified to be important for the phenomenon of activation induced cell death (AICD) in T lymphocytes (14). The transgenic overexpression of *Cyclon* enhances the activation-induced cell death of CD4+ and CD8+ T lymphocytes and targeted deletion of *Cyclon* allele leads to resistance to AICD and lower expression of Fas (CD95) (14). These experiments demonstrate that *Cyclon* modulates the activation induced cell death and that this effect is mediated through induction of the expression of the cell membrane pro-apoptic death receptor Fas. This receptor is a member of the Tumor Necrosis Factor Receptor Superfamily, member 6, and plays pivotal role in regulation of programmed cell death and the proper physiological functioning of the immune system (19). *Cyclon* is also expressed in the central nervous system – in neurons and glia, and probably in these cells it also regulates the Fas expression and hence the process of programmed cell death. Neuronal and glial apoptosis are processes definitely associated with the pathogenesis of the disease and therefore it is possible that *Cyclon* also plays important role in the pathogenesis of schizophrenia.

Another factor associated with the cell differentiation and apoptosis of cells in the nervous system is BDNF. Concerning the genetic link of BDNF with the pathogenesis of schizophrenia of interest is a study of Agartz et al. (1). The authors found an association between a polymorphism of BDNF and brain morphology in patient with schizophrenia. In a MRI study of the volume of cerebellar tonsils, hemispheres and subregions of *vermis*, striatal structures, hippocampus and corpus callosum differences between patients and healthy controls were found. A significant difference in the volume of frontal gray matter is associated with BDNF 11757 G/C polymorphism, and 270 C/T polymorphism is associated with caudate nucleus total volume (1). In other studies a link between BDNF gene and neural development displayed as a modulation of activity dependent synaptic plasticity in differentiating neurons, particularly in the hippocampal region and neocortex were found (7, 12, 18).

There are studies indicating that the rs6265 methionine/valine single nucleotide polymorphism (SNP) is associated with memory alterations (5, 6, 9, 16). Moreover, in previous cross-sectional MRI studies the presence of methionine allele is associated with lower volume of frontal and temporal gray matter compared to the volume in valine homozygotes (1, 9, 17).

Many evidences point towards the genetic link between the neurotrophins and schizophrenia. Role in the pathogenesis of schizophrenia also play other neurotrophins besides BDNF and its allelic variants. Neurotrophins like NT-3 and its polymorphisms are probably also connected to schizophrenia (4, 8). Later observations show that the level of expression of BDNF is lowered even before the first

schizophrenic episode and correlates with stress (15) and estrogen level (13). These observations are in unison with many hypotheses about schizophrenia which view biochemical and genetic factors as key entities in disease pathogenesis and are associated with BDNF (2).

Considering all these observations our aim was to assess the mRNA expression of *Cyclon* and the level of BDNF in peripheral blood in patients suffering from schizophrenia.

PATIENTS:

16 patients were recruited to the study, 4 male with average age 28,3 years and 12 female with average age 38,2 years. The patients were sampled twice - during disease relapse and after at least 21 days after accomplishing a drug induced remission. The patients were on standard treatment with second generation antipsychotics.

METHODS:

Expression of Cyclon mRNA in peripheral blood leucocytes. 6 ml of anticoagulated venous blood was collected in K2 EDTA vacuum closed system. Total RNA isolation from blood leukocytes was performed with QIAamp RNA Blood Mini Kit (QIAGEN, Germany) according to manufacturer's instructions and stored at -80°C until further analysis. Reverse transctiption of total RNA to cDNA was performed with Precision^{TM} Reverse Transcription Kit (PrimerDesign, United Kingdom) with random nonamer primers according to manufacturer's instructions. Quantitative polymerase chain reaction was performed with SYBR Green Precision^{TM} 2X Real-Time qPCR MasterMix (PrimerDesign, United Kingdom) and gene specific primers for the target gene *CCDC86* and the *ACTB* reference gene (PrimerDesign, United Kingdom) according to manufacturer's instructions. qPCR was performed on StepOne Plus (Applied Biosystems, USA). The relative expression of the *CCDC86* target gene mRNA compared to reference gene *ACTB* mRNA was calculated using ΔΔCt method by the instrument software (StepOne Software v2.0) and presented as ratio to a reference sample.

Plasma BDNF. 6 ml of anticoagulated venous blood was collected in K2 EDTA vacuum closed system. The sample was transported on ice (4°C) in less than 30 minutes and blood plasma was separated after centrifugation at 1000 x g for 15 minutes at (4°C). The separated blood plasma was stored at -80°C until further analysis. Plasma BDNF was quantified using Quantikine Human BDNF Immunoassay ELISA Kit, catalog number DBD00 (R&D Systems, USA) according to manufacturer's instructions.

Statistical analysis: t-test and correlation analysis.

RESULTS:

The relative expression of *Cyclon/CCDC86* in relapse and remission are shown in fig. 1. The results show statistically significant difference (higher in relapse) in the expression between relapse and remission in schizophrenia patients (t-test, P<0,01).

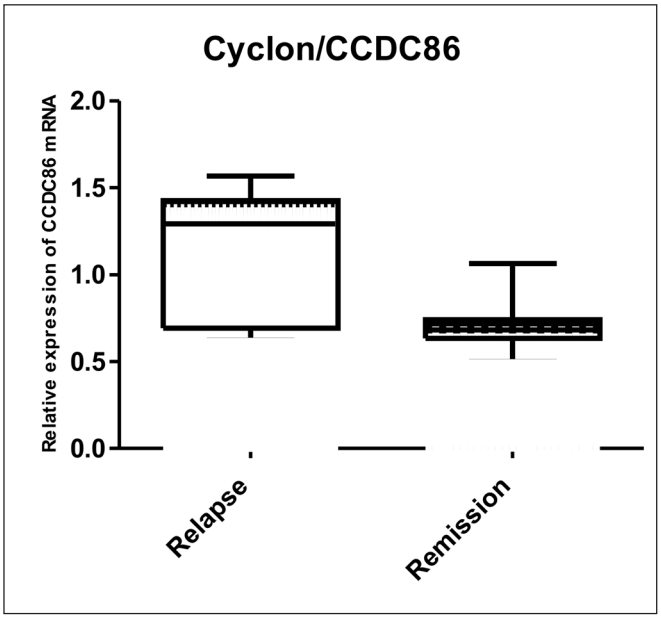

Fig. 1. Relative expression of Cyclon/CCDC86 mRNA in relapse and remission of schizophrenia.

The plasma levels of BDNF in relapse and remission are shown in fig. 2. There is trend for higher level of BDNF in remission compared to BDNF in relapse, but the difference is not statistically significant.

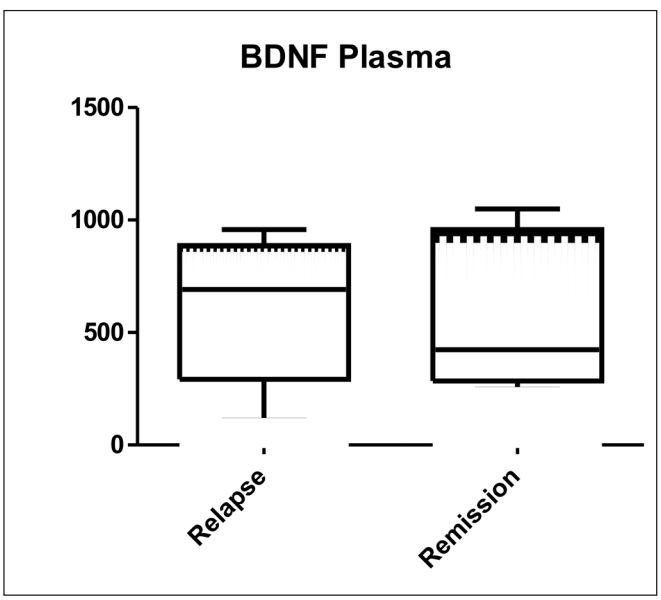

Fig. 2. Plasma level of BDNF in relapse and remission of schizophrenia.

We did not find correlation between *Cyclon* mRNA and plasma BDNF level in relapse (r=0,154, P=0,691) and in remission (r=0,107, P=0,818).

DISCUSSION:

Considering the multitude of different theories trying to explain the pathogenesis of schizophrenia and the many unclarities we sought to assess the dynamics of Cyclon gene mRNA in peripheral blood leukocytes. To our knowledge there is no data in the literature. Because *Cyclon* is induced by IL-3 and there are many studies linking IL-3 and schizophrenia we propose that *Cyclon* may be involved in the pathogenesis of schizophrenia. Our result show dynamics, i.e. statistically significant difference in the expression between period of relapse and remission.

Concerning the levels of BDNF in blood plasma the concentration is higher in remission compared to relapse but the difference is not statistically significant.

The results show dynamics in the parameters tested but without proven correlation between *Cyclon* and BDNF in relapse and remission.

According to the data for the role of BDNF in the processes of apoptosis and neuronal plasticity and the processes of neuroinflammation (1) and the link between IL-3 and *Cyclon* (15) probably both play important role in the pathogenesis of schizophrenia and their effects on apoptosis, plasticity and glial mediated inflammation are synergistic(1).

As for BDNF there are many confirmations for its link with schizophrenia in literature for *Cyclon* the data is scarce and these are first reports for its link with schizophrenia. *Cyclon* is involved with immune system regulation and autoimmunity and its link with schizophrenia is point for a long considered hypothesis - that schizophrenia is in fact an autoimmune disease.

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