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1. Introduction

Psychopharmacotherapy of patients with schizophrenia or bipolar disorder is a challenge because of the frequent occurrence of side effects or treatment failure, which can lead to poor adherence and to increased risk of recurrence of illness and readmissions to hospitals. The therapeutic failure or the appearance of adverse effects is partly attributed to the differences or changes in drug metabolism. A large part of the psychopharmacons that are currently used in psychiatry, are metabolized by cytochrome P450 (CYP) enzymes; therefore, a patient's drug-metabolizing capacity is highly influenced by the hepatic CYP activities. Patients' psychopharmacon-metabolizing capacity can be estimated by the evaluation of CYP genotypes and CYP phenotypes. Although the genetic factors resulting in poor or extensive (and ultra-rapid) metabolism can be simply identified by CYP-genotyping, the crucial task is the assessment of hepatic CYP activities (CYP phenotype). The individuals with functional wild type alleles may become transient poor (or extensive) metabolizers as an effect of internal (e.g. diseases, hormonal status, age) or environmental factors (e.g. nutrition, medication, smoking). This means that CYP genotype determines the potential for the expression of functional or non-functional CYP enzyme, whereas the homozygous wild genotype, predicted to be translated to an intermediate metabolizer phenotype, may be switched into poor (or extensive) metabolism due to phenoconversion, which eventually influences the patient's response to a drug. We have described a complex diagnostic system (CYPtest^{TM}) that determines CYP-metabolizing capacity by CYP-genotyping and the current CYP expression in leukocytes (Temesvári *et al*, 2012). CYP mRNA levels in leukocytes were proven to inform about the hepatic activities of CYP1A2, CYP2C9, CYP2C19 and CYP3A4 enzymes.

The goals of the present project were to investigate the patients' CYP-status (CYP genotype and CYP expression) predicting potential poor or extensive metabolism and to analyse the influence of CYP-status on blood concentrations of psychopharmacons and patients' dose-requirements. We attempted to provide evidence for that CYP genotypes are not the only determinant factors in drug-metabolizer status, but the CYP expression can highly influence a patient's drug-metabolizing capacity, which is eventually associated with the therapeutic outcome. Therefore, we investigated the possibilities of personalized psychopharmacotherapy adjusted to the patients' CYP-status.

2. Specific tasks and results

2.1. Identification of psychopharmacons and their metabolites

For the present project, the antipsychotics and adjuvant therapeutics (mood stabilizers, anticonvulsants) most frequently used in psychiatry have been selected: aripiprazole, clozapine, haloperidol, olanzapine, quetiapine, risperidone, carbamazepine, clonazepam and valproic acid. Liquid chromatographic – mass spectrometric (LC-MS/MS) method was developed for identification of each drug and its metabolite(s) in biological matrices (e.g. in patients' sera and in incubation mixtures with hepatic microsomes or hepatocytes).

2.2. *In vitro* metabolism of psychopharmacons (Tóth *et al*, 2016a)

In vitro pharmacokinetic parameters ($t_{1/2}$ elimination half-life, Cl_{int} intrinsic clearance) of psychopharmacons were determined in human hepatocytes, and the bioavailability values (F) were calculated. The predicted bioavailability obtained from human hepatocytes showed an excellent rank order with the clinical bioavailability data. Identification of the key enzymes involved in biotransformation of the drugs was carried out using human liver microsomes and chemical

inhibitors selective for the major drug-metabolizing CYP isoforms. The results of *in vitro* pharmacokinetic and metabolic studies (Table 1) contributed to the knowledge of the overall metabolic pathways of the drugs investigated and to the identification of the key enzymes participate in their biotransformation. The key CYP enzymes were focused on during the patients CYPtesting, whereas the psychopharmacons and their metabolites identified in *in vitro* studies were followed and quantified in patients' blood samples.

Table 1. *In vitro* pharmacokinetic and metabolic parameters of psychopharmacons

	Pharmacokinetic parameters			Metabolites	CYPs involved in metabolism
	$t_{1/2}$ (min)	Cl_{int} (ml/min/kg)	F (%)		
Aripiprazole	311.9	3.66	76.3	dehydro-aripiprazole hydroxy-aripiprazole	CYP2D6, CYP3A4
Clozapine	155.6	7.34	61.6	clozapine N-oxide norclozapine	CYP3A4 CYP1A2, CYP2D6, CYP3A4
Haloperidol	221.6	5.16	69.6	reduced haloperidol piridiniom ion N-desalkyl haloperidol	- CYP2D6, CYP3A4 CYP2D6, CYP3A4
Olanzapine	128.8	8.87	56.9	N-desmethyl olanzapine olanzapine N-oxide hydroxy-olanzapine I hydroxy-olanzapine II	CYP2D6, CYP3A4
Quetiapine	42.9	26.64	30.7	7-hydroxy-quetiapine quetiapine sulfoxide N-desalkyl quetiapine 7-hydroxy-N-desalkyl quetiapine	CYP2D6, CYP3A4 CYP3A4 CYP3A4 CYP2C19, CYP2D6, CYP3A4
Risperidone	165.2	6.92	63.1	9-hydroxy-risperidone	CYP2D6, CYP3A4
Carbamazepine	450	2.54	86.1	carbamazepine epoxide	CYP3A4
Clonazepam	450	2.54	86.1	7-aminoclonazepam	CYP3A4
Valproic acid	450	2.54	86.1	3-hydroxy-valproic acid 4-hydroxy-valproic acid 5-hydroxy-valproic acid	CYP2C9, CYP2C19, CYP3A4 CYP1A2, CYP2C9, CYP3A4 CYP1A2, CYP2C9, CYP3A4

$t_{1/2}$ elimination half-life, Cl_{int} intrinsic clearance, F predicted bioavailability

2.3. Method development for *CYP2D6* and *NAT2* genotyping (Kiss *et al*, 2016; Tóth *et al*, 2016b)

Since CYP2D6 is one of the major enzymes responsible for the metabolism of plenty of psychopharmacons, the methodology of CYPtest™ for CYP2D6-genotyping has been supplemented with the identification of loss-of-function *CYP2D6*10* and *CYP2D6*41* alleles as well as with the novel method for the identification of *CYP2D6* duplication. To distinguish the duplication of the functional *CYP2D6*1* and the non-functional *CYP2D6*4* alleles, a real-time PCR method was developed combining quantitative PCR and genotyping PCR with TaqMan probes. *CYP2D6*4* genotyping in parallel with *RNase P* reference gene assay were performed, then relative copies of the wild type (*CYP2D6*1*) and *CYP2D6*4* alleles were obtained based on the relative quantity of each alleles compared to a control sample carrying exactly one wild type allele and one *CYP2D6*4* allele.

Since the highly polymorphic N-acetyl transferase 2 (NAT2) is also involved in clonazepam metabolism, we have developed genotyping methods for identification of slow and rapid acetylation phenotypes. NAT2 acetylation phenotype was inferred from the four-SNP panel of 191G>A (rs1801279), 341C>T (rs1801280), 590G>A (rs1799930) and 857G>A (1799931), distinguishing the slow acetylator *NAT2*5, NAT2*6, NAT2*7* and *NAT2*14* alleles from the rapid acetylator alleles (Doll and Hein, 2001; Hein and Doll, 2012). Patients with two slow acetylator alleles were assigned to the slow acetylator phenotype category, whereas all the others were considered to display rapid/intermediate acetylator phenotype.

2.4. CYP allele frequencies in patients

The polymorphic CYP alleles resulting in clinically relevant alteration in CYP activities were identified in 217 inpatients at the Department of Psychiatry and Psychotherapy, Semmelweis University. The CYP allele frequencies in the patients were similar to those in Caucasian white populations except for *CYP2C19*17* (Table 2). The gain-of-function *CYP2C19*17* allele occurred significantly less frequently in the patients of the present study than in Caucasian white individuals.

Table 2. Frequencies of polymorphic *CYP* alleles in the patients and in Caucasian populations

CYP	Allele	Frequency (%) in the present study	Frequency (%) in Caucasian populations*
CYP2C9	**2*	12.0	8-19
	**3*	9.4	6-10
CYP2C19	**2*	13.1	6-15
	**3*	0.7	>1
	**4*	0.7	>1
	**17*	7.4	20-25
CYP2D6	**3*	1.6	2
	**4*	20.9	12-21
	**5*	1.9	2-4
	**6*	0.7	1
	**10*	1.4	1-2
	**41*	7.0	8
	duplication	3.3	2-3
CYP3A5	**3*	94.9	90-95

* http://www.cypalleles.ki.se/

2.5. Patients' CYP-status and their psychopharmacotherapy

For personalized psychopharmacotherapy, it is essential to identify the major key factors that can influence patients' drug-metabolizing capacity and the elimination rates of psychopharmacons.

2.5.1. Clinical relevance of CYP enzymes in clozapine therapy (Tóth *et al*, 2016c)

The atypical antipsychotic clozapine is effective in treatment-resistant schizophrenia; however, the success or failure of clozapine therapy is substantially affected by the variables that impact the clozapine blood concentration. Thus, elucidating the inter-individual differences in clozapine pharmacokinetics can facilitate the personalized therapy. Since potential role in *in vitro* metabolism of clozapine is assigned to CYP1A2, CYP2C19, CYP2D6 and CYP3A enzymes, the association between the patients' CYP-status (*CYP* genotypes, CYP expression) and clozapine clearance was

evaluated in 92 psychiatric patients. The patients' *CYP2C19* or *CYP2D6* genotypes and CYP1A2 expression seemed to have no effect on clozapine serum concentration, whereas CYP3A4 expression was found to be the major determinant of normalized clozapine concentration (185.53±56.53 in low expressers *vs* 78.05±29.57 or 66.52±0.25 (ng/ml)/(mg/kg) in normal or high expressers, $P<0.0001$), in particular that the patients expressed CYP1A2 at relatively low level (Fig. 1). The functional *CYP3A5*1* allele seemed to influence clozapine concentrations in those patients who expressed CYP3A4 at low levels.

The dose-requirement for the therapeutic concentration of clozapine was substantially lower in low CYP3A4 expresser patients than in normal/high expressers (2.18±0.64 *vs* 4.98±1.40 mg/kg, $P<0.0001$) (Fig. 1). Overdosing of the low CYP3A4 expresser patients was found to be more frequent than of the CYP3A4 normal/high expressers. Furthermore, significantly higher plasma concentration ratios of norclozapine/clozapine and clozapine N-oxide/clozapine were observed in the patients displaying normal/high CYP3A4 expression than in the low expressers. The active metabolite norclozapine is a more potent 5-HT_{2C} antagonist than clozapine; therefore, the risk of side effects, such as weight-gain and seizure, is increased for the patients expressing CYP3A4 at normal/high concentration or for those carrying *CYP3A5*1*. Prospective assaying of CYP3A-status (CYP3A4 expression, *CYP3A5* genotype) can better identify the patients with higher risk of inefficiency or adverse reactions, and can facilitate the improvement of personalized clozapine therapy.

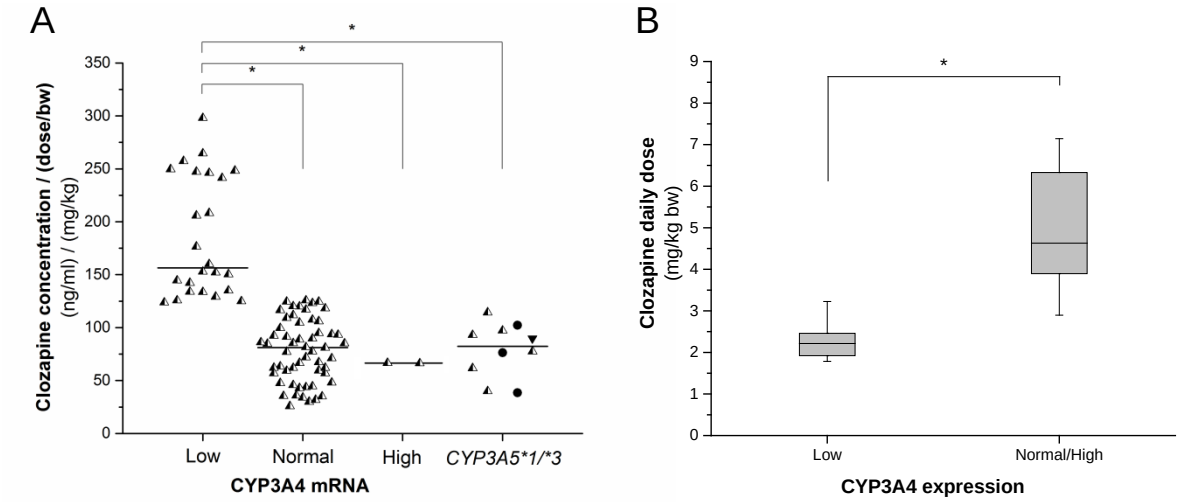

Fig. 1 The influence of the patients' CYP3A4 expression and *CYP3A5* genotype on serum clozapine concentrations (A) and dose-requirements (B). (A) Clozapine concentrations were normalized by the dose and the bodyweight in CYP3A5 expressers (*CYP3A5*1/*3*) and non-expresser patients expressing CYP3A4 at low, normal and high levels, and (B) dose-requirements were calculated from applied doses in the patients displaying therapeutic concentrations of clozapine (200-600 ng/ml). The black points (A) indicate low CYP3A4 expresser patients carrying *CYP3A5*1*. The black triangle (A) indicates a high CYP3A4 expresser patient carrying *CYP3A5*1*.
* $P<0.0001$

2.5.2. Optimization of clonazepam therapy adjusted to patient's CYP3A-status and *NAT2* genotype (Tóth *et al*, 2016b)

Clonazepam, the benzodiazepine type drug was initially introduced as an antiepileptic agent; however, it is a useful therapeutic adjunct in psychiatric disorders. The shortcomings of clonazepam

therapy include tolerance, withdrawal symptoms and adverse effects, such as drowsiness, dizziness and confusion leading to increased risk of falls. Inter-individual variability in the incidence of adverse events in patients partly originates from the differences in clonazepam metabolism due to genetic and non-genetic factors. Since the prominent role in clonazepam nitro-reduction (to 7-amino-clonazepam) and in acetylation of 7-amino-clonazepam is assigned to CYP3A and NAT2 enzymes, respectively, the association between the patients' CYP3A-status (*CYP3A5* genotype, CYP3A4 expression) or NAT2 acetylator phenotype and clonazepam metabolism (plasma concentrations of clonazepam and 7-amino-clonazepam) was evaluated in 98 psychiatric patients suffering from schizophrenia or bipolar disorders. The patients' CYP3A4 expression was found to be the major determinant of clonazepam plasma concentrations normalized by the dose and the bodyweight, whereas the presence of functional CYP3A5 enzyme (patients carrying at least one allele of *CYP3A5*1*) did not contribute to clonazepam pharmacokinetics. The patients expressing CYP3A4 at low levels displayed much higher blood concentrations of clonazepam than those with normal CYP3A4 levels (1263.5±482.9 and 558.5±202.4 (ng/ml)/(mg/kg) in low and normal expressers, respectively, $P<0.0001$). Consequently, the dose-requirement for the therapeutic concentration of clonazepam was substantially lower in low CYP3A4 expresser patients than in normal expressers (0.029±0.011 *vs* 0.058±0.024 mg/kg, $P<0.0001$). Furthermore, significantly higher (about 2-fold) plasma concentration ratio of 7-amino-clonazepam and clonazepam was observed in the patients displaying normal CYP3A4 expression and slow N-acetylation than all the others. 7-Amino-clonazepam seems to accumulate in normal CYP3A4 expresser patients with slow NAT2 acetylator phenotypes. Prospective assaying of CYP3A4 expression and NAT2 acetylator phenotype can better identify the patients with higher risk of adverse reactions and can facilitate the improvement of personalized clonazepam therapy and withdrawal regimen.

2.5.3. Clinical significance of CYP2C9-status guided valproic acid therapy (Tóth *et al*, 2015; Bűdi *et al*, 2015; Nagy *et al*, 2015; Monostory *et al*, 2016)

Valproic acid is both an option for anticonvulsant adjuvant therapy for schizophrenia or bipolar disorder and the first option of choices for patients with epilepsy. Significant variations in valproate pharmacokinetics and shifting the metabolic pathways towards CYP2C9-dependent metabolism seem to play some role in the age-related differences in the incidence of adverse events. It was clearly demonstrated that the CYP2C9-catalyzed oxidation can become the principal route of valproate metabolism in those special cases when glucuronidation or mitochondrial β-oxidation pathways are compromised or poorly developed. Strong association between the patients' CYP2C9-status (*CYP2C9* genotype and CYP2C9 expression) and their dose-requirements for optimal valproate blood concentration was found. Thus, CYP2C9-status driven valproate therapy was suggested for patients: 1) non-valproate therapy was proposed to the patients with two mutated *CYP2C9* alleles, 2) and valproate therapy adjusted to the patients' CYP2C9-status was applied in those with one or two wild-type alleles. Normal dose (30-40 mg/kg) was given to the normal CYP2C9 expresser patients carrying CYP2C9 homozygous wild genotype (*CYP2C9*1/*1*), reduced dose (10-20 mg/kg) was administered to the patients with heterozygous genotypes (*CYP2C9*1/*2* or *CYP2C9*1/*3*), or to low CYP2C9 expressers, while increased dose (>40 mg/kg) was targeted in high expresser patients with *CYP2C9*1/*1* genotype (Tóth *et al*, 2015).

The benefits of CYP2C9-status driven therapy over conventional clinical practice were evaluated in 99 patients. CYP2C9-guided treatment significantly reduced valproate-misdosing and consequently

decreased the ratio of patients out of the range of target valproate blood concentrations. In CYPtest group of patients receiving CYP2C9-status adapted dose, serum alkaline phosphatase (ALP) and the ratio of patients with abnormal ALP levels were substantially lower than in the control group. The incidence of serious side effects, notably hyperammonemia, was reduced in CYPtest group; however, some other side effects, such as weight changes and somnolence, could not be avoided (Bűdi *et al*, 2015; Monostory *et al*, 2016). We have also presented a case report of a patient displaying serious side effects under valproate therapy, which was attributed to the loss-of-function mutations in *CYP2C9* (*CYP2C9*3/*3* genotype) (Nagy *et al*, 2015).

2.5.4. Impact of the *CYP2D6* genotype on serum concentrations of aripiprazole, risperidone and quetiapine

The atypical antipsychotics, aripiprazole, risperidone and quetiapine are frequently used in psychiatry. Similarly to clozapine, these drugs are effective in treatment of schizophrenia and bipolar disorder and less likely to cause extrapyramidal symptoms in patients; however, they still have side effects, such as weight gain, tardive dyskinesia, agranulocytosis or increased blood glucose concentration. Since blood concentrations are associated with the frequency of adverse effects, patients' antipsychotic-metabolizing capacity highly influences the therapeutic outcomes. Several CYP enzymes (CYP2D6, CYP3A) have been reported to be involved in the metabolism of aripiprazole, risperidone and quetiapine that was also confirmed by the results of our *in vitro* studies. The association between the patients' *CYP2D6* genotype or CYP3A-status (*CYP3A5* genotype, CYP3A4 expression) and antipsychotic clearance was evaluated in psychiatric patients (Fig. 2 and Fig. 3).

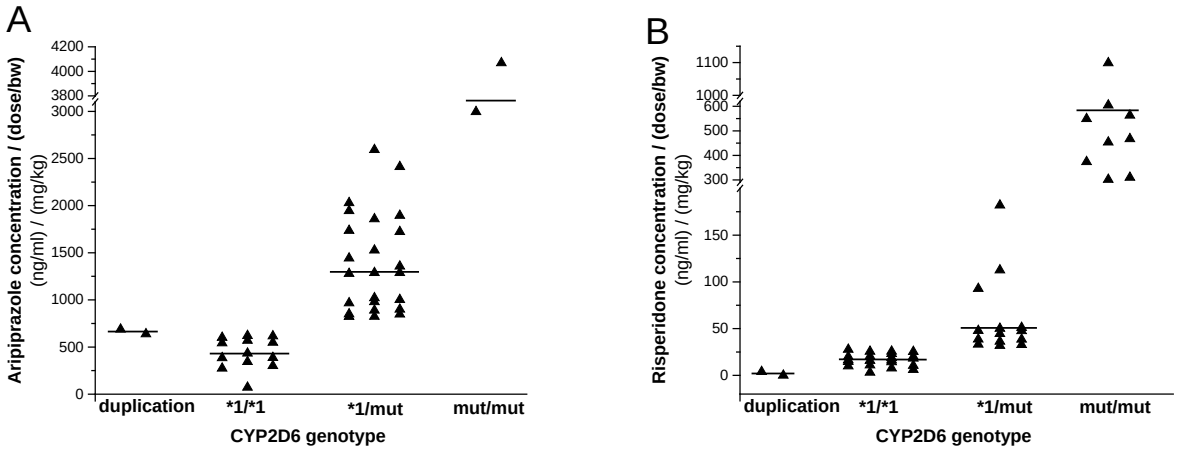

Fig. 2. The influence of the patients' *CYP2D6* genotypes on serum aripiprazole (A) and risperidone concentrations (B).
*CYP2D6*1* wild type allele; CYP2D6mut loss-of-function and reduced-function alleles *(*3, *4, *5, *6, *10, *41*)

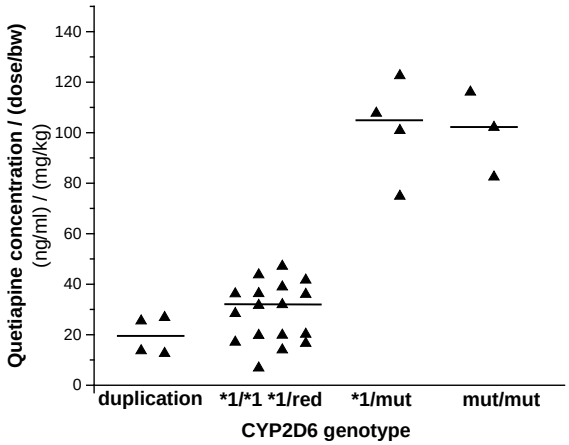

Fig. 3. The influence of the patients' *CYP2D6* genotype on serum quetiapine concentrations. *CYP2D6*1* wild-type allele; CYP2D6mut loss-of-function alleles (**3, *4, *5, *6*); CYP2D6red reduced-function alleles (**10, *41*).

The patients' *CYP2D6* genotype seems to be the major determinant of normalized aripiprazole, risperidone or quetiapine concentrations. One or two copies of loss-of-function or reduced-function alleles (**3, *4, *5, *6, *10, *41*) resulted in increased serum concentrations of both aripiprazole and risperidone (Fig. 2). Normalized serum concentrations of quetiapine were found to be influenced by the loss-of-function alleles (**3, *4, *5, *6*), whereas reduced-function alleles did not lead to an increase in serum concentration comparing to homogenous wild genotype (*CYP2D6*1/*1*) (Fig. 3). On the other hand, duplication of *CYP2D6* gene did not increase the antipsychotic clearance. For drawing reliable consequences, more patients on aripiprazole, risperidone or quetiapine therapy should be involved.

3. Conclusion

The patients' CYP-status and drug-metabolizing capacity, influenced by genetic and non-genetic factors, were found to determine the normalized blood concentrations of psychopharmacons; therefore, prospective assaying of patients' CYP-status can identify poor metabolizers with higher risk of side effects or extensive metabolizers displaying therapeutic failure. Although a preliminary identification of the drug-metabolizing enzyme(s) was found to be essential, the clinical relevance of these enzymes and the association between the patients' CYP-status and psychopharmacotherapy had to be elucidated. CYP-status driven psychopharmacotherapy was suggested for the patients: 1) CYP3A4 expression and *CYP3A5* genotype seemed to influence clozapine therapy; 2) the prospective knowledge of patients' CYP3A4 expression and NAT2 acetylation phenotype can facilitate the improvement of clonazepam treatment; 3) CYP2C9-status guided valproic acid therapy could reduce misdosing and consequently decrease the ratio of patients out of the range of target valproate blood concentrations in those special cases when glucuronidation or mitochondrial β-oxidation pathways were compromised or poorly developed; 4) the patients' *CYP2D6* genotype appeared to be the major determinant of aripiprazole, risperidone and quetiapine clearance. Tailored medication controlled by patients' drug-metabolizing capacity can facilitate the improvement of psychopharmacotherapy, leading to the dosage optimization for a more effective therapy, and minimizing the risk of side effects.

4. Deviation from the Research Plan

4.1 Personal changes

In 2013, the pregraduate student Borbála Soltész (Eötvös Loránd University) was replaced to Cintia Juhász (Budapest University of Technology and Economics). Juhász C. was involved in development of *CYP2D6* genotype methodology. She has presented the results (entitled: 'A *CYP2D6* polimorfizmusai: genotípustól a fenotípusig') at the National Undergraduate Research Conference in 2014 and won of the 3rd prize.

An additional pregraduate student Ágnes Emília Kiss (Budapest University of Technology and Economics) was involved in *in vitro* pharmacokinetic studies of psychopharmacons. She defended her MSc thesis based on these *in vitro* results (entitled: 'Pszichofarmakonok farmakokinetikai viselkedésének becslése májsejteken végzett *in vitro* vizsgálatokból') in 2015.

4.2 Financial restructuring

Financial resources planned for conference participation were not fully applied because the participation of the PhD students was free of charge and the principal investigator was invited to IHSS conference in 2016; thus, these finances were allocated to daily allowance, consumables and miscellaneous costs (e.g. publication, participation at national conferences). The allocation facilitated the dissemination of the results.

4. References and Publications

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