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THE LINK BETWEEN HYPERHOMOCYSTEINEMIA AND METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM IN CHILDREN AND ADOLESCENTS WITH PSYCHOTIC DISORDERS

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CONTENT

1. INTRODUCTION

1.1. The topicality and novelty of the research

In 2010, the first disability was found for 163 children with psychic and behavioural disorders [1].

The precise schizophrenia-causing factors still have not been found, therefore there is an opinion on the multi-factorial genesis of the illness [2].

However, none of the researched indices has proved to be the determinant.

The conception of illness etiology can be ensured developing the research of illness causes [3;4].

In last 5 – 6 years, there have been studies and the data are obtained on the possible role of Hcy in pathogenesis of schizophrenia and affective disorders. The given disorders of Hcy metabolism are often together with changes of concentration of several vitamins (B_6, B_{12}, folic acid) in blood serum.

In connection with Hcy, the concentration of plasma B_{12} vitamin and folic acid is researched, since they have an ability significantly to decrease Hcy level and in such way, probably, to prevent or to weaken the caused neurodegenerative changes [5;6;7].

Basing upon the above-mentioned, it is significant to set which psychic illnesses have changes of Hcy level in blood plasma; whether the changes of its concentration depend on the clinic signs of the illness (what kind of syndromes or complexes of syndromes are characteristic to the patients with increased Hcy level) and the factors of pace (the illness develops, there is a remission or continuous pace of progredient).

1.2. The set hypothesis of the research

Hypothesis – if the patient whose carrier of mutation C677T of the gene *MTHFR* in heterozygotic form becomes ill with disorders of schizophrenia spectrum, they have a trend to be clinically more serious, with explicit affective saturation.

In the future, it could allow more precisely forecasting the illness form and to set the diagnosis.

1.3. The aim of the research

The aim of the research I is to get information on Hcy level, polymorphism of *MTHFR* gene C677T (rs1801133), as well as the frequency of T allele incidence for children and teenagers with disorders of schizophrenia spectrum and affective disorders. As well as to set correlation between Hcy level and the above-mentioned disorders and changes of Hcy level depending on changes of the illness.

1.4. The necessary tasks of the studies for reaching the aim of the research

It is necessary to carry out the following tasks for reaching the aim:

1. To set the levels of folic acid and B12 vitamin in blood serum for clinical patients with disorders of schizophrenia spectrum, as well as for patients with affective disorders and the control group.

2. To set the Hcy level for the patients involved in the research and the control group, as well as to set the correlation between the studied groups and Hcy level.
3. To set the correlation between Hcy level and disorders of schizophrenia spectrum, taking into account their clinical form, the level of symptoms and affect.
4. To set the correlation between Hcy level and illnesses of affective disorders.
5. To study *MTHFR* gene C677T polymorphisms and frequency of T allele incidence for the patients involved in the study and the control group.
6. To set the correlation between the Hcy level in blood, diagnosis for the patients and *MTHFR* gene C677T polymorphism.
7. To set the changes of Hcy level in connection with the changes of clinical signs of the patients.
8. To evaluate the influence of medicine used in the therapy (anti-psychotic, anti-depressive, mood stabilizers) on Hcy level, setting repeatedly Hcy level in blood serum.

1.5. Personal input

Analysis of literature, development of study design and coordination in the Ethics committee of Riga Stradins university, blood sample collection, the evaluation of the psychic condition of the patients with diagnostic scales, patients treatment, analyzis of the results, writing of articles, thesis and promotional work.

2. THE MATERIAL AND METHODS OF THE RESEARCH

2.1. The material of the research

118 out of 170 patients in the State Ltd. BKUS Children's Psychiatric Clinic Department No. 7 with disorders of schizophrenia spectrum (schizophrenia, schizoaffective disorders, schizotypal disorders), as well as patients with affective disorders (depression episode, recurrent depressive disorders; bipolar affective disorders – mixed affective episode) were involved in the research. The disorders for the patients were with or without anxiety; the patients were in the clinic from November 1, 2007 until January 31, 2011. The patients were elected according to their diagnosis (according to SSK – 10 *Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research*) [8] and the current clinic signs of the illness with respect to gender. 94 patients from the control group – mentally healthy, were involved in the research as well. The patients of the control group were taken from the preschool educational institutions (34 children), 26 children were from the primary school, but 34 children from the classes of elementary and high schools. The patients were from the age of 3 to 18. The wide range of the age is chosen since the majority to agree to carry out biochemical and genetic blood analyses were the parents of smaller children. In the group of teenagers, numerically more parents and teenagers themselves did not agree carrying out clinical analyses; supposedly, it is connected with the ruling stigma in the society – fears that prognostically there could be set a probable genetic or psychic illness.

The patients with serious somatic pathologies (inability of kidneys, usage of glicocorticoides), the patients with proven genetic illnesses, non-

compliance patients; as well as in the case if the parents or the guardians of a juvenile did not agree to involving a child in the research.

The set therapy schema of the patients involved in the research by the attending physician was not changed. The medicine was appointed according to the registered indications and dosing principles, as well as the possible contraindications and side effects were evaluated.

In the research, the patients of schizophrenia spectrum were divided into 3 groups depending on the current diagnosis – taking into account the clinical signs and pace factors of the illness: 1. Continuous paranoid schizophrenia (20 patients); 2. Episodic pace paranoid schizophrenia with progredient pace and schizoaffective disorders (40 patients); 3. Simple schizophrenia and schizotypal disorders (29 patients). The fourth group includes (28 patients) patients with affective disorders. Affective disorders were more detailed in the following way – depressive disorders and recurrent depressive disorders with or without alert, bipolar affective disorders, mixed affective condition.

The demographic data of the patients involved in the research are in the chart 2.1.

Chart 2.1.

The demographic data of the patients involved in the research

	Schizophrenia spectrum disrders	**Affective disorders**	**Control**
Gender			
• Male	50 (61%)	6 (20%)	54 (57%)
• Female	32 (39%)	16 (80%)	40 (43%)
Age (years)			
• 3-7	0	0	34 (36%)
• 7-12	12 (15%)	5 (23%)	26 (28%)
• 12-15	23 (28%)	6 (27%)	12 (13%)
• 15-18	47 (57%)	11 (50%)	22 (23%)
Level of education			
• elementary school	39 (48%)	14 (64%)	35 (37%)
• secondary school	26 (32%)	5 (23%)	19 (20%)
• special elementary school	17 (20%)	3 (13%)	0
• no school	0	0	40 (43%)
Disability	24 (29%)	1 (4%)	0
Duration of illness (years)			
• 1	37 (45%)	11 (50%)	0
• 2-3	24 (29%)	8 (36%)	0
• >3	21 (26%)	3 (14%)	0
• no illness	0	0	94 (100%)

2.2. Research methods

The data of the patients were registered in the questionnaires specially made for the goal. The questionnaires included information on the anamnesis of the family, children's' growing and development until the beginning of the illness, the beginning of the illness, its pace and factors, about all the previous therapy methods and results, as well as the current treatment in the hospital and efficiency.

All the analyses are taken in the morning, on an empty stomach.

The examination of the patients is carried out in dynamics – both, repeatedly evaluating psychic condition and laboratorial examinations.

The level of B_{12} vitamin and folic acid in blood serum was set in NMS laboratories, using immunochemiluminescent method / Immulite 2000.

Hcy level in blood serum is set is set in the research laboratory of RSU.

2.2.1. Setting of homocysteine concentration in blood plasma

The equipment and parameters: liquid chromatography of high efficiency Shimadzu LC-20 Prominence, fluorimetric detector RF-10AxL.
Diapason of detection - EX: 385 nm, EM: 515 nm. The time of analyses - 5 min. the speed of mobile phase flow – 1.3 ml/min. The temperature of the column – 25 °C.
Chromsystems GmbH commercial reagent kit and method:
1. The plasma sample of 100 µl is filled with 25 µl of inner standard and 25 µl of reduction reagent.
2. Everything is blended in vortex rotator for 2 sec and then 5 min it is incubated in the room temperature.
3. 100 µl of precipitation reagent is added.
4. Blended in vortex rotator for 30 sec.
5. Centrifugate in 9000 turns in 5 min.
6. In a new test-tube of 50 µl of supernatant add 100 µl of derivatization reagent and slightly shake.
7. Incubate in 55 °C for 10 min.
8. 20 µl of mixture is filled in HPLC sample panel.

2.2.2. Setting of C677T SNP

The genetic analyses were carried out at RSU scientific laboratory of human molecular genetics.

DNS is extracted from the venous blood (with EDTA preservative) with a standard phenol/chloroform method [9].

C667T (rs1801133) polymorphism in *MTHFR* gene was analyzed using reaction chain and the following polymorphism of restriction fragment length (PCR-RFLP) analyses, according to the previously signed protocol [10].

PCR was carried out in 20 µl total capacity: 2 µl 10X Taq *Buffer with* $(NH_4)_2SO_4$, 1,6 µl 25mM $MgCl_2$, 0.5 µl 10mM dNTP, 0.5 µl 10 pmol of synthetic oligonucleotide (the sequence is in the chart 2.3.) and 2.5 U recombinant *Taq* polymerase, adding 1 µl of DNA sample (concentration 50ng/mkl). All reagents are from Fermentas (Lithuania), the synthetic oligonucleotide from MWG (Germany).

For carrying out PCR reaction, an automatic thermocycler (Tprofessional Thermocycler (Biometra), Germany) was used, applying the following program – 95 °C 3 min; 30 cycle 94 °C, 65 °C, 72 °C each stage for 1 minute; one cycle 72 °C 10 min and 4 °C interruption of the reaction.

In the result of PCR reaction, a 198 bp long product was obtained consisting of a specific cord of polymorphism. To recognize the site, the restriction was made: 4 µl PCR product was added a restriction mix (1 mkl of restrictaze buffer 0.75 mkl ddH_2O, 0.25 mkl *HinfI* restrictaze (Fermentas, Lithuania)). The mixture was put in 37 °C for 12 hours.

To check whether the obtained genotyping results are true the direct secvenation (ABI Prism 300 genetic analyzer, using *Big Dye* v.3.1. reagents (*Applied Biosystems*)). 10 samples of each genotype group with one of the

synthetic nucleotides. In all the cases, the result complied with the obtained after the restriction [11].

2.2.3. The evaluation of the psychic condition of the patients

The clinical condition of the patients and its changes was evaluated according to diagnostic scales. The evaluation of the diagnostic scales was set in marks; the total score complied with the firm severity level of the clinical condition. In the case of schizophrenia and schizophrenia spectrum disorders the BPRS (*Brief psychiatric rating scale*) and HAM-A (*Hamilton anxiety scale*) was used. BPRS scale provides information on the characteristic positive and negative symptoms of schizophrenia and schizophrenia spectrum disorders [12]. HAM-A scale, however, reflects the anxiety level of the patients [13]. For description of the patients with affective disorders the HAM-D (*Hamilton depression scale*) was applied [14], but for setting the level of anxiety - HAM-A scale. (See diagnostic scales in the supplement).

2.3. The statistic processing of the data obtained in the study

The results obtained within the framework of the study are recorded in a specially made protocol of the study, as well as in electronic database.

The common descriptive statistic methods are applied for characterising the groups. Depending on the type of the variable, the average and dispersion interval is calculated, as well as the median. The Fisher's exact test and chi-squared test are applied for comparing the groups.

Spearman's rank correlation coefficient (r) is used for the analyses of the correlation.

ANOVA (Analysis of Variance) tests are applied to evaluate the association between the quantitative variables (Hcy, B12, folic acid) and genotypes for the groups involved in the study.

The T test is applied to set whether there are statistically significant differences between the averages of the two selections.

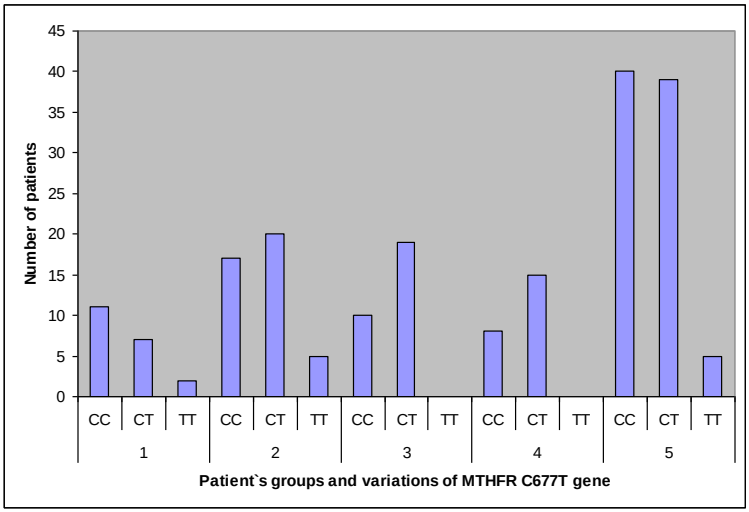
P values less that $p<0.05$, is regarded a statistically significant one.

The statistic data are processes applying the software package SPSS (*Statistical package for the social sciences, SPSS Inc., Chicago, IL*) version 13.0, 2004.

3. THE RESULTS OF THE RESERCH

The level of B_{12} vitamin and folic acid were norms limit for all the patients involved in the study.

In the molecular analyses with DNA, 84 people were found with CC genotype, 100 people with CT genotype and 10 people with TT genotype. There was no more frequent occurrence of any genotype in the current patient groups ($p<0.01$) (Picture 3.1).



Picture 3.1. The polymorphismus of MTHFR 677C → T in patient groups

Diagnostic cods:

1 – Continuous paranoid schizophrenia

2 – Episodic pace paranoid schizophrenia with progredient pace and schizoaffective disorders

3 – Simple schizophrenia and schizotypal disorders

4 – Affective disorders (depression with anxiety, bipolar affective disorders, depression without anxiety

5 – Control (intact)

The more uncommon alleles T frequency in the group of patients is 0.29, but in the control group – 0. 24.

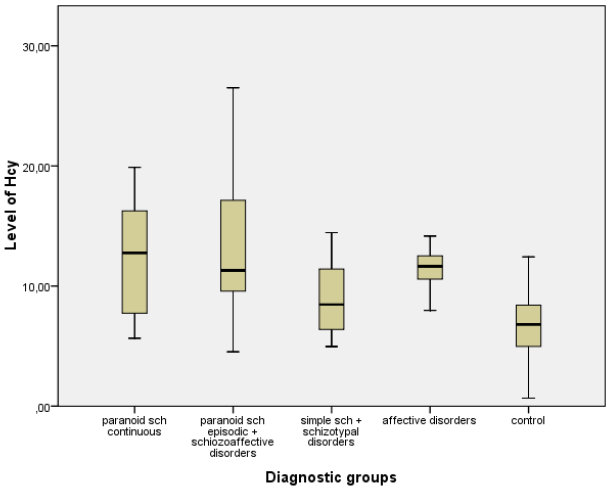
The correlation of Hcy concentration between control group and diagnostic groups in chart 3.1.

Chart 3.1.

The correlation of Hcy concentration between control group and diagnostic groups

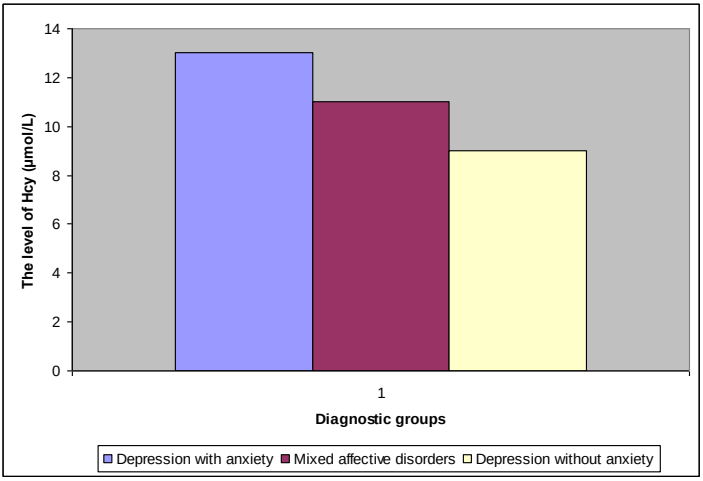
Control / Diagnose	r	p
Schizophrenia spectrum disorders	-0.46	<0.01
Continuous paranoid schizophrenia	-0.58	<0.01
Episodic pace paranoid schizophrenia with progredient pace and schizoaffective disorders	-0.53	<0.01
Simple schizophrenia and schizotypal disorders	-0.19	<0.01
Pacients with affective spectrum disorders	-0.45	<0.01

Higher Hcy level is observed to patients with more serious schizophrenia illness pace (continuous pace of paranoid schizophrenia (12.76 ± 5.25 μmol/L), episodic pace paranoid schizophrenia and schizoaffective disorders (11.30 ± 7.75 μmol/L) (r = -0.56; p <0.01). The patients with simple schizophrenia and schizotypal disorders had Hcy level of 8.47 ± 3.26μmol/L (Picture 3.2).



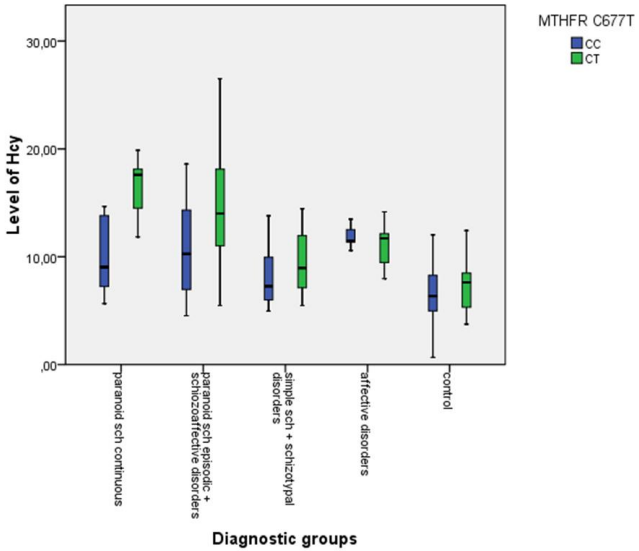
Picture 3.2.The meen level of Hcy (µmol/L) for patients with schizophrenia spectrum disorders and affective spectrum disorders

In the group of affective disorders, the patients were divided into 3 subgroup depending clinical symptomatic (Picture 3.3).



Picture 3.3. The level of Hcy for patients with affective disorders

For the patients of the first two diagnostic groups (continuous pace paranoid schizophrenia and episodic pace paranoid schizophrenia, schizoaffective disorders) Hcy level is influenced by the presence of *MTHFR* gene variants. For the patients with CT genotype, Hcy level is significantly higher thatn for the patients with CC genotype. In the other groups, changed of Hcy level depending on the genotype were not stated (Picture 3.4).



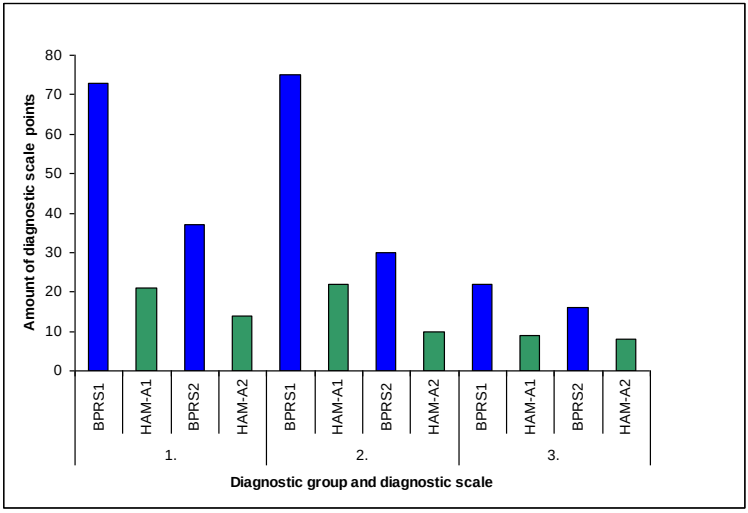
Picture 3.4. The mean level of Hcy (µmol/L) in patients with affective disorders and schizophrenia spectrum disorders depending on MTHFR C677T polymorphism

According to the picture (Picture 3.4), the difference of average Hcy levels is obvious within the groups 1 and 2, in the other groups the difference of the average levels is not so significant, however, taking into account dispersion of the data, the hypothesis on the diagnosis of the average Hcy level similarities depending on CC and CT genotypes.

T test is applied for checking the hypothesis, since the amounts of selection subgroups are small and different.

The results of the tests show that the difference of average values is statistically significant depending on CC and CT only in cases of the groups 1 and 2 (accordingly $p<0.001$ ND $p<0.028$). The difference of average Hcy levels for the diagnosis and the control groups 3 and 4 depending on CC and CT is not statistically significant (accordingly $p<0.28$ and $p<0.43$) .

In the picture 3.5, the evaluation of schizophrenia and illnesses of schizophrenia spectrum patients is shown according to diagnostic scales.

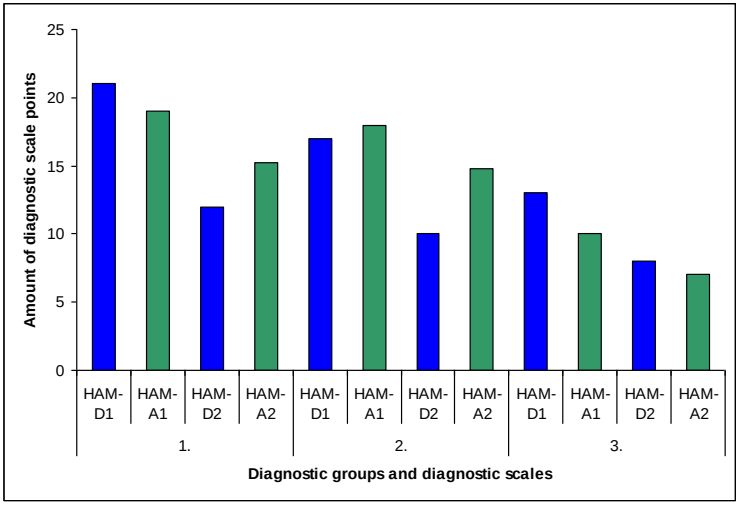


Picture 3.5. The scores of diagnostic scales for patients with schizophrenia spectrum disorders

1 – Continuous paranoid schizophrenia
2 – Episodic pace paranoid schizophrenia with progredient pace and schizoaffective disorders
3 – Simple schizophrenia and schizotypal disorders
BPRS-1 – admitted in hospital
BPRS-2 – discharging from hospital
HAMA-1 – admitted in hospital
HAMA-2 – discharging from hospital
Hcy-1 – level of Hcy (μmol/L) admitted in hospital
Hcy-2 – level of Hcy (μmol/L) discharging from hospital

In the picture 3.6, a patient group with illnesses of affective disorders spectrum is reflected. The severity level is measured according to the Hamilton

depression scale, but the anxiety level of patients is set according to the Hamilton anxiety scale.



Picture 3.6. The scores of diagnostic scales for patients with affective spectrum disorders

1. Depression with anxiety
2. Mixed affective disorders
3. Depression without anxiety

HAM-D1 –HAM-D before therapy
HAM-A1- HAM-A before therapy
HAM-D2 –HAM-D after therapy
HAM-A2 - HAM-A after therapy

According to the given chart (chart No 3.2.) it is seen that Hcy level is higher for patients of schizophrenia of first to diagnostic groups with CT genotype. These patients have significantly higher indices of diagnostic scales that show that these patients have more serious pace of schizophrenia, they

have significantly higher indices of Hamilton anxiety scale, which indicate that illness is with explicit affective saturation, anxiety.

The Hcy level indices for patients of schizophrenia spectrum disorders with CC genotype diagnostic scale were significantly lower.

Chart 3.2.

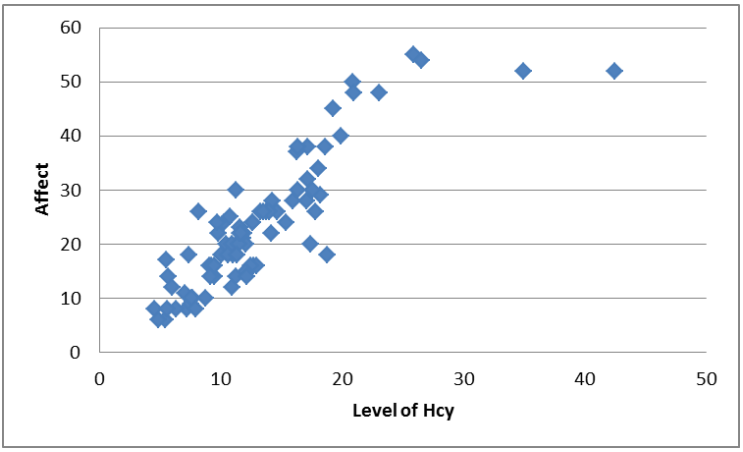
The mean level of Hcy (µmol/L), MTHFR C677T gene polymorphism and indices of diagnostic scales in patients with affective disorders and schizophrenia spectrum disorders

Diagnostic group	Genotype	Hcy (µmol/L)	BPRS	HAM-A
1	CC	9,03	40	12
	CT	17,6	90	28
2	CC	10,27	45	14
	CT	14,01	86	26
3	CC	7,26	20	10
	CT	8,96	28	12
4	CC	11,47	-------	18
	CT	11,71	-------	19
5	CC	6,35	-------	------
	CT	7,62	-------	------

The picture 3.7. shows reflects the following – the connection between Hcy level and explicit level of the affect that is set according to Hamilton anxiety scale for patients of schizophrenia. Here there are two observations that do not satisfy quality criteria; we did not analyze them further on. In the result, 79 observations remain from 81 observations.

It is graphically seen that the connection between Hcy level and the affect is positive; it means that a hypothesis can be set that at the higher Hcy level the level of the affect is higher. To verify solidity of these connections, the correlation analysis was carried out.

According to the picture (Picture 3.7), we can conclude that the patients with more serious affective saturation (especially anxiety level), which is measured according to HAMA-A scale, is stated higher Hcy level.



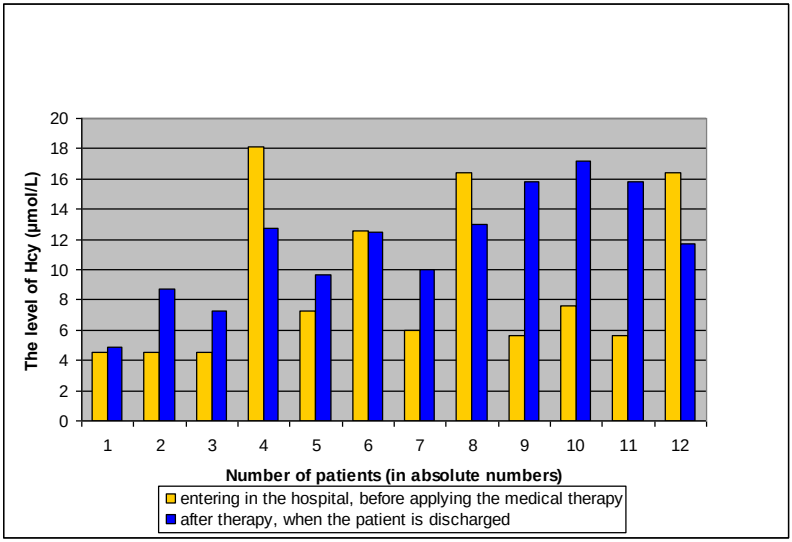
Picture 3.7. The mean level of Hcy (µmol/L) and level of affect (HAM-A – amount of scores) for diagnostic group patiens

The correlation analysis confirms previously set hypothesis. The connection between Hcy level and affect level is very tight (the ratio of correlation is 89%) and this connection is equally set.

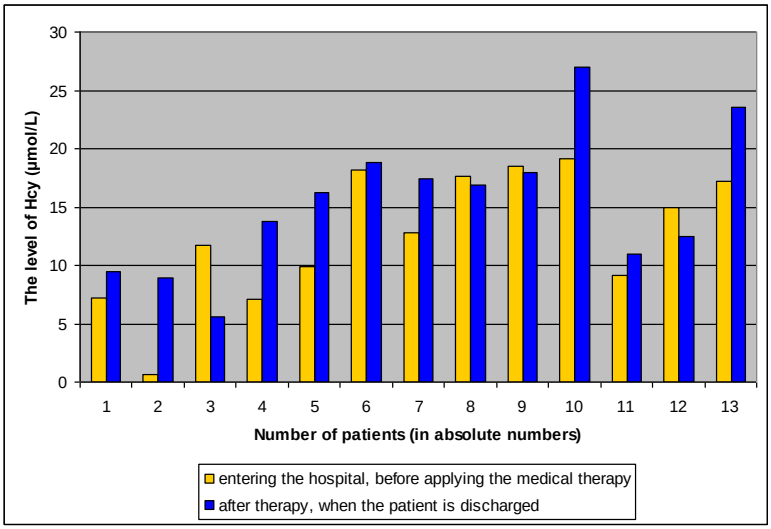
In the dynamics, Hcy level is set for 43 patients (Picture 3.8). According to the results it can be concluded that Hcy level in the dynamics slightly increases practically for all patients of diagnostic groups. Only in the control group it is observed that Hcy level is constant. Most of all Hcy level increases in the diagnostic group 1. It can be explained with the specifics of clinical sign and therapy for the patients of this group. Illness for the patients is serious; intensifications are permanent and remission-partial. The patients get massive, combined psychopharmacotherapy both in the hospital and at home.

Supposedly, this condition is the one that sets the increase of Hcy level in the dynamics.

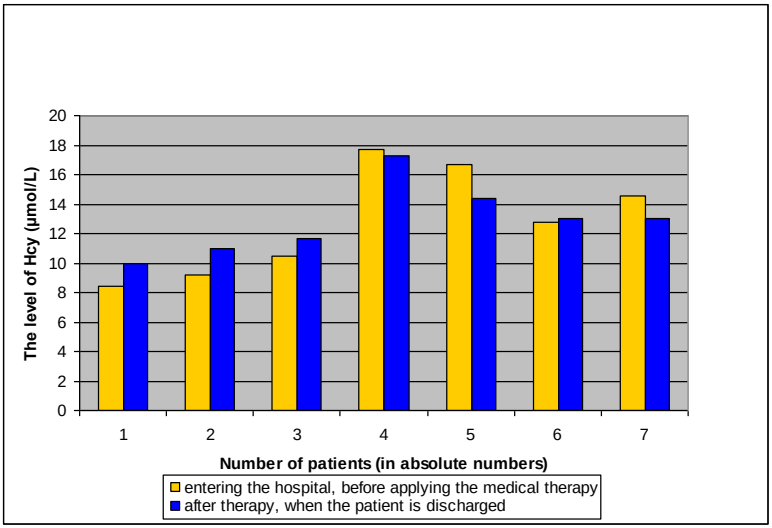
In the following pictures (Pictures 3.8.-3.11.) changes of Hcy level in the dynamics are reflected, after carrying out the recurrent analyses. The first time Hcy level was set when entering the hospital, before applying the medical therapy. The second time the level is set after the therapy, when the patient is discharged.



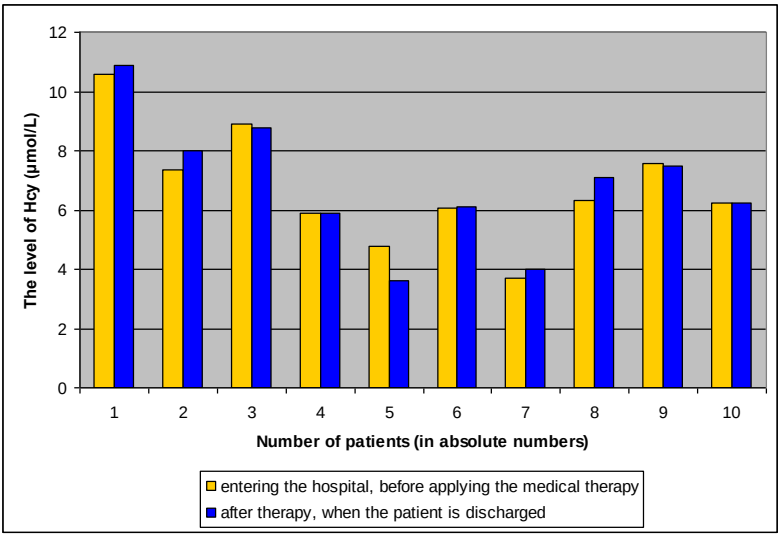
Picture 3.8. The changes of Hcy level (µmol/L) for patients who get anti-psychotic monotherapy



Picture 3.9. The changes of Hcy level (µmol/L) for patients who get anti-psychotic polytherapy and / or is therapy resistant



Picture 3.10. The changes of Hcy level (µmol/L) for patients who get anti-depressiv therapy



Picture 3.11. The changes of Hcy level (µmol/L) for patients without medicines

4. DISSCUSSION OF THE RESULTS OF THE RESEARCH

Still diagnostics of schizophrenia and schizophrenia spectrum disorders is problematic, since there are no specific laboratory examinations to diagnose them. The diagnostics of schizophrenia always firs of all is based on the clinical signs – specific negative symptoms: autism, emotional dullness, the decrease of activity, peculiar changes of mentality. The crucial criteria in the case of schizophrenia of all forms is a criteria of dynamics which characterises with a progressive pace and appearance of qualitatively new symptoms, the dilation of their psychopathological diapason and with the increase of the above-mentioned negative symptoms [15].

Different biological markers are being studies to find new, more proof confirming analyses, examinations of schizophrenia. Hcy is mentioned as one of such markers.

The of the study was to clarify the role of Hcy in the case of schizophrenia spectrum disorders, as well as to clarify the influence of medicine on the changes of Hcy level.

In Latvia, for the first time the study is worked out in children's psychiatry on the possible ethiology, illness pace and forecasts of schizophrenia and illness of affective disorders spectrum.

Now, there are no practical studies on hyperhomocisteinemia in connection with psychic disorders in psychiatry of children. There are separate studies with numerically few patient groups on the connection of Hcy level with schizophrenia illness for teenagers (age 14 - 21). It was stated that Hcy level is higher for the patients with schizophrenia than to in the control group. However, this connection was observed almost concerning boys [16].

Up to now, in the described studies it is set that higher Hcy level is for women with depression or BAT, but for men with schizophrenia [17]. In the study it was stated that Hcy level is increased equally to BAT and in the case of schizophrenia. It is known that out of psychic disorders women more often have a mood and anxiety illnesses, but men more often than women have schizophrenia or schizophrenia spectrum disorders / illnesses. This fact directs to think that Hcy in not specific marker of schizophrenia or affective disorders, but general marker of psychic disorders.

Therefore we included in the study patients with schizophrenia and depressive disorders. It is proved that many biological fields for schizophrenia and mood disorders are similar. However, the clinical sign, pace and therapy of the illness differs significantly.

It can be found in the literature that a high Hcy level for younger patients - men could be due to the pathophisiological aspects of the illness. For example, it is known that the beginning of schizophrenia is early than to women. It is also proved that that the illness has a trend to become chronicle, if a man gets ill at a younger age.[87;88]. It is also described that the Hcy level is higher for those whose illness progresses while under examination (or it has started recently) [18;19;20].

In study, there were mostly boys in the group of schizophrenia patients (61 %) (the boys have characteristics of illness with schizophrenia at the age of teenagers, the girls – closer to maturity and after the age of 20), however, in the group of affective disorders, there were mostly girls (80%) (Women with affective disorders get ill more often).

Comparing Hcy level for boys and girls, the difference was not found.

Up to now, there is no information in the studies on the different schizophrenia or nosological groups of affective disorders with significant increase of Hcy level. After setting the Hcy level, its concentration in blood

plasma was not analysed in connection with the pace of the illness, forecast and the invalidity level of patients.

All the studies mostly were carried out in the groups of younger patients with schizophrenia and affective disorders, as well as of those patients who got ill recently; the biggest part of the patients it was the first or the second time of hospitalization. With certainty higher Hcy level was set in the groups of young men, both with schizophrenia and affective disorders.

At the same time, there is a little information on the connection between the anxiety and the increased Hcy level. Up to now, the interaction of anxiety and Hcy level and mutual connection is not studies.

According to Hcy level differences between the diagnostic groups of our study, we have to think that its level depends of the clinical features of the illness. Hcy level is higher for the patients with an acute start of the illness, and it characterises with affective saturation and more serious general psychic and somatic condition. In those diagnostic groups were patients with continuous pace paranoid schizophrenia with the average Hcy level of 12.76 ± 5.25 μmol/L and episodic pace paranoid schizophrenia and schizoaffective disorders with the average Hcy level in blood plasma of 11.30 ± 7.75 μmol/L. The increased Hcy level during aggravation of the illness could be connected with a stress in the condition of acute psychosis and in such way causing different neurodegenerative impacts and clinical worsening.

Up to now, there is not proved mechanism which is connected with Hcy level with psychic illnesses. It is described that Hcy causes vascular damages, therefore influencing CNS blood supply and the disorders of CNS development and functions appear [21].

It is possible that a subclinic deficit of folic acid and B_{12} vitamin causes a bigger chance that a child will have schizophrenia [22].

However, this news are also on another mechanism, when in the result of neurodegenerative processes the shrinking and death of brain cells is

observed, which causes the increase of permeability of cell membranes and Hcy gets in space of cells. At the same time, a reparation process is observed in tissues, which is along with reactions of methylation. In the result, the Hcy forms [23]. The above-mentioned cases on the increased Hcy level are also as markers of pathological condition not as an ethiological factor of the illness [24].

Another mechanism is described as well, when homocysteine and homocysteine acid have ability to increase the level of intracellular calcium ions and amount of active oxygen connection in the brains of rats, similar like NMDA (N – metil – D - aspartat acid). It causes the premature death of cells. These mechanisms are in the base of Hcy and their neurotoxic impact of its derivates [25]. In such a way, the metabolic and toxic encephalopathies are explained. The studies show that in the result of increased Hcy level there is stress condition in tissues, followed up by increase of permeability of hematoencephalic barrier for neurotoxic substances [26;27]. In the result of this process, worsening of clinical symptomatic, neurodegenerative processes are observed, the pace of the illness has a trend to become chronical.

The women with schizophrenia and better reaction to therapy, more often had T allele [28]. It allows thinking that, possibly, there are some genetic changes which would have been to men with schizophrenia. It was not stated in the study that T allele is more often to women. Due to a small number, TT genotype was set only to 10 people involved in the study; therefore, the data are not included in the analysis. More often, CT genotype was stated; however, the dominance of the mentioned genotype was not set in any of the diagnostic groups. When analysing the patients of schizophrenia more deeply, I stated that there are differences in the clinical sign of the illness and pace for patients with CT and CC genotype and different *MTHFR* C677T genotypes. The patients who have *MTHFR* gene C677T polymorphism in heterozygotic form, a more serious illness pace is observed – there are more characteristic forms of

"serious" schizophrenia (continuous pace or episodic with a progress).the illness is with more serious condition of general patients (both, somatic and psychic). The patients are more often in the hospital, they have polypharmacotherapy. On this score, the data of our study did not agree with the literature, where the existence of T allele is connected with a more positive reaction to the therapy of medicine. After mutation of *MTHFR* gene C677T for heterozygotic patients the resistance to therapy; they received bigger doses of medicine than patients with CC genotype did. Patients with CT genotype got more side effects (cardiological the changes of QTcinterval, side effects of endocrine and extrapyramidal system). To a certain extent, related to the study carried out in Israel, where young men (under the age of 50) were observed. They were ill with schizophrenia or schizoaffective disorders, they had tardive movement disorders (tardive dyskinesia and parkinsonism), which are the side-effects of neuroleptic treatment. It was stated that in the patient group with tardive movement disorders, there was a higher HCY level in the blood. It makes us believe that possibly the higher HCY level can be connected with a risk that in the result of the therapy the movement disorders could appear [39].

Currently, it is not possible to say precisely what causes more side effects for the patients with CT genotype – extreme sensitivity, bigger doses of medicine or both the factors. However, we can conclude that the patients with CT genotype have to use medicine more carefully, it is necessary to follow the somatic condition of the patients to discover and treat the possible side effects.

It is described that there is a possible connection between the gene which is responsible for disorders of Hcy metabolism and positive reaction to usage of neuroleptic medicine.

In Canada, the study was carried out, where the reaction of schizophrenia patients was analysed to the therapy of conventional neuroleptic medicine and indices of illness remissions with a patient group where the resistant forms of the illness's therapy and "bad" remissions were observed; as

well as a control group of healthy people was compared. It was observed that in the group with a good reaction to therapy of conventional neuroleptic medicine there were more women; better illness indices in a longer period, “better” remissions; more often it neurotransmission of dopamines was stated [30]. There is an opinion that neuroleptic medicine often helps reducing the intensity of symptoms, but significantly do not reduce the psychotic episode [31].

The results of another study testify that if *MTHFR* gene mutation is involved in the pathogenesis of schizophrenia, the illness has a fast and consistent positive reaction to typical therapy of neuroleptic medicine and / or good forecast of permanent illness. It was also stated that the patients who reacted well on the therapy of conventional neuroleptic medicine, were observed at least one recidive of the illness, in case when a dose of neuroleptic medicine was decreased or it was completely cancelled [32].

It is characteristic in the recurrent Hcy level analysis that Hcy level has increased in the dynamics for the patients which had continuous pace paranoid schizophrenia ($r = 0.82$; $p<0.01$). In the other diagnostic groups, the correlation between the diagnosis of the patient and increase of Hcy level was not so tight. I think that the given changes can be connected with the following factors. Firstly, it could testify that the changes in the molecular level go on, despite the fact that the clinical sign improves and stabilizes. Therefore, it can be concluded that the therapy appointed for the patients is only symptomatic – it decreases the existing symptoms, but it does not influence the neurodegenerative process, it does not stop it. Secondly, it is possible, that the sense is in the therapy appointed for the patients, which for the patients of continuous pace paranoid schizophrenia is complex. It consists of several antipsychotic medicine, correctors, mood stabilizers.

Up to now, there are no data on the used medicine for psychic disorders (neuroleptic medicine, antidepressive medicine, antiparkinsonism medicine, stabilizers of mood) impact of Hcy level.

It is proved that many anticonvulsants decrease the level of folic acid in blood plasma and in the result, almost a half of the patients getting the anticonvulsants in the therapy, have comparatively high Hcy level and therefore, the risk of development of vascular illnesses improves [33;34].

However, the patients involved in our study the higher Hcy level was not stated, if there was valproic acid in the therapy, the level of folic acid, B_6 and B_{12} vitamins in serum plasma was normal.

According to the results of the pilot study [*Susser E., et al., 1998*], where Hcy level for the patients of schizophrenia and healthy individuals with low an normal level of folic acid was compared, it can be concluded that for the patients with a low level of folic acid, the level of Hcy was higher than for the healthy individuals with a low level of folic acid. In the group with a normal level of folic acid, it did not differ. The data of the study confirmed the hypothesis that the sensitive defect of folic acid in Hcy metabolism in some cases causes development of schizophrenia [35].

Therefore, in the future more attention has to be paid to studies, how much usage of B_6 and B_{12} vitamins, folic acid and trimetilglicine could change the schizophrenia and mood disorders, and pace [36]. It is possible that the additional appointing of the mentioned vitamins could prevent intensification of the illness or to change the pace of the illness to easier way.

A clinical case is described on the young patient with significantly higher concentration o Hcy serum. In the therapy, additionally to neuroleptic medicine, the injections of B_{12} vitamin were appointed and the patient`s clinical state get better, but after the treatment of vitamins – clinical state deteriorated [37].

In the study with depressive patients, it was stated that the reaction to antidepressive therapy is worse, if the level of folic acid is low in blood and to improve the clinical condition it was suggested to appoint additionally also folic acid [38].

It is possible that additional therapy with these vitamins could worsen schizophrenia to many patients [16;39]. In the studies where the schizophrenia patients (with significantly higher Hcy level) were appointed vitamin therapy (for 12 weeks they took 2 mg of folic acid along with 400 μg of vitamin B_{12} and 25 mg of vitamin B_6), after some time the decrease of Hcy level ws observed, as well as reducing of schizophrenia symptomatic [40].

5. CONCLUSIONS

1. For children and teenagers in all the studies and control groups the level of B_{12} vitamin and folic acid was within the limits.
2. Recognised a correlation between the continuous pace paranoid schizophrenia, schizoaffective disorders and Hcy level (respectively r = 0.58 and r = 0.53; $p<0.01$). At the same time among the simple schizophrenia and schizoaffective disorders one the one hand and the increased Hcy level from the other hand, the correlation was not found (r = 0.19; $p<0.01$).
3. There is a correlation between the increased Hcy level and affective disorders (r = 0.45; $p<0.01$), but relatively less that for illnesses of schizophrenia spectrum.
4. The more serious is the pace of schizophrenia and a significant affective saturation is observed, the higher the level of Hcy is detected.
5. In the case of continuous and episodic pace paranoid schizophrenia, as well as schizoaffective disorders, Hcy level is higher, is the patient has *MTHFR* C677T gene CT genotype.
6. In the case of *MTHFR* gene CT genotype, continuous and episodic pace paranoid schizophrenia, as well as schizoaffective disorders is clinically more serious.
7. The prevalence of *MTHFR* gene C677T TT genotype was not found in any group, the frequency of T allele in the patient group was 0.29, but in the control group 0.24.
8. The increase of Hcy level in the dynamics at the this moment can not be explaned. The increase can be attributed with the illness process, which continues despite the improvement of the clinical sign and/ or received therapy with medicines.

6. PRACTICAL RECOMMENDATIONS

In the clinical practice, it had to suggested to set Hcy level, as well as polymorphism of *MTHFR* C677T gene and concentration of vitamins that influence Hcy level (folic acid, B group vitamins) for the patients with illnesses of schizophrenia spectrum and mood disorders. The level of vitamins has to be set each 3-5 years, since the changes in the diet and / or therapy of medicaments could cause their deficit.

If a patient is stated *MTHFR* gene C677T CT genotype – it would be necessary to evaluate the therapy of medications more carefully and to follow the possible side effects of the therapy (tardive dyskinesia, tremor). It has to be taken into account that these patients have a higher risk for another illness with the increased level of Hcy level. The main is heart – blood vessels illnesses (cardiac infarction, cerebral thrombosis).

It would be desirable to continue the started study, involving a greater number of patients to set whether there is prevalence of any genotype in any diagnostic group, as well as to analyse the influence of the therapy of medications (medicaments and their doses) and Hcy level for schizophrenia patients.

7. PUBLICATIONS (SCIENTIFIC ARTICLES), ABSTRACTS AND PRESENTATIONS ON THE RESEARCH TOPIC

7.1.Publications (scientific articles) on the study research topic

1. L. Kevere, S. Purvina, D. Bauze et al. „Homocysteine and MTHFR C677T polymorphism in children and adolescents with psychotic and mood disorders" manuscript is accepted for publication in Nordic Journal of Psychiatry 05.04.2013.
2. L. Kevere, S. Purvina, D. Bauze et al. „"Elevated serum levels of homocysteine as an early prognostic factor of psychiatric disorders in children and adolescents," Schizophrenia Research and Treatment 2012; 2012:373261 Published Online 2012 October 2. doi:10.1155/2012/373261
3. L. Kevere, S. Purvina, D. Bauze et al. "Hyperhomocysteinemia in children and adolescents with psychotic and mood disorders". Bridging Eastern and Western Psychiatry". 2009; 7: (2): 23-30.
4. L. Ķevere, S. Purviņa, D. Bauze et al. "Hiperhomocisteinēmija kā riska faktors un marķieris psihiatrisko slimību norisē", RSU scientific papers 2008. p.345-249.
5. L. Ķevere, S. Purviņa, D. Bauze et al. "The link between hyperhomocysteinemia and methylenetetrahydrofolate reductase 677C→T polymorphism in children and adolescents with psychotic disorders". RSU scientific papers 2011. p.166-170.

7.2.Abstracts on the research topic

1. L. Ķevere, S. Purviņa, D. Bauze et al. "The link between hyperhomocysteinemia, methylenetetrahydrofolate reductase 677 C→T polymorphism and psychiatric disorders in children and adolescents" Neuropsychiatrie de l`enfance et de l`adolescence. IACAPAP 2012 20th World Congress – Paris. 2012; 60: (5): 227.
2. L. Ķevere, S. Purviņa, D. Bauze et al. "MTHFR gēna polimorfisms un homocisteīna līmeņa atšķirības bērniem un pusaudžiem ar šizofrēnijas spektra saslimšanām", RSU Scientific conference thesis 2012, p. 232.
3. L. Kevere, S. Purvina, D. Bauze et al. "Hyperhomocysteinemia, methylenetetrahydrofolate reductase 677C→T polymorphism and psychiatric disrders in children". European Neuropsychopharmacology. 2012; 22: (1): 64-65.
4. L. Kevere, S. Purvina, D. Bauze et al. "Influence of homocysteine on psychiatric disorders". 2011. 10th World Congress of Biological Psychiatry. P-14-007.
5. L. Kevere, S. Purvina, D. Bauze et al „Hyperhomocysteinemia in children and adolescents with psychotic and mood disorders" „2nd Young Psychiatrists Network Meeting", Riga, Latvia: April 6-8 2011,abstract book p. 39-40.
6. L. Kevere, S. Purvina, D. Bauze et al "Elevated level of homocystein for children and adolescents with psychotic and mood disorders". European Neuropsychopharmacology. 2011; 21: (2): 169-170.
7. L. Kevere, S. Purvina, D. Bauze et al. " Children and adolescents with psychotic and mood disorders and level of homocysteine" Chinese medical Journal 2010; 123: (2): 41-42.

8. L. Ķevere, S. Purviņa, D. Bauze, M. Zeibārts, A. Riževs, M. Caune, I. Purviņš, R. Andrēziņa. "Paaugstināts homocisteīna līmenis bērniem un pusaudžiem ar psihotiskiem un afektīviem traucējumiem. " RSU Scientific conference thesis 2011, p. 191.
9. L. Ķevere, S. Purviņa, D. Bauze u.c. „Hiperhomocisteinēmija bērniem un pusaudžiem ar šizofrēnijas spektra un afektīviem traucējumiem." RSU Scientific conference thesis 2010, RSU, 243.
10. S. Purvina, L. Kevere, D. Bauze et al. „Hyperhomocysteinemia in children and adolescents with psychotic and mood disorders" European Psychiatry The journal of the European Psychiatric Association Abstracts on CD-Rom. 2010; 25: (1): PW01-191.
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13. L. Ķevere, S. Purviņa, D. Bauze, M. Zeibārts, A. Riževs, M. Caune, I. Purviņš, R. Andrēziņa. „Homociateīna līmeņa asinīs saistība ar šiozofrēnijas norises gaitu un tās klīniskām izpausmēm." RSU Scientific conference thesis 2009, p.75. lpp.
14. D. Bauze, L. Piekuse, B. Lāce, L. Ķevere, S. Purviņa, M. Zeibārts, A. Riževs. „Homocsteīna līmeņa atkarība no MTHFR C677T polimorfisma Latvijas populācijā." RSU 2009. gada zinātniskā konference. RSU Scientific conference thesis 2009, p. 117.lpp.

15. Laura Ķevere, Santa Purviņa, Daiga Bauze, Mārcis Zeibārts, Arnis Riževs, Indulis Purviņš. Hiperhomocisteinēmija bērnu psihiatrijas klīnikā. RSU Scientific conference thesis 2008.
16. L. Ķevere, S. Purviņa, D. Bauze u.c. „MTHFR gēna polimorfisms un homocisteīna līmeņa atšķirības bērniem un pusaudžiem ar šizofrēnijas spektra saslimšanām" RSU Scientific conference thesis 2012.

7.3.Oral and poster presentations at congresses and conferences on the search topic

1. L. Ķevere, S. Purviņa, D. Bauze et al. "The link between hyperhomocysteinemia, methylenetetrahydrofolate reductase 677 C→T polymorphism and psychiatric disorders in children and adolescents" Neuropsychiatrie de l`enfance et de l`adolescence. IACAPAP 2012 20th World Congress – Paris. (poster presentatione).
2. L. Ķevere, S. Purviņa, D. Bauze et al. "MTHFR gēna polimorfisms un homocisteīna līmeņa atšķirības bērniem un pusaudžiem ar šizofrēnijas spektra saslimšanām", RSU Scientific conference 2012 (oral presentatione).
3. L. Kevere, S. Purvina, D. Bauze et al. "Hyperhomocysteinemia, methylenetetrahydrofolate reductase 677C→T polymorphism and psychiatric disrders in children". Congress of European Neuropsychopharmacology. Marth 2012. (poster presentatione).
4. L. Kevere, S. Purvina, D. Bauze et al. "Influence of homocysteine on psychiatric disorders". 2011. 10th World Congress of Biological Psychiatry. (poster presentatione).

5. L. Kevere, S. Purvina, D. Bauze et al „Hyperhomocysteinemia in children and adolescents with psychotic and mood disorders" „2nd Young Psychiatrists Network Meeting", Riga, Latvia: April 6-8 2011. (oral presentatione).
6. L. Kevere, S. Purvina, D. Bauze et al "Elevated level of homocystein for children and adolescents with psychotic and mood disorders". Congress of European Neuropsychopharmacology. April 2011. (poster presentatione).
7. L. Kevere, S. Purvina, D. Bauze et al. "Children and adolescents with psychotic and mood disorders and level of homocysteine" IACAPAP 2010 19th World Congress – Beijing. (oral presentatione).
8. L. Ķevere, S. Purviņa, D. Bauze, M. Zeibārts, A. Riževs, M. Caune, I. Purviņš, R. Andrēziņa. "Paaugstināts homocisteīna līmenis bērniem un pusaudžiem ar psihotiskiem un afektīviem traucējumiem. " RSU Scientific conference 2011 (poster presentatione).
9. L. Ķevere, S. Purviņa, D. Bauze u.c. „Hiperhomocisteinēmija bērniem un pusaudžiem ar šizofrēnijas spektra un afektīviem traucējumiem." RSU Scientific conference 2010 (poster presentatione).
10. S. Purvina, L. Kevere, D. Bauze et al. „Hyperhomocysteinemia in children and adolescents with psychotic and mood disorders" Congress of European Psychiatric Association. 2010. (poster presentatione).
11. S. Purvina, L. Kevere, D. Bauze et al. "The link between Hyperhomocysteinemia and psychotic disorders in childhood and adolescents". Congress of European Psychiatric association 2009. (poster presentatione).
12. L. Kevere, S. Purvina, D. Bauze et al. „Hyperhomocysteinemia and individuality of psychotic disorders in childhood and adolescents".

European congress of Neuropsychopharmacology. 2009. (poster presentatione).

13. L. Ķevere, S. Purviņa, D. Bauze, M. Zeibārts, A. Riževs, M. Caune, I. Purviņš, R. Andrēziņa. „Homociateīna līmeņa asinīs saistība ar šiozofrēnijas norises gaitu un tās klīniskām izpausmēm." RSU Scientific conference 2009 (poster presentatione).
14. D. Bauze, L. Piekuse, B. Lāce, L. Ķevere, S. Purviņa, M. Zeibārts, A. Riževs. „Homocsteīna līmeņa atkarība no MTHFR C677T polimorfisma Latvijas populācijā." RSU Scientific conference 2009 (poster presentatione).
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16. L. Ķevere, S. Purviņa, D. Bauze u.c. „MTHFR gēna polimorfisms un homocisteīna līmeņa atšķirības bērniem un pusaudžiem ar šizofrēnijas spektra saslimšanām" RSU Scientific conference 2012 (poster presentatione).

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