Correlations Between Neuroendocrine Disorders and Biochemical Brain Metabolites Alterations in Antidepressant Treatment

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We approach the theme of modern investigation and treatment strategies, based on biochemical, clinical-biological, metabolic, pharmacogenetic, neuro-imagistic, and neuroendocrine integrative correlations in the management of depressive disorders. Our main objective was to investigate: the biochemical brain metabolites [N-acetyl-aspartate (NAA), gamma-aminobutyric acid (GABA), aspartate (Asp), creatine (CR), glutamine (Gln), glicerophosphocholine (GPC), phosphocholine (PC), phosphocreatine (PCr), taurine (Tau), N-methyl-D-aspartate (N-MDA), serine, glycine, choline (Cho)]; the neuroimagistic and neurobiological markers and the metabolic abnormalities in correlation with the molecular pharmacogenetic testing in children and adolescents treated with antidepressant medication. Our research was conducted between 2009-2016 on 90 children and adolescents with depressive disorders -45 children-G1, who benefited of pharmacogenetic testing tailored pharmacotherapy, and 45 without pharmacogenetic testing-G2. The patients were also evaluated by MR spectroscopy at baseline and after pharmacotherapy. The efficacy of the chosen therapy in correlation with the pharmacogenetic testing was evaluated by the mean change in the CDRS (Child Depression Rating Scale) total scores, in the CGI-S/I (Clinical Global Impression Severity/ Improvement), CGAS (Clinical Global Assessment of Functioning) and by the change of the relevant neurobiological markers and MR spectroscopy biochemical brain metabolites. Our results showed statistically significant differences in the clinical scores between the studied groups. Our research could represent a proof that the biochemical brain metabolites registered in depressive disorders modified values in the MR spectroscopy and the administration of antidepressants could determine metabolic and neuroendocrine abnormalities (changed lipid profiles, high insulin and plasma glucose levels, weight gain, obesity), especially when chosen without prior pharmacogenetic testing.

Keywords: biochemical metabolites, N-acetyl aspartate (NAA), gama-aminobutyric acid (GABA), metabolic abnormalities, antidepressants, pharmacogenetic testing

Antidepressants are first choice medication in depressive disorders, being used in the treatment of bipolar disorders, as well. Despite the advantages, they present considerable side effects – metabolic (changed lipid profiles, high insulin and plasma glucose levels, weight gain, obesity), and neuroendocrine abnormalities, which can result in discontinuation of treatment [1, 2].

Antidepressant drugs, in particular some of them, influence cellular lipogenesis and are associated with metabolic side effects,including weight gain, body mass index (BMI) and blood insulin level increase [3-6]. Antidepressant-induced weight gain has important effects on long-term health. The weight gain being one of the most undesirable antidepressants side effects, poses the patients to other significant risks: diabetes, metabolic syndrome, lipid abnormalities, and cardio-vascular events [7-14]. Due to the increasing use of antidepressants in children and adolescents, their metabolic and endocrine adverse effects are of particular concern, especially within the paediatric population that appears to be at greater risk [15-17].

Our main objective was also to investigate the biochemical brain metabolites through MR spectroscopy [N-acetyl-aspartate (NAA), gamma-aminobutyric acid (GABA), aspartate (Asp), creatine (CR), glutamine (Gln), glicerophosphocholine (GPC), phosphocholine (PC), phosphocreatine (PCr), taurine (Tau), N-methyl-D-aspartate (N-MDA), serine, glycine, choline (Cho)] [18].

Glutamate and glutamine, which can be clearly identified and, in part, quantified in MR spectroscopy of the brain, play important roles in normal and pathological biochemistry. Pathways of glutamate metabolism include transamination, dehydrogenation, deamination and decarboxylation (to GABA). Glutamine is notable in hepatic encephalopathy, but is also a significant metabolic fuel in several other organs and tissues, including neoplasms. Myo-inositol is a 6-carbon alcohol, which acquires new interest from its detection and quantitation in MR spectroscopy. One of its roles–is the biochemical relationship to messenger-inositol polyphosphates. The new perspectives in the field of neuroimagistics and pharmacogenetics give us the opportunity to make some connections between the clinical features, the neurobiological, pharmacogenetic and neuroimagistic markers and the further clinical evolution and prognostic in depressive disorders [19-24]. The treatment of choice in the management of depression should be chosen in correlation with the biochemical, neurobiological, pharmacogenetic, neuroimagistic and clinical profile of the target patients. When choosing the suitable pharmacotherapy, the pharmacogenetic markers should be carefully analyzed [25-27].

In our present research–we approach the theme of modern treatment strategies, correlated to the pharmacogenetic testing, the neurobiological, neuroendocrine and imagistic markers, in the management

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of depressive disorders in children and adolescents [1, 2, 19-25].

We will capture the clinical significance of modern pharmacologic treatment approaches correlated to the evaluation of the neuroimagistic markers, especially through MR spectroscopy [21, 25-27]. The genetic variations in the P450 cytochrome system (CYP) is correlated to the response to medication in depressive disorders. The CYP polymorphism determines different metabolic phenotypes [1, 3, 4, 7]. Most studies and guidelines mention the serotonin reuptake inhibitors (SSRI) antidepressant treatment as the first choice treatment in depressive disorders.

Experimental part

Patients, material and methods

The present research was performed between the years 2009 and 2016, in the University Hospital for Child and Adolescent Psychiatry and Neurology Timi'oara, in collaboration with the Center of Genomics. Our actual study was focused especially on biochemical, neuro-biological, neuroendocrine, neuroimagistic, respectively, clinical aspects and on specific pharmacogenetic correlations.

The study population included 90 patients (children and adolescents) with depressive disorders. We obtained the informed assent for each patient and the informed consent from the parents/legal guardians. Our study was done in accordance to the Ethical Committee regulations of the Victor Babes University of Medicine and Pharmacy Timisoara, with the ICH-GCP (Good Clinical Practice) regulations and guidelines, taking also into account ethical models proposed for healthcare institutions [28].

Our study population was divided in 2 groups: in 45 patients the antidepressant treatment was guided by the pharmacogenetic testing (group I) and 45 patients were treated without pharmacogenetic testing prior to the treatment election.

Biochemical metabolites and neuroimagistic investigations (MR spectroscopy)

We investigated key aspects of the cerebral function and metabolism by MR spectroscopy. We quantified the following neurometabolites: [N-acetyl-aspartate (NAA), gamma-aminobutyric acid (GABA), aspartate (Asp), creatine (CR), glutamine (Gln), glicerophosphocholine (GPC), phosphocholine (PC), phosphocreatine (PCr), taurine (Tau), N-methyl-D-aspartate (N-MDA), serine, glycine, choline (Cho)].

We used the MR Spectroscopy Software Package for the MR spectral quantification, which automatically calculated a matrix of the correlation quotients and of concentrations of the cerebral biochemical metabolites. So that we evaluated the efficacy of the chosen pharmacotherapy in correlation with the pharmacogenetic testing and the variation of the cerebral metabolites, quantified by MR Spectroscopy, by the change of the mean total scores of the scales (CDRS-Child Depression Rating Scale, CGI-S/I-Clinical Global Impression of Severity/ Improvement, CGAS-Clinical Global Assessment of Functioning, CSSRS-Columbia Suicide Severity Rating Scale) from baseline till endpoint in different timepoints.

Metabolic abnormalities investigations

We investigated the metabolic parameters – lipid profiles (plasma cholesterol levels, triglycerides), insulin plasma levels, plasma glucose levels, blood pressure, BMI change, weight gain. For every patient treated with antidepressants, a clinical examination, a set of hematological, biochemical, lipid, coagulation blood tests and electrocardiogram (ECG) (especially investigating the QTc complexes prolongation) were performed. Blood pressure, heart rate and vital signs, and plasma glucose were monitored. Glucose (molecular formula: $C_6H_{12}O_6$) is a mono-saccharide existing in nature only as D-isomer form.

During the antidepressant treatment is also important to monitor the renal function, so we also evaluated the renal function through the serum urea, creatinine and uric acid. Urea (CH_4N_2O) is an organic compound with a carbonyl (C=O) functional group linked to two NH_2 groups. Creatinine- 2-Amino-1-methyl-5H-imidazol-4-one, an important indicator of renal function.

Uric acid (7,9-Dihydro-1H-purine-2,6,8(3H)-trione)-a diprotic aromatic acid- is a product of purine nucleotides metabolism, leading to metabolic abnormalities in case of high values.

The lipid profiles of the patients were assessed by determining the high-density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), total cholesterol (T-Chol) and triglyceride plasma levels.

HDL is the smallest and densest lipoprotein particle- and plays an anti-atherogenic role by removing the excess of cholesterol from cells. The non-HDL cholesterol lipoproteins are atherogenetic and represent a good predictor of cardiovascular events. LDL-Chol particles represent the most important cardiovascular risk predictors.

Cholesterol (=(3beta)-cholest-5-en-3-ol) is an organic molecule with 256 stereo-isomers, being a crucial component of cell membranes and a precursor for steroid hormones, vitamin D, and bile acids.

Triglycerides are esters derived from the combination of glycerol and three fatty acids (RCO_2H, $R'CO_2H$ and $R"CO_2H$), according to the formula:

$$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R"CO_2H = RCO_2CH_2CH(O_2CR')CH_2CO_2R" + 3H_2O$$

Plasma levels of thyroid stimulating hormone (TSH), free T4 (FT4), free T3 (FT3), and insulin were also assessed. Inflammatory biomarkers [C-reactive protein (CRP), interleukine 3 (IL-3), interleukine 6 (IL-6), fibrinogen] were also tested.

Clinical evaluation of the patients

In order to analyze the clinical evolution of the patients in each study group, we applied the following instruments and scales: CDRS-Child Depression Rating Scale, CGI-S/I-Clinical Global Impression of Severity/Improvement, CGAS-Clinical Global Assessment of Functioning, CSSRS-Columbia Suicide Severity Rating Scale.

Pharmacogenetic testing

The pharmacogenetic testing was done through the genotyping - SNP – Single Nucleotide Polymorphisms, through RT-PCR, after the DNA prelevation. The SNP's, the *star-alelleles/* haplotypes and the sum of *star-alelleles*, enherited from the parents, were identified.

The genotypes of the allelic variants CYP * have been determined through the specific allelic fluorescence measurement, using the software for allelic discrimination. The identification of the alleles CYP2D6 *3, *4, *5, *41, responsible for the medication metabolizing types, was significant. Also the panel including CYP2C19*2,3,4 as major metabolic pathway is relevant. Genomic DNA was extracted from EDTA blood using QIAamp DNA Mini Kit (Qiagen, Germany). DNA samples were stored at -80°C.

The CYP genotyping was performed, so that the laboratory staff was blinded to the patients' data. CYP allele identification was performed by using TaqMan Drug Metabolism Genotyping Assay for Allelic Discrimination CYP2D6* and TaqMan® PCR Master Mix (Applied Biosystems) according to the protocol provided by the producer. Allelic discrimination was carried out on Applied Biosystems 7900HT Fast Real-Time PCR System in a reaction volume of 25 μL, containing TaqMan Drug Metabolism Genotyping Assay for Allelic Discrimination CYP and TaqMan® PCR Master Mix and DNA probe. Genotypes were determined by measuring allele-specific fluorescence using the software for allelic discrimination (Applied Biosystems). Based on the CYP genotype, three groups of metabolizers were identified: WT (wild type), SNP (Single Nucleotide Polymorphisms) and the mixed type WT/SNP.

Statistical analysis

All analyses were carried out using SPSS software (version 17.0, Chicago, IL, USA) and Microsoft Excel. For comparing the clinical scales scores (CDRS, CGI-S/I, CGAS, CSSRS) and also the MR Spectroscopy brain metabolites values at different time points, the Friedman non-parametric test for pair values was performed. For comparing the clinical response, evolution between the groups-GI (patients with depressive disorders who benefited of pharmacogenetic testing in choosing the proper medication) and the group GII (without prior pharmacogenetic testing), the Mann-Whitney non-parametric test was applied. For comparing the mean total clinical scales scores and also the MR Spectroscopy biochemical brain metabolites values at different times, the nonparametric test Wilcoxon signed Ranks was used.

Results and discussions

We identified some relevant metabolic abnormalities - hypercholesterolemia, high plasma triglycerides, insulin and glucose levels, BMI increase, weight gain, modified urea and creatinine values, especially in the group of patients without pharmacogenetic testing prior to the treatment election [29]. CYP testing allowed us to choose the proper medication and also to adjust the medication doses accordingly. In the second group (GII = 45 depressive disorders patients with or without comorbidities) pharmacogenetic non tested, the medication has been assigned according to the clinical symptoms and not using a personalized approach according to patients pharmacogenetic profile. The major CYP metabolizing pathways for the principal antidepressant medication groups are for the SSRI's-CYP2D6 or/and CYP2C19 and for the SNRI's (Serotonin-Norepinephrine Reuptake Inhibitors) also CYP2D6 (table1).

Sertraline Hydrochloride, $C_{17}H_{17}Cl_2N$ or (1S, 4S)-4-(3, 4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine, is an antidepressant medication, a selective serotonin reuptake inhibitor (SSRI). Sertraline has one active metabolite and, like other SSRIs, have less sedative, anticholinergic, and cardiovascular effects than the tricyclic antidepressant drugs because it does not have clinically important anticholinergic, antihistamine, or adrenergic blocking activity [3, 7, 10, 20].

Fluoxetine Hydrochloride, $C_{17}H_{18}F_3NO$ or N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine, is also a SSRI antidepressant.

Paroxetine Hydrochloride, $C_{19}H_{20}FNO_3$ or (3S, 4R)-3-(1,3-benzodioxol-5-yloxymethyl)-4-(4-fluorophenyl)piperidine, is also a SSRI antidepressant.

Table 1

CYP450 MAJOR METABOLIZING PATHWAYS FOR ANTIDEPRESSANTS

Antidepressant Medication	CYP450		
	CYP2D6	CYP42C19	CYP3A4
Sertraline $C_{17}H_{17}Cl_2N$	+++	+++	
Fluoxetine $C_{17}H_{18}F_3NO$	+++	+++	
Paroxetine $C_{19}H_{20}FNO_3$	+++		
Fluvoxamine $C_{15}H_{21}F_3N_2O_2$	+++		
Citalopram $C_{20}H_{21}FN_2O$		+++	
Escitalopram $C_{20}H_{21}FN_2O$		+++	
Venlafaxine $C_{17}H_{27}NO_2$	+++		
Agomelatine $C_{15}H_{17}NO_2$			+++

Fluvoxamine Hydrochloride, $C_{15}H_{21}F_3N_2O_2$ is a SSRI antidepressant as well, or 2-[(E)-[5-methoxy-1-[4-(trifluoromethyl) phenyl] pentylidene] amino] oxyethanamine.

Citalopram Hydrochloride, $C_{20}H_{21}FN_2O$ or 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile, is a SSRI antidepressant.

Escitalopram, $C_{20}H_{21}FN_2O$ is the (S)-stereoisomer (Left-enantiomer) of the earlier drug citalopram, hence the name escitalopram [20, 25, 27].

Venlafaxine, $C_{17}H_{27}NO_2$ or 1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol, is an antidepressant of SNRI class.

Agomelatine, $C_{15}H_{17}NO_2$ or N-[2-(7-methoxynaphthalen-1-yl) ethyl]acetamide is an atypical antidepressant. The chemical structure of agomelatine is very similar to melatonin. While melatonin has an indole ring system, agomelatine has a naphthalene bioisostere instead. It is a melatoninergic agonist and selective serotonin 5-HT2B and 5-HT2C receptor antagonist, metabolically stable analogue of melatonin [3, 7, 10, 20].

In the group where the pharmacogenetic testing was not performed (G2 = 45 patients with depression) the antidepressant medication has been assigned according to the clinical symptoms and not according to personalized pharmacogenetic profile of the patients. Compared to pharmacogenetic tested group, in pharmacogenetic non tested group highly modified and abnormal lipid profiles, weight gain, BMI increase and abnormal metabolic values were found.

We applied Friedman nonparametric test for pair values to compare each group values at different times. For group II patients - without pharmacogenetic testing prior to the treatment election, the BMI values increased significantly from baseline, due to the CYP polymorphisms of the patients ($p < 0.001$, $\alpha = 0.001$). For insulin values, the differences recorded at different times were also statistically significant ($\alpha = 0.001$). The results are presented in table 2.

For group II patients, without pharmacogenetic testing prior to the treatment election, the increase of insulin values from baseline until 18 months was statistically significant ($\alpha = 0.001$). For these patients the antidepressant treatment was not adapted to their genotype; we assume they were most prone and exposed to the adverse effects of the antidepressants-increased insulin values or even

		BMI (kg/m^2)				Insulin values (μU/mL)			
Timepoint	**N**	**Mean**	**Std. Deviation**	**Min.**	**Max.**	**Mean**	**Std. Deviation**	**Min.**	**Max.**
Baseline	45	19.28	2.92	16.00	25.00	6.40	2.11	4.4	12.60
3 Months	45	23.45	2.78	17.40	27.70	13.26	1.42	5.6	17.80
6 Months	45	25.38	1.99	21.50	28.90	17.55	3.76	7.9	24.90
1 Year	45	27.47	1.93	23.80	30.00	27.69	1.20	24.9	28.90
18 Months	45	29.83	2.14	24.50	33.00	30.18	1.78	25.3	30.78

Table 2
DIFFERENT TIMES RECORDED VALUES COMPARISON FOR GROUP II PATIENTS - WITHOUT PHARMACOGENETIC TESTING PRIOR TO THE TREATMENT ELECTION

Table 3
DIFFERENT TIMES RECORDED VALUES COMPARISON FOR GROUP I PATIENTS - WITH PHARMACOGENETIC TESTING PRIOR TO THE TREATMENT ELECTION

		BMI (kg/m^2)				Insulin values (μU/mL)			
Time	**N**	**Mean**	**Std. Deviation**	**Min.**	**Max.**	**Mean**	**Std. Deviation**	**Min.**	**Max.**
Baseline	45	21.53	2.44	16.00	26.00	6.80	3.53	4.4	17.7
3 Months	45	22.25	2.58	16.30	27.30	7.99	4.12	4.9	18.3
6 Months	45	22.48	2.67	17.00	28.90	11.21	3.76	4.8	20.5
1 Year	45	22.57	2.75	17.10	29.50	12.27	10.26	4.9	35.4
18 Months	45	22.95	3.13	17.30	29.99	12.44	3.94	4.9	19.9

hyperinsulinism, with high morbidity consequences.

The increased BMI values from baseline until 18 months in group II patients was also statistically significant, with a threshold of significance $\alpha = 0.001$, and was much higher than for group I patients. Different times recorded values comparison for group I patients is summarized in table 3.

The increase of the BMI values from baseline until 18 months was not statistically significant; threshold of significance $\alpha = 0.05$ for the comparison baseline-18 months. BMI increase was much lower than for group II patients; we assume that patients from group I were not so prone and exposed to adverse effects such as weight gain, because their antidepressant treatment has been chosen according to their pharmacogenetic profile.

Patients in this study had pro-inflammatory mediators such as tumor necrosis factor-TNF and Interleukin-6 elevated plasma levels. We mention also the neuroendocrine disorders that were detected in the study groups of patients with antidepressive treatments: metabolic disorders (diabetes mellitus, hyperinsulinism), thyroid disorders. A high percentage of patients presented modified plasma levels of TSH-Thyroid Stimulating Hormone, FT3-free T3, FT4-free T4, insulin, glycemia, triglycerides, cholesterol and inflammatory tests - CRP-Reactive Protein C, Interleukine 3, 6 and also EEG changes. We obtained interesting results, when comparing the study groups (with and without pharmacogenetic testing prior to the treatment election) in terms of clinical evolution, captured through the clinical psychiatric scales scores assessment from baseline till endpoint and also in terms of the brain metabolites variation evaluated by MR Spectroscopy at different times.

Through the MR Spectroscopy we found modified values and concentrations of cerebral metabolites for both groups of patients with depressive disorders [30-32].

We noticed some patterns of glutamatergic abnormalities such as Glx level decrease, specific for depressive disorders, in anterior cingulate cortex (ACC) and in medial frontal cortex [33, 33]. On the other side, the levels of glutamate in the occipital cortex were increased. The hyppocampal bilateral metabolic evaluation showed some changes of NAA/Cho, NAA/Cr, NAA/Cho + Cr ratio and high values of Ino/Cr. We also noticed: increase cerebral levels of Lactat, Glutamat, Glicine, Glx and Myo-Inositol; decrease GABA levels in both prefrontal and occipital cortex; very high Glutamat levels especially in frontal cortex, identifying brain lesion; very low NAA and NAAG (NAcetylAspartiglutamat) levels (fig. 1).

There have been shown correlations between neurometabolites' pathways and treatment response: patients who had a good clinical response showed metabolites' levels normalization identified by MR Spectroscopy. Our results proved that patients with

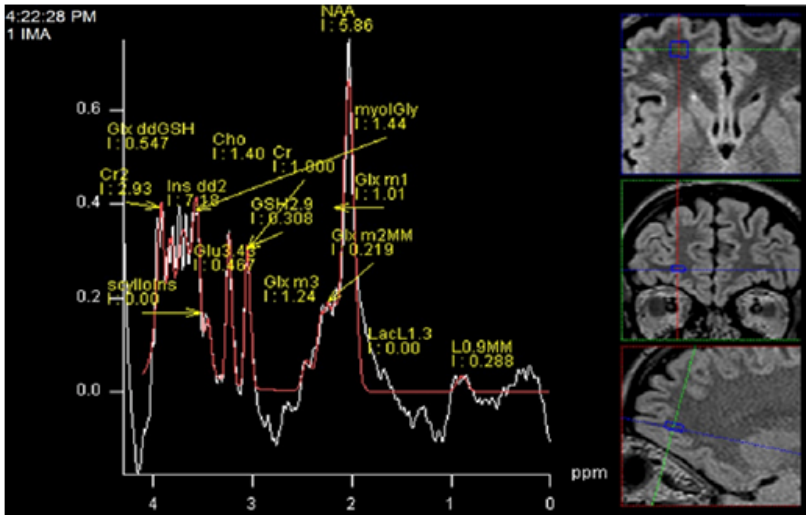

Fig. 1. Concentrations, peaks and correlations of MR Spectroscopy biochemical brain metabolites of depressive disorders and antidepressant treatment patients

antidepressant treatment giuded by pharmacogenetic testing registered improvement metabolites quantified by MR Spectroscopy, as a positive response to the chosen pharmacotherapy. It is also important to detect and to treat neuroendocrine comorbidities and to prevent abnormal metabolic events. The approach have to be ethical, personalized, avoiding as much as possible the emergence of adverse events [35-41]. The glutamatergic dysfunction is increasingly involved in depressive disorders. The Glutamate, being a brain metabolite with significant role in the neurotransmission, has very high values in depressive patients. The glutamatergic pathways are involved in cognition and memory processes, and excessive concentrations of Glutamate in brain become neurotoxic [42-44]. In depressive disorders, myo-inositol levels being elevated, Lithium would be indicated as mood stabilizer, relying on this principle, being a noncompetitive inhibitor of inositol.

Conclusions

Our research supports the implementation of an integrative approach by means of pharmacogenetic testing in choosing the proper antidepressant therapy. It also highlights the importance of investigating the relevant biochemical, neurobiological and neuroimagistic markers in clinical practice for a personalized and taylored therapy in pediatric depressive disorders. Antidepressants are very useful when properly and carefully administered, but can determine some relevant metabolic adverse events and abnormalities, when chosen without prior pharmacogenetic testing.

The pharmacogenetic testing, the fingerprinting of the biochemical, neurobiological and spectroscopic, MR spectroscopy markers represent strongly predictive factors of the clinical evolution after the administration of antidepressant medication.

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