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Integrative clinico-biological, pharmacogenetic, neuroimagistic, neuroendocrinological and psychological correlations in depressive and anxiety disorders

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Abstract

We approach the theme of modern treatment strategies, based on clinico-biological, pharmacogenetic, neuroimagistic, neuroendocrinological and psychological integrative correlations in the management of depressive and comorbid anxiety disorders. We target to evaluate the efficacy of the pharmacogenetic testing and the evolution, functioning of patients in correlation with specific neurobiological, neuroimagistic and neuroendocrinological markers. Our research was conducted between 2010–2016 on 80 children and adolescents with depressive and comorbid anxiety disorders – 40 children (G1 group), who benefited in choosing the pharmacotherapy from pharmacogenetic testing and 40 children without testing (G2 group). Also, the patients were evaluated through magnetic resonance (MR) spectroscopy at baseline and after pharmacotherapy. The efficacy of the chosen therapy in correlation with the pharmacogenetic testing was evaluated through the mean change in the CDRS (Children's Depression Rating Scale) total scores, in the CGI-S/I (Clinical Global Impression – Severity/Improvement), CGAS (Children's Global Assessment Scale) and through the change of the relevant neurobiological markers and MR spectroscopy metabolites. We evaluated the side effects through the PAERS (Pediatric Adverse Events Rating Scale)-Clinician. Our results show statistically significant differences of the clinical scores between the studied groups: for those subjects who benefited of pharmacogenetic testing, the CDRS, the global functioning scores prove a higher clinical improvement, a better compliance and lower PAERS side effects scores and also improvement concerning the MR spectroscopy dosed metabolites values. Our research was a proof sustaining the use of the pharmacogenetic testing in clinical practice and the value of investigating relevant neurobiological, neuroimagistic and neuroendocrinological markers for a personalized therapy in depressive disorders.

Keywords: depressive disorders, comorbid anxiety disorders, pharmacogenetic testing, spectroscopy, neuroimagistic, neuroendocrinological markers.

Introduction

Nowadays, depression is a common illness worldwide, with more than 300 million people affected, so that 800 000 people die yearly due to suicide. Suicide is the second leading cause of death today. The burden of depression and other mental health comorbid conditions is on the rise globally. In general, psychopharmacological treatment in depressive disorders is characterized by long treatment courses, frequent drug changes, lack of compliance, numerous relapses, a high incidence of adverse events and marked interindividual differences in drug response [1–5].

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 [6–9].

Also, these neuroimagistic markers are helpful in quantifying the medication response, the clinical evolution in depressive disorders. The election treatment in the

management of depression should be chosen in correlation with the neurobiological, pharmacogenetic, neuroimagistic and clinical profile of the target patients. When choosing the suitable pharmacotherapy, the pharmacogenetic markers should be analyzed carefully [10–12].

In our present research, we approach the theme of modern treatment strategies, correlated with the pharmacogenetic testing, the neurobiological, neuroendocrinological and imagistic markers, in the management of depressive and comorbid anxiety disorders in children and adolescents [1, 2, 13–17].

We will capture the clinical significance of modern pharmacological treatment approaches, correlated with the evaluation of the neuroimagistic markers, especially through magnetic resonance (MR) spectroscopy [15, 17–20].

The main objectives of our study were: the evaluation of the clinical utility of the pharmacogenetic testing, of the neurobiological, neuroimagistic, neuroendocrinological markers; the efficacy of the different pharmacological interventions in the child and adolescent depressive disorders; analyzing the integrative pharmacogenetic, neurobiological, neuroendocrinological and neuroimagistic correlations; the dynamic evaluation of the efficacy of the pharmacogenetic testing in the treatment of depressive and anxiety disorders in children and adolescents; the dynamic evaluation of the clinical evolution, of the global functioning and of the adverse events in depressive disorders, in correlation with specific markers. Also, the evaluation, through neuroimagistics (MR spectroscopy), of the modification of the metabolites/activation of different pathways in correlation with the chosen pharmacotherapy, after and without pharmacogenetic testing [21–23].

The MR spectroscopy is a versatile, non-invasive instrument, which permits the *in vivo* identification and quantification of the biochemical substances and neurometabolites in the brain. It is very useful for the clinical evaluation, longitudinal monitoring and for the evaluation of the efficacy of the administered treatment [17, 24–26].

Nucleotide polymorphism within different genes can alter the metabolism, efficacy and adverse events of psychiatric drugs, including medication needed in treating depressive disorders [27, 28].

The genetic variations in the cytochrome P450 system is correlated with the response to medication in depressive disorders. There are different metabolic phenotypes in function of those CYPs polymorphism [1, 3, 4, 6].

The studies and guides mention the selective serotonin reuptake inhibitors (SSRIs) antidepressant treatment, as first line treatment in depressive disorders. The genotypes CYP2D6 and CYP2C19 are strongest correlated with the response to the antidepressant medication, especially from the SSRI category [8, 9, 27].

The aim of our research was to put an integrative approach in evidence, correlating relevant markers with clinical aspects in depressive disorders, especially in the pediatric population.

In the actual general context, our research offers new perspectives, especially because of the lack of consistent studies for children and adolescents with depressive disorders, concerning the modern molecular, pharmacogenetic testing correlated with modern neuroimagistic investigations.

Our study is especially valuable in the light of a multidisciplinary approach, implying complex correlations between the clinical, neurobiological, pharmacogenetic, neuroendocrinological and neuroimagistic markers.

Patients, Materials and Methods

The research was performed between the years 2010 and 2016, in the Clinic of Psychiatry and Neurology for Children and Adolescents, "Louis Ţurcanu" Emergency Hospital for Children, Timişoara, Romania. We recruited patients, children and adolescents with depressive disorders, with or without relevant comorbidities.

Our actual study is focusing especially on neurobiological, neuroendocrinological, neuroimagistic, respectively clinical aspects and specific pharmacogenetic correlations.

The diagnoses of the studied patients were put according to DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition) and in the last two years through DSM-5 (5th edition) and reconfirmed by a child and adolescent psychiatrist through the Kiddie–Schedule for Affective Disorders and Schizophrenia for school age children–Present and Lifetime Version (K-SADS-PL) application.

The study samples consisted of 80 patients, children and adolescents with depressive disorders with or without comorbidities. The patients included in the study were aged between 12 and 20 years (median age 13.78±4 years).

We obtained for each patient the informed assent and the informed consent from the parents/legal guardians. Our study was done in accordance with the Ethical Committee regulations of the "Victor Babeş" University of Medicine and Pharmacy, Timişoara, with the *International Conference on Harmonization–Good Clinical Practice* (ICH-GCP) regulations and guidelines.

Our study sample was divided in two groups: 40 patients took treatment after pharmacogenetic testing and 40 patients without the pharmacogenetic testing before the treatment election.

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 (Children's Depression Rating Scale), CGI-S/I (Clinical Global Impression – Severity/Improvement), CGAS (Children's Global Assessment Scale), C-SSRS (Columbia-Suicide Severity Rating Scale). The CDRS was applied by an authorized rater, in order to evaluate the psychopathology. In order to quantify the presence of adverse events in correlation with the administered medication, we applied PAERS (Pediatric Adverse Events Rating Scale).

Pharmacogenetic testing

The pharmacogenetic testing was done through the single-nucleotide polymorphisms (SNPs) genotyping, through reverse transcription–polymerase chain reaction (RT-PCR), after the DNA prelevation. The SNPs, the "star alleles"/haplotypes and the sum of "star alleles", inherited 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 CYP2D6*3, *4, *5, and *41 alleles, responsible for the medication metabolizing types, was significant. Also, the panel including CYP2C19*2, *3, and *4 as major metabolic pathway is relevant. Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) blood using QIAamp® DNA Mini Kit (Qiagen, Germany). DNA samples were stored at -80^{0}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 and the WT/SNP mixed type.

Neuroimagistic investigations (MR spectroscopy)

For the correlation of clinical data with the cerebral biological changes, we performed the neuroimagistic investigations. The patients have been evaluated through MR spectroscopy at baseline and after the chosen pharmacotherapy with or without pharmacogenetic testing before. Through MR spectroscopy, we investigated key aspects of the brain function and metabolism.

We quantified the following neurometabolites: NAA (N-acetylaspartate), GABA (gamma-Aminobutyric acid), Asp (Aspartate), CR (Creatine), Gln (Glutamine), Glx (Glutamate + Glutamine), GPC (Glycerophosphocholine), PC (Phosphocholine), PCr (Phosphocreatine), Tau (Taurine), N-MDA (N-methyl-D-aspartate), Ino (Inositol), Serine, Glycine, Cho (Choline).

We used the MR spectroscopy software package for the MR spectral quantification, which automatically calculates a matrix of the correlation quotients of the cerebral metabolites.

The efficacy of the chosen therapy in correlation with the pharmacogenetic testing has been evaluated through the modification of the applied clinical scales total scores and through the change registered for the relevant neurobiological markers and neurometabolites, from the initial values until endpoint, in each timepoint. So that, we evaluated the efficacy of the chosen pharmacotherapy in correlation with the pharmacogenetic testing and the variation of the cerebral metabolites, quantified through the MR spectroscopy, through the change of the mean total scores of the scales (CDRS, CGI-S/I, CGAS, PAERS, C-SSRS) from baseline until endpoint in different timepoints.

Statistical analysis

All analyses were carried out using SPSS (Statistical Package for the Social Sciences) software (version 17.0, Chicago, IL, USA) and Microsoft Excel. For comparing the clinical scales scores (CDRS, CGI-S/I, CGAS, PAERS, C-SSRS) and also the MR spectroscopy brain metabolites values at different time points, the Friedman's non-parametric test for pair values was used. For comparing the clinical response, evolution between the groups – G1 (patients with depressive disorders who benefited of pharmacogenetic testing in choosing the proper medication) and G2 group (without pharmacogenetic testing) –, the Mann–Whitney non-parametric test was applied.

For comparing the mean total clinical scales scores and also the MR spectroscopy brain metabolites values at two different time points and in each two with two different timepoints, the non-parametric Wilcoxon signed-rank test was used. We also applied the Pearson's test for the correlation of the obtained results.

Results

We must mention that in our study groups of patients with depressive disorders, we identified the following comorbidities (Figure 1):

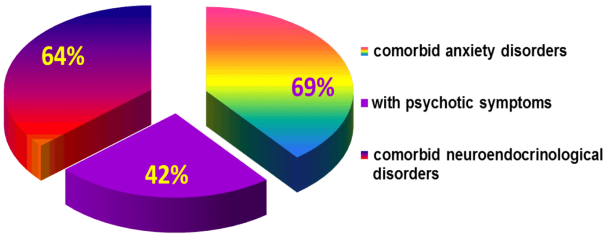

Figure 1 – ***Psychiatric comorbidities of the study groups with depressive disorders.***

Knowing these comorbidities is very important for the clinician when choosing the proper pharmacotherapy: antidepressant, anxiolytic, antipsychotic or mood stabilizing medication.

We obtained significant results through our research. We identified for the G1 group – 40 patients with depressive disorders, with or without comorbidities, where the pharmacogenetic testing was applied –, pharmacogenetic polymorphisms at the level of CYP450 enzymes. Therefore, we observed in our studied samples the WT or normal type metabolizer, the patients who had SNPs, who need in the clinical practice the adjustment of the doses of the administered pharmacotherapy, as well as careful choosing of the medication and the WT/SNP mixed type, who encounter also some difficulties in this area (Figures 2 and 3).

Therefore, the pharmacogenetic CYP testing permitted us to choose the proper medication and also to adjust the medication doses accordingly.

In the G2 group, where the pharmacogenetic testing was not performed (40 patients also with depressive disorders, with or without comorbidities), the medication has been assigned according to the clinical symptoms but not to the personalized pharmacogenetic profile of the patients.

Therefore, when prescribing medication for pediatric depressive disorders, we must pay attention to the following obtained information (Figure 4):

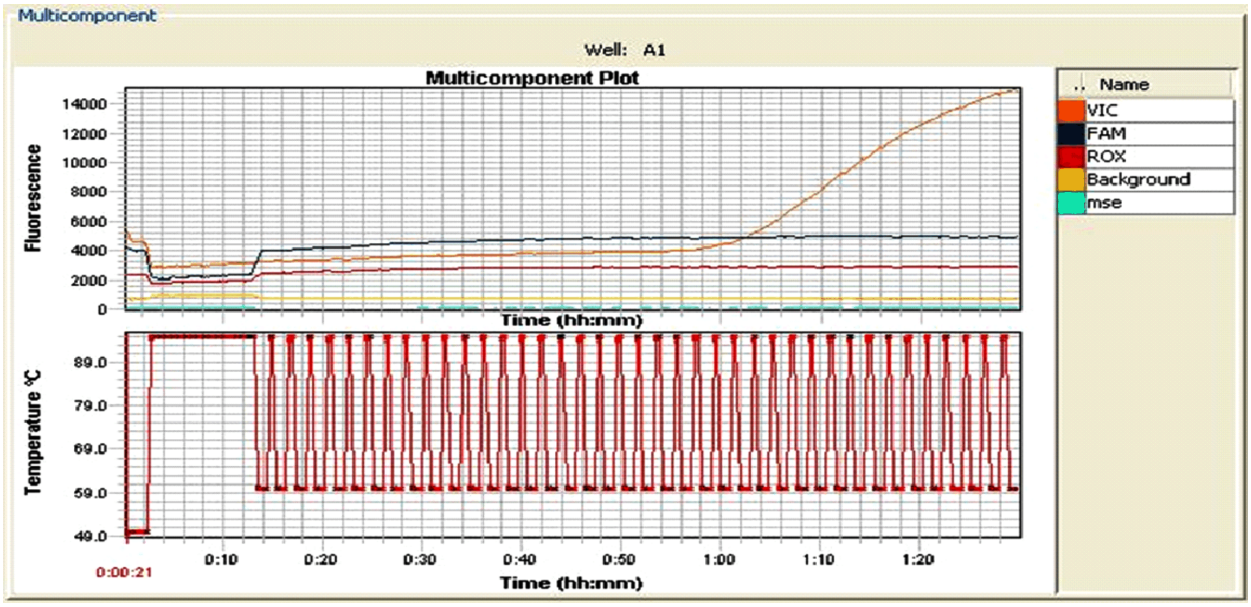

Figure 2 – *Results of the pharmacogenetic testing for the WT or normal type metabolizer patient group. WT: Wild type.*

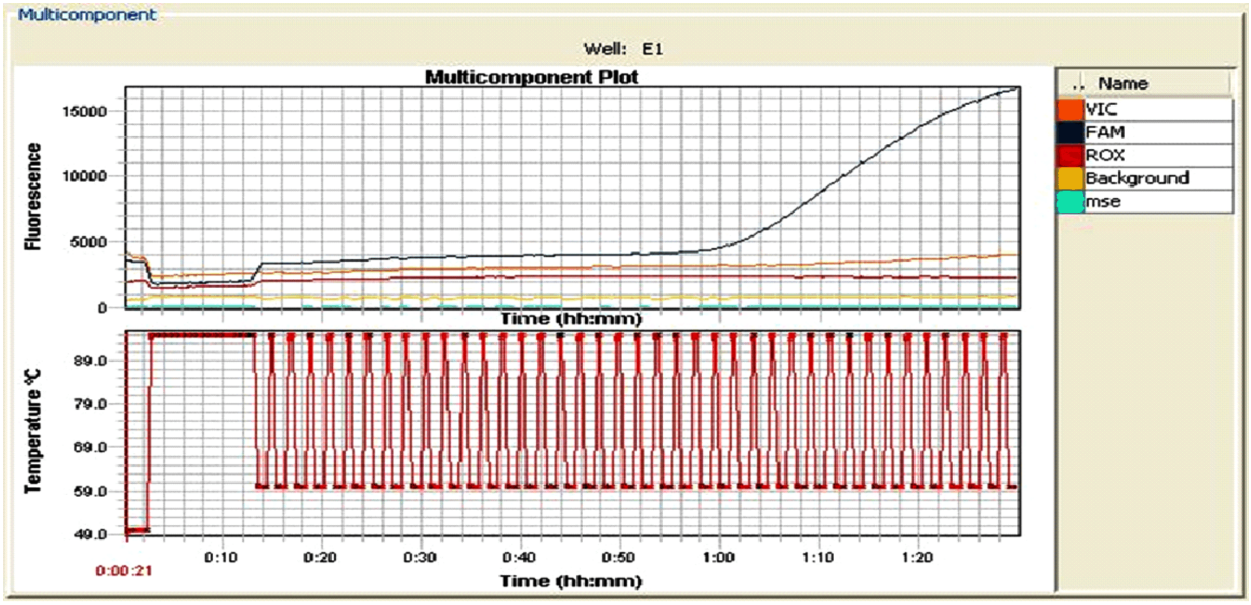

Figure 3 – *Results of the pharmacogenetic testing for the patient group with CYP450 SNPs. SNP: Single-nucleotide polymorphism.*

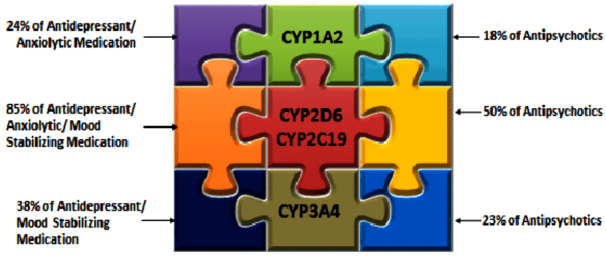

Figure 4 – *Results of the pharmacogenetic testing correlated with the major CYP metabolizing pathways for relevant medication categories.*

So that, the major CYP metabolizing pathways for the principal antidepressant medication groups are for the SSRIs – CYP2D6 or/and CYP2C19 – and for the serotonin and norepinephrine reuptake inhibitors (SNRI's) also CYP2D6 (Table 1).

Table 1 – *CYP450 major metabolizing pathways for antidepressant medication*

Antidepressant medication	CYP450		
	CYP2D6	*CYP2C19*	*CYP3A4*
Sertraline	+++	+++	
Fluoxetine	+++	+++	
Paroxetine	+++		
Fluvoxamine	+++		
Citalopram		+++	
Escitalopram		+++	
Venlafaxine	+++		
Agomelatine			+++

CYP: Cytochrome P.

So that, for the patients with SNP or WT/SNP polymorphisms, the clinician must avoid the antidepressant,

anxiolytic, antipsychotic or mood stabilizing medication metabolized through that CYP.

We also must avoid the antidepressant medication Paroxetine, Fluvoxamine, Venlafaxine for the patients with SNPs CYP2D6, Sertraline and Fluoxetine, in case of SNPs CYP2D6 and/or CYP2C19 and Citalopram for CYP2C19.

We obtained interesting results, when comparing the study samples (with and without pharmacogenetic testing), concerning the clinical evolution, captured through the clinical psychiatric scales scores from baseline until endpoint but also concerning the variation of the brain metabolites values of the MR spectroscopy, in time.

Through the MR spectroscopy, we found modified values and concentrations of the cerebral metabolites for both groups of patients with depressive disorders.

We observed some patterns of glutamatergic abnormalities, consistent with Glx level reductions, specific for depressive disorders. Also, a decrease of the Glx levels in the anterior cingulate cortex (ACC) and in the medial frontal cortex, was detected. On the other side, the levels of glutamate in the occipital cortex were increased.

Through the hippocampal bilateral metabolic evaluation, some modifications of the ratios NAA/Cho, NAA/Cr, NAA/Cho + Cr and high values for Ino/Cr were detected. Also, the increase of the cerebral levels of Lactate, Glutamate, Glycine, Glx and myo-Inositol were observed.

Concerning the cerebral perfusion, especially for the lesion zones, there were detected some right>left asymmetries, with right hyperperfusion zones.

The global neuroimagistic aspect was that of a global affection, with global cerebral hypovascularization, with a predominance in the frontal lobes, hippocampus, parietal lobes bilaterally and also affected components of the anaerobic metabolism (Lactate). Through the MR spectroscopy, also following aspects were captured:

- reduced GABA levels in both the prefrontal and occipital cortex;
- very high Glutamate values especially in the frontal cortex, identifying brain lesions;
- very low NAA and NAAG (N-acetyl-aspartyl-glutamate) values.

We also observed high values for the Lactate/NAA, Glutamate/Cr, Cho/Cr, NAA/Cr, NAA/Cho and reduced Glutamine/Glutamate ratios (Figures 5 and 6).

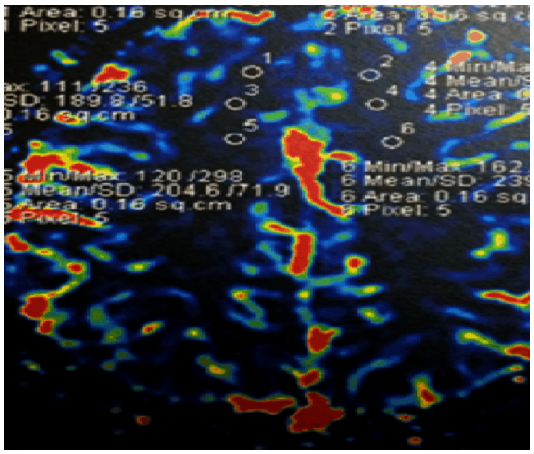

Figure 5 – ***Results of the MR spectroscopy brain metabolites concentrations for the patients with depressive disorders.***

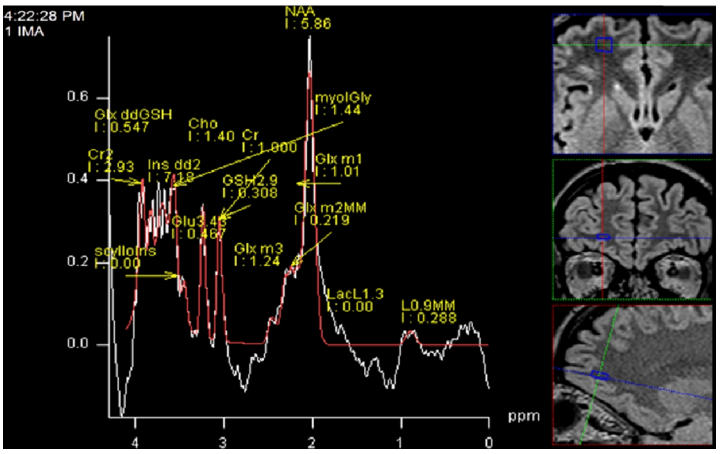

Figure 6 – ***Concentrations, peaks and correlations of MR spectroscopy brain metabolites for the patients with depressive disorders.***

We also obtained interesting results concerning the MR spectroscopy quantified metabolites and their variation from baseline until endpoint.

So that, we observed the "normalization" of the brain metabolites – the decrease of Glutamate and the increase of GABA, NAA and NAAG and the normalization/ decrease of the pathological values of the metabolites' reports after the treatment with correctly chosen medication (antidepressive, antipsychotic, anxiolytic, mood stabilizing) in the G1 group, who benefited of prior pharmacogenetic testing.

We also made some correlations concerning the neurometabolites' pathways and the treatment response – the patients who had good clinical response showed also the normalization of the metabolites' levels identified through the MR spectroscopy.

We obtained statistically significant differences of the clinical scales scores, between the patient group who benefited of pharmacogenetic testing, when choosing the proper pharmacotherapy and the other group, in each timepoint and also between baseline and endpoint values of the evaluation for all the scales (p<0.001, significance level α=0.001). The CDRS, CGI-S and PAERS adverse event scores registered a statistically significant decrease, the GAS functioning scores showed an improvement and the MR spectroscopy metabolites values improved, implying a good clinical evolution in the pharmacogenetically tested group. We took into account the fact that high CDRS and CGI-S scores mean a poor clinical evolution and decreased scores are correlated with a clinical improvement.

We quantified a good response to medication if we found a decrease and improvement with more than >30% of the CDRS scores from baseline.

Also, the suicidal ideation and behavior captured through the C-SSRS scale, diminished in the G1 patient group (with pharmacogenetic testing).

Through comparing the total clinical scale scores (CDRS, CGI-S/I, CGAS, C-SSRS) and the values of the MR spectroscopy brain metabolites in each two with

two different timepoints, through the application of the Wilcoxon signed-rank non-parametric test, we obtained statistically significant differences for the G1 group (who benefited of pharmacogenetic testing), with choosing the suitable (antidepressant, antipsychotic, anxiolytic, mood stabilizing) medication, proving a good clinical evolution in time (p<0.001, significance level α=0.001).

The obtained results proved that the patients who took medication chosen after the prior pharmacogenetic testing registered the improvement of the spectroscopy metabolites, as a positive response to the chosen pharmacotherapy.

In the G2 group, without pharmacogenetic testing, we could observe clinical poor or non-response, lack of improvement of the MR spectroscopy captured brain metabolites correlated with multiple adverse effects in the PAERS.

Comparing the differences between the two analyzed groups (G1 and G2), concerning the total mean clinical scales scores (CDRS, CGI-S/I, CGAS) for each analyzed moment, applying the Mann–Whitney non-parametric test, we observed the decrease of the CDRS and CGI-S scores and increased CGAS scores, meaning good clinical evolution in the G1 group (with pharmacogenetic testing) and poor clinical evolution with non-response in the G2 group (without pharmacogenetic testing) (Table 2).

Table 2 – ***Comparison of the CDRS scores between the G1 group (with pharmacogenetic testing) and G2 group (without pharmacogenetic testing), in different timepoints***

Timepoint	Group	Mean CDRS	SD	SEM
T0 (baseline)	G1	86.7	28.16	3.87
	G2	86.4	35.19	7.04
T1 (after six months)	G1	75.4	23.6	3.24
	G2	95.2	32.62	6.52
T2 (after one year)	G1	70.8	19.4	2.67
	G2	98.4	30.37	6.07
T3 (after 18 months)	G1	65.8	14.48	1.99
	G2	108.9	30.84	6.17
T4 (after two years)	G1	56.7	12.29	1.69
	G2	111.2	26.14	5.23

CDRS: Children's Depression Rating Scale; SD: Standard deviation; SEM: Standard error of the mean.

Through applying the Pearson's test, we obtained as correlations' results, in the G1 group (with pharmacogenetic testing), the following statistically significant positive correlations between the improvement of the brain metabolites' values in MR spectroscopy and the pharmacogenetic testing application for choosing the suitable pharmacotherapy, and the good clinical response and evolution captured through the improvement of the clinical psychiatric scales scores – CDRS, CGI-S and CGAS (Table 3).

Table 3 – ***Spearman's correlations transformed z, between the psychiatric clinical scale scores and the MR spectroscopy metabolites improvement for the studied groups***

Correlations	Patients with depressive disorders					
	G1 group (with pharmacogenetic testing)			*G2 group (without pharmacogenetic testing)*		
	r^*	z^{**}	*z-SD***	r^*	z^{**}	*z-SD***
Lower total CDRS scores – Metabolite values improvement	.969	.521	.373	.313	.365	.362
Lower CGI-S scores – Metabolite values improvement	.977	.355	.301	.221	.310	.252
High functioning CGAS scores – Metabolite values improvement	.983	.846	.675	.687	.723	.701

MR: Magnetic resonance; CDRS: Children's Depression Rating Scale; CGI-S: Clinical Global Impression – Severity; CGAS: Children's Global Assessment Scale; *r*: Spearman's correlation coefficient; *z*: Transformed values; SD: Standard deviation; *: Coefficient of determination; **: Coefficient of non-determination.

We must mention also the neuroendocrinological comorbidities that we detected in the study groups of patients with depressive disorders: 64% of the studied patients presented comorbid neuroendocrinological disorders – metabolic disorders (diabetes mellitus, hyperinsulinism), thyroid disorders, polycystic ovary syndrome (PCOS). So that, those patients presented modified values through the blood dosing of thyroid-stimulating hormone (TSH), free T3 (FT3), free T4 (FT4), insulin, glycemia, triglycerides, cholesterol levels and the inflammatory probes – C-reactive protein (CRP), interleukin (IL) 3, IL6, in function of their present comorbidity.

Discussion

To our knowledge, there is a lack of studies assessing whether the pharmacogenetic-guided selection of treatment is more effective than unguided treatment in improving patients' with depressive disorders response and tolerability. In our study, also the present comorbidities were not excluded [1, 2, 4, 6, 29].

In the actual general context, our research offers new perspectives, especially because of the lack of consistent studies for children and adolescents with depressive disorders, concerning the modern molecular, pharmacogenetic testing correlated with modern neuroimagistic investigations and up to date clinical psychiatric scales. We also studied these aspects in the patients with psychoses and in ultra-high risk for psychosis patients [20, 27, 28]. Some of the pharmacogenetic and neuroimagistic aspects have been approached in some studies in adults and also the effects of combinatorial pharmacogenomics testing but there is a lack of research concerning the pediatric population [14, 29, 30]. So that, Winner *et al.* found a statistical trend for better outcomes in a trial conducted in 51 study subjects – 26 pharmacogenetic-guided *versus* 25 unguided [11]. The pharmacogenetic studies in general in Romanian population are rare.

Our study is especially valuable in the light of a multidisciplinary approach, implying complex correlations between the clinical, neurobiological, pharmacogenetic, neuroendocrinological and neuroimagistic markers [8, 17, 18, 31, 32].

Our research opens the perspective of the personalized pharmacotherapy for children and adolescents, which is tailored to the genetic variability, the neuroimagistic and neurobiological particularities.

Our obtained results of the present study are in line with some in the adult population existing researches concerning pharmacogenetic testing and the neuroimagistic modifications of the brain metabolites in depressive disorders but as far as we know, there is a lack of information about the integrative correlations of the variables and markers [1, 2, 6, 18, 24, 33].

We obtained good clinical evolution for the pharmacogenetic-guided treatment group, our results being in line with the results obtained through the study of Singh [12]. Our results are in agreement with previous studies reporting that pharmacogenetic tools are effective in patients with depressive disorders [1–6].

Also, in the pediatric population, there is a lack of information and studies in this area of research.

Non-adherence is a global challenge for psychiatry, while improved tolerability obtained through the pharmacogenetic-guided chosen treatment facilitates long-term adherence [6, 8].

Some of the modifications and pathological values of the brain metabolites are reversible and can be corrected through the proper neuropsychopharmacological interventions applied [17, 24, 34, 35].

Also, for the patients with already installed depressive disorders, some of the cerebral metabolites' modifications are reversible, if proper, carefully chosen pharmacotherapy, in function of the pharmacogenetic, neuroimagistic and clinical profile of the patient, is administered [17, 20].

The clinicians must pay greatest attention also to the comorbidities of the patients with depressive disorders – psychiatric or neuroendocrinological disorders. The administered treatment is different in function of these present comorbidities. Therefore, that, in depressive disorders with psychotic symptoms, also antipsychotics, are useful. We must be careful to detect and treat also the neuroendocrinological comorbidities. Therefore, that hypo- or hyperthyroidism or the autoimmune thyroid disorders must be carefully investigated and treated. These autoimmune disorders aspects are correlated with inflammation as common root etiology. Some studies incriminate this inflammatory theory as etiology for the depressive disorders also [36, 37].

New studies sustain the utility of the triiodothyronine (T3) hormone administration in some categories of depressive disorders [38]. If the clinician encounters these comorbidities, dosing of TSH, FT3, FT4, insulin, glycemia, triglycerides, cholesterol levels and markers describing inflammation – CRP, IL3, IL6 –, is necessary. The therapeutic scheme must be adjusted with synthetic thyroid hormones – thyroxine (T4) or/and T3 – and Metformin in hyperinsulinism, metabolic syndrome or PCOS [36, 37].

Analyzing the modifications captured for the depressive disorders categories, through the MR spectroscopy, we observed some relevant aspects, some of them being in line with the existing literature [17, 25, 26, 33].

The most relevant vulnerability markers in depressive disorders were NAA, NAAG, GABA and Glutamate, consistent with the data obtained for psychosis in other studies [32, 33]. However, in comparison with the data obtained in the studies about psychosis, in depressive disorders GABA presented through MR spectroscopy decreased values and not increased like for psychotic disorders. GABA having a neuromodulating and also role in neurotransmission, its decreased value determines dysfunctions in these areas, specific for depression [17, 20, 33–35].

The NAA, which has a neurotrophic role, was very low for the patients with depressive disorders. On this fact relies the value of some antidepressant treatments, which have a neuroprotective, neurotrophic role, because they prevent the decrease of NAA in the brain [17].

The glutamatergic dysfunction is increasingly implicated in depressive disorders.

Glutamate, being a brain metabolite with significant role in the neurotransmission, has very high values in depressive patients. The glutamatergic pathways are implied in the cognition and memory processes and the excessive concentrations of Glutamate in the brain are neurotoxic. On this principle relies the efficacy of some antidepressant treatments and of the Lithium, as neurostabilizers, which decrease the brain Glutamate values [17, 24].

In line with other studies, we found in unipolar depression patients a decrease of Glx [15, 17, 24–26]. In contrast with depression associated with bipolar disorder, the opposite effect, elevated Glx, tends to be observed [31, 33–35]. This pattern of contrast is supposed to be region specific and is clearest observed in medial frontal cortex. Interestingly, in bipolar disorders, during episodes of mania an elevation in the ratio of Glutamate to Glutamine, in both anterior cingulate and parieto-occipital cortex is observed [15].

Interestingly, in schizophrenia, a trend toward a similar elevation is also seen. This similarity of MR spectroscopy findings between bipolar disorders and psychosis echoes growing evidence of overlap from the genetic level through the clinical presentation [26].

In depressive disorders and also bipolar disorders, myo-Inositol levels being elevated, Lithium would be indicated as mood stabilizer, relying on this principle, being a non-competitive inhibitor of Inositol.

The observed low values for NAA and NAAG in the frontal and temporal lobe, in the thalamus, these metabolites representing neuronal integrity markers, with relevant roles in mediating and modulating the superior mental functions, are in line with the data obtained by Brugger *et al.*, in 2011, also concerning disorders like psychosis, multiple sclerosis and Alzheimer, having a common etiology expressed also through inflammation [17, 24, 26].

Some of the neurometabolic, neurochemical, neurobiological, neuroimagistic modifications persisted even after the clinical remission of the patients with depressive disorders, as significant vulnerability markers and scar of the past depressive episodes. In depressive disorders, it appears that glutamatergic findings tend to persist after clinical recovery, so Glx elevations are observed also in recovered euthymic people, in accordance with the studies of Bhagwagar *et al.* (2007) and Soeiro-de-Souza *et al.* (2013) and Brugger *et al.* (2011) [26].

The neuroendocrinological correlations and also the inflammation theories in the etiology of depressive disorders are significant. Interestingly, these theories tie in with growing interest in the possible role of inflammatory mechanisms in the pathophysiology of depressive disorders [36]. Across several studies, it appears that depressed patients on average have elevated plasma levels of pro-inflammatory mediators, such as tumor necrosis factor (TNF) and IL6, results also obtained through our study [37].

Further research is needed in the field of child psychiatry/psychiatry, pharmacogenetics and neuroimagistics, in order to develop a genetically informed, personalized medicine, although some promising researches concerning the genetic liability and its clinical application have already been done.

The pediatric patients with depressive disorders, being in development, their whole developmental trajectory could be compromised because of the lack of efficacy of the intervention and medication [19, 20, 27, 28].

For this category, particularly, issues like medication safety are crucial. So that the suitable evaluation of the neurobiological, neuroendocrinological, neuroimagistic markers, can bring significant benefits, helping the clinician to choose the best adapted medication.

The clinical implications of the pharmacogenetic testing are very significant. We must keep in mind the fact that, in the case of more than 50% of the patients with depressive disorders, the treatment is a failure because of the CYP polymorphisms. So that, for the patients with SNP or WT/SNP polymorphisms, the clinician must avoid the antidepressant, anxiolytic, antipsychotic or mood stabilizing medication metabolized through that CYP.

We also must avoid the antidepressant medication Paroxetine, Fluvoxamine, Venlafaxine – for the patients with SNPs CYP2D6, Sertraline and Fluoxetine, in case of SNPs CYP2D6 and/or CYP2C19 and Citalopram for CYP2C19 [1–4].

From the antidepressant medication classes, we avoided especially the SSRIs for the patients with SNPs CYP2D6 or with -795C/T. When the CYP polymorphisms appear, a medication not extensively metabolized through that CYP level would be indicated. The careful monitoring of the plasmatic concentrations is also needed.

Therefore, the decrease of medication dose or the administration of an alternative medication is of clinical utility. In other cases, it could be of clinical utility to decrease the medication dose with 50%, in order to avoid the encountered adverse events [9–12].

This represents a valuable future perspective in the clinical practice, because a personalized therapy adapted in function of the genetic, pharmacogenetic, neurobiological, spectroscopic profile, could be chosen as first line indication. The results of our research and clinical practice plead for the utility of this modern integrative approach in child depressive disorders [5–8].

Personalization of psychiatric treatments using pharmacogenetic information is emerging as a valuable tool to identify which medications will be more effective, which will require dose adjustments or which may cause serious adverse reactions.

Conclusions

Our research was a proof that sustains the implementation of the pharmacogenetic testing and the value of investigating the relevant neurobiological and neuroimagistic markers, in the clinical practice, for a personalized, individualized therapy also in pediatric depressive disorders, as a fruitful path of care and intervention. As future perspective, the CYP prescreening, the emergence of pharmacogenetics and of the vulnerability markers and of the neuroimagistic, spectroscopic fingerprints, as modern approaches, announce a new stage in the clinical psychiatry, in which the genotype and the biomarkers influence the election of therapy, increasing the safety and efficacy of medication. Clinicians must integrate the clinical but also the genetic dates and also a combined multigenic pharmacogenomic test is needed. Also, the conceptualization of guides which make the translation of the pharmacogenetic results into a predictable phenotype must capture great attention in the future approach.

Conflict of interests

The authors declare that they have no conflict of interests.

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Author contribution

Lavinia Maria Hogea and Daniela Veronica Chiriac have equal contribution and thus share first authorship.

References

[1] Pérez V, Salavert A, Espadaler J, Tuson M, Saiz-Ruiz J, Sáez-Navarro C, Bobes J, Baca-García E, Vieta E, Olivares JM, Rodriguez-Jimenez R, Villagrán JM, Gascón J, Cañete-Crespillo J, Solé M, Saiz PA, Ibáñez Á, de Diego-Adeliño J; AB-GEN Collaborative Group, Menchón JM. Efficacy of prospective pharmacogenetic testing in the treatment of major depressive disorder: results of a randomized, double-blind clinical trial. BMC Psychiatry, 2017, 17(1):250.

[2] Tansey KE, Guipponi M, Hu X, Domenici E, Lewis G, Malafosse A, Wendland JR, Lewis CM, McGuffin P, Uher R. Contribution of common genetic variants to antidepressant response. Biol Psychiatry, 2013, 73(7):679–682.

[3] Perlis RH. Pharmacogenomic testing and personalized treatment of depression. Clin Chem, 2014, 60(1):53–59.

[4] Fabbri C, Serretti A. Pharmacogenetics of major depressive disorder: top genes and pathways toward clinical applications. Curr Psychiatry Rep, 2015, 17(7):50.

[5] Hicks JK, Bishop JR, Sangkuhl K, Müller DJ, Ji Y, Leckband SG, Leeder JS, Graham RL, Chiulli DL, LLerena A, Skaar TC, Scott SA, Stingl JC, Klein TE, Caudle KE, Gaedigk A; Clinical Pharmacogenetics Implementation Consortium. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. Clin Pharmacol Ther, 2015, 98(2):127–134.

[6] Vieta E. [Personalised medicine applied to mental health: precision psychiatry]. Rev Psiquiatr Salud Ment, 2015, 8(3): 117–118.

[7] Jürgens G, Jacobsen CB, Rasmussen HB, Werge T, Nordentoft M, Andersen SE. Utility and adoption of CYP2D6 and CYP2C19 genotyping and its translation into psychiatric clinical practice. Acta Psychiatr Scand, 2012, 125(3):228–237.

[8] Drozda K, Müller DJ, Bishop JR. Pharmacogenomic testing for neuropsychiatric drugs: current status of drug labeling, guidelines for using genetic information, and test options. Pharmacotherapy, 2014, 34(2):166–184.
[9] Lohoff FW, Ferraro TN. Pharmacogenetic considerations in the treatment of psychiatric disorders. Expert Opin Pharmacother, 2010, 11(3):423–439.
[10] Rosenblat JD, Lee Y, McIntyre RS. Does pharmacogenomic testing improve clinical outcomes for major depressive disorder? A systematic review of clinical trials and cost-effectiveness studies. J Clin Psychiatry, 2017, 78(6):720–729.
[11] Winner JG, Carhart JM, Altar CA, Allen JD, Dechairo BM. A prospective, randomized, double-blind study assessing the clinical impact of integrated pharmacogenomics testing for major depressive disorder. Discov Med, 2013, 16(89):219–227.
[12] Singh AB. Improved antidepressant remission in major depression *via* a pharmacokinetic pathway polygene pharmacogenetic report. Clin Psychopharmacol Neurosci, 2015, 13(2):150–156.
[13] Espadaler J, Tuson M, Lopez-Ibor JM, Lopez-Ibor F, Lopez-Ibor MI. Pharmacogenetic testing for the guidance of psychiatric treatment: a multicenter retrospective analysis. CNS Spectr, 2017, 22(4):315–324.
[14] Altar CA, Carhart JM, Allen JD, Hall-Flavin DK, Dechairo BM, Winner JG. Clinical validity: combinatorial pharmacogenomics predicts antidepressant responses and healthcare utilizations better than single gene phenotypes. Pharmacogenomics J, 2015, 15(5):443–451.
[15] Yüksel C, Öngür D. Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. Biol Psychiatry, 2010, 68(9):785–794.
[16] Outhred T, Das P, Dobson-Stone C, Felmingham KL, Bryant RA, Nathan PJ, Malhi GS, Kemp AH. Impact of 5-HTTLPR on SSRI serotonin transporter blockade during emotion regulation: a preliminary fMRI study. J Affect Disord, 2016, 196:11–19.
[17] Stagg CJ, Rothman DL (eds). Magnetic resonance spectroscopy: tools for neuroscience research and emerging clinical applications. 1st edition, Academic Press–Elsevier, San Diego, 2014, 84–107.
[18] Zhang JP, Malhotra AK. Pharmacogenetics of antipsychotics: recent progress and methodological issues. Expert Opin Drug Metab Toxicol, 2013, 9(2):183–191.
[19] Nussbaum LA, Andreescu N, Nussbaum L, Gradinaru R, Puiu M. Ethical issues related to early intervention in children and adolescents with ultra high risk for psychosis: clinical implications and future perspectives. Rev Rom Bioet, 2014, 12(3):64–81.
[20] Nussbaum LA, Hogea LM, Andreescu NI, Grădinaru RC, Puiu M, Todica A. The prognostic and clinical significance of neuroimagistic and neurobiological vulnerability markers in correlation with the molecular pharmacogenetic testing in psychoses and ultra high-risk categories. Rom J Morphol Embryol, 2016, 57(3):959–967.
[21] Sheffield LJ, Phillimore HE. Clinical use of pharmacogenomic tests in 2009. Clin Biochem Rev, 2009, 30(2):55–65.
[22] Zhang JP, Malhotra AK. Pharmacogenetics and antipsychotics: therapeutic efficacy and side effects prediction. Expert Opin Drug Metab Toxicol, 2011, 7(1):9–37.
[23] Malhotra AK, Zhang JP, Lencz T. Pharmacogenetics in psychiatry: translating research into clinical practice. Mol Psychiatry, 2012, 17(8):760–769.
[24] de la Fuente-Sandoval C, Reyes-Madrigal F, Mao X, León-Ortiz P, Rodríguez-Mayoral O, Solís-Vivanco R, Favila R, Graff-Guerrero A, Shungu DC. Cortico-striatal GABAergic and glutamatergic dysregulations in subjects at ultra-high risk for psychosis investigated with proton magnetic resonance spectroscopy. Int J Neuropsychopharmacol, 2015, 19(3):*pyv*105.
[25] Ashwal S, Bartnik-Olson B, Holshouser B. Proton magnetic resonance spectroscopy: applications in neonatal medicine. In: Blüml S, Panigrahy A (eds). MR spectroscopy of pediatric brain disorders. 1st edition, Springer-Verlag, New York, 2013, 77–93.
[26] Brugger S, Davis JM, Leucht S, Stone JM. Proton magnetic resonance spectroscopy and illness stage in schizophrenia – a systematic review and meta-analysis. Biol Psychiatry, 2011, 69(5):495–503.
[27] Nussbaum L, Grădinaru R, Andreescu N, Dumitraşcu V, Tudor A, Suciu L, Ştefănescu R, Puiu M. The response to atypical antipsychotic drugs in correlation with the CYP2D6 genotype: clinical implications and perspectives. Farmacia, 2014, 62(6):1191–1201.
[28] Nussbaum LA, Dumitraşcu V, Tudor A, Grădinaru R, Andreescu N, Puiu M. Molecular study of weight gain related to atypical antipsychotics: clinical implications of the CYP2D6 genotype. Rom J Morphol Embryol, 2014, 55(3):877–884.
[29] Mackenzie B, Souza RP, Likhodi O, Tiwari AK, Zai CC, Sturgess J, Müller DJ. Pharmacogenetics of antipsychotic treatment response and side effects. Therapy, 2010, 7(2): 191–198.
[30] Adkins DE, Åberg K, McClay JL, Bukszár J, Zhao Z, Jia P, Stroup TS, Perkins D, McEvoy JP, Lieberman JA, Sullivan PF, van den Oord EJCG. Genomewide pharmacogenomic study of metabolic side effects to antipsychotic drugs. Mol Psychiatry, 2011, 16(3):321–332.
[31] Müller DJ, Chowdhury NI, Zai CC. The pharmacogenetics of antipsychotic-induced adverse events. Curr Opin Psychiatry, 2013, 26(2):144–150.
[32] Allen P, Chaddock CA, Egerton A, Howes OD, Barker G, Bonoldi I, Fusar-Poli P, Murray R, McGuire P. Functional outcome in people at high risk for psychosis predicted by thalamic glutamate levels and prefronto-striatal activation. Schizophr Bull, 2015, 41(2):429–439.
[33] Marsman A, Mandl RCW, Klomp DWJ, Bohlken MM, Boer VO, Andreychenko A, Cahn W, Kahn RS, Luijten PR, Hulshoff Pol HE. GABA and glutamate in schizophrenia: a 7 T ^{1}H-MRS study. Neuroimage Clin, 2014, 6:398–407.
[34] Toga AW, Mazziotta JC (eds). Brain mapping: the systems. Academic Press, London, 2000.
[35] Kraguljac NV, White DM, Reid MA, Lahti AC. Increased hippocampal glutamate and volumetric deficits in unmedicated patients with schizophrenia. JAMA Psychiatry, 2013, 70(12): 1294–1302.
[36] Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol, 2006, 27(1):24–31.
[37] Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. A meta-analysis of cytokines in major depression. Biol Psychiatry, 2010, 67(5):446–457.
[38] Touma KTB, Zoucha AM, Scarff JR. Liothyronine for depression: a review and guidance for safety monitoring. Innov Clin Neurosci, 2017, 14(3–4):24–29.

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