Pharmacological research in the last 10 years has led to the synthesis of new antiepileptic drugs (AED) widely used to treat the disease. However, epilepsy remains a condition only partially controlled by medication at a significant number of patients [1]. Several hypotheses concerning the etiology of resistance to anti-epileptic therapy have been advanced. Epilepsy is known to be a heterogeneous condition with variable causes; therefore the lack of response to treatment may be multifactorial and variable, including both genetic and environmental factors [2]. Structural changes [3] or abnormalities in the neural network and in the functioning of neurotransmitters [4] can cause resistance to antiepileptic treatment.

One of the hypotheses put forth recently claims that there is another mechanism involved in the pathogenesis of treatment-refractory epilepsy, which affects drug penetration into the brain through transporters [5]. Patients with drug-refractory epilepsy are non-responsive to several AED which have different molecular mechanisms of action. These clinical observations suggest a nonspecific mechanism limiting the effectiveness of AED [6]. The overexpression of efflux transporters at the blood brain barrier, preventing optimal AED levels in the brain in the context of an appropriate plasma drug level could be such a mechanism. The prototype of these transporters is P-glycoprotein (P-gp).

ABC transporter family and P-glycoprotein

P-gp was first identified in 1976 by Juliano and Ling as a surface protein at the level of ovary cells in Chinese hamsters expressing the MDR phenotype [7]. P-gp belongs to the superfamily of ABC...
transporters [8] which includes more than 100 such proteins identifiable in all types of organisms [9]. So far 48 genes have been identified in humans. These genes were classified as belonging to several subfamilies: ABCA (ABC1), ABCB (MDR/TAP), ABCC (MRP/CFTR), ABCCD (ALD), ABCE (OABP), ABCF (GCN20) and ABCG (White) [10]. Most of the transporters in this superfamily act as membrane pumps activated by ATP hydrolysis, directing the flow of specific substrates against a concentration gradient. The substrate of these proteins is extremely diverse, including drugs, food nutrients, amino acids, peptides, sugars, pigments and metals [11]. Mutations in these proteins may be the cause of genetic disorders such as CFTR protein in cystic fibrosis, MRP2 in the Dubin Johnson syndrome, ALD protein in adrenoleukodystrophy. The P-gp protein is encoded by a small family of 2 human genes, called MDR1 (ABCB1) and MDR2 (ABCB4). These genes are located next to each other on chromosome 7 (7q21) [12]. The MDR1 gene encodes the specific drug transporter responsible for the drug multidrug resistance phenotype and the MDR2 gene encodes a transporter for phosphatidylcholine in the bile canaliculi.

Human glycoprotein P-gp encoded by the MDR1 gene is a membrane protein containing 1280 amino acids [13], comprised of 2 homologous halves, each one with a portion consisting of 6 transmembrane segments and a nucleotide binding domain, or unit ABC [14]. The 3D structure and the exact mechanism of action of P-gp are not yet fully known. What it is known is the fact that it acts as a pump that removes the specific substrate from the cell into the extracellular space, thus decreasing its accumulation in the cell [15].

### Locating P-gp continent tissue and P-gp substrate

Histopathology studies revealed the presence of this glycoprotein in humans in various tumors (phenotype MDR) and at the apical pole of the cell membrane in normal tissues with an excretion function (liver, kidney, adrenal glands) or a barrier function (intestine, blood-brain barrier, placenta, testicular, and ovarian barrier). This presence suggests the important role of these proteins, that of body detoxification and protection against toxic metabolites and xenobiotics, helping their excretion in the bile, urine, in the intestinal lumen and preventing the accumulation of these toxins in the brain, testis and fetal body [16].

P-gp has an increased transport capacity and very broad substrate specificity, so that a large variety of drugs, with different structures and belonging to several classes can constitute P-gp substrate. These drugs include anticancer chemotherapy drugs, anti-HIV protease inhibitors, H2 antagonists, cardiac glycoside, calcium channel blockers, immunosuppressant drugs, corticosteroids, antiemetic and anti diarrheal drugs, antibiotics, anthelmintics, anti-epileptics, sedatives and antidepressants [17]. Some of these substances act as P-gp inhibitors, while others as inducers of this transporter [18] (Table 1). The typical P-gp substrate is actually a high-molecular-weight (over 400 kDa), hydrophobic, amphipathic molecule, with a flat ring-type system

<table>
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<tr>
<th>P-gp substrate</th>
<th>Other</th>
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<td>Cancer chemotherapy</td>
<td>Ca channel blockers</td>
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<tr>
<td>Doxorubicin</td>
<td>Verapamil</td>
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<tr>
<td>Daunorubicin</td>
<td>Nifedipine</td>
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<td>Achtynomicine D</td>
<td>Diltiazem</td>
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<tr>
<td>Vincristine</td>
<td>Protease inhibitors</td>
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<td>Etoposide</td>
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<td>Teniposid</td>
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<td>Paclitaxel</td>
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Table 1. Examples of P-gp substrates and inhibitors
and weak ionic charge at physiological pH levels [19]. But linear nonaromatic or circular compounds that are neutral or weak acids can also be substrates of the same transporter [5]. Some AED are matching these criteria of structure.

In conclusion we can say that P-gp is a major determining factor for the availability and effect of in vivo drugs, being involved in drug interactions that may become clinically relevant for drugs with narrow therapeutic index [20].

Genetic variations of MDR1

To date there is ample data showing that MDR1 gene variations affect P-gp expression and function. Most cases in which a single nucleotide polymorphism (SNP) is determined involve non-encoding gene regions, thus not affecting the P-gp amino acid sequence. However genetic variants involving the ABCB1 gene encoding region determine changes in the amino acid sequence affecting P-gp expression and activity [12]. MDR1 is a highly polymorphic gene with at least 105 variants of the ABCB1 (MDR1) gene, with significant differences in frequency in individuals of different ethnic groups [21]. The first genetic polymorphism to be identified was the G2677T variant, isolated from adrenal, liver and kidney samples, leading to a change of Ala893Ser in the P-gp structure [22]. The polymorphism at the level of exon 26 (C3435T) in the MDRI gene, decreasing P-gp in the duodenum, determines the increase of the peak plasma concentration of its substrate, digoxin, in healthy volunteers [23]. Also, other substances which are substrates for the transporter, such as fexofenadine, tacrolimus, irinotecan, cyclosporin A12, have been shown to increase plasma concentrations associated with certain mutations of ABC1 gene. Recent studies have shown that C3435T-type ABCB1 gene polymorphism is associated with the effectiveness of anti-emetic treatment with 5HT3 antagonists in patients with cancer [24], and with postural hypotension as a side effect of nortriptiline therapy in patients with depression [25].

Many P-gp data were obtained from oncology patients due to the role it plays in chemotherapy resistance, revealing an association between C3435T and G3677T ABCB1 gene polymorphism and risk of drug-resistance in patients with lymphoproliferative disorders [26]. The association of single polymorphisms commonly found in ABCB1 gene and the risk of relapse after treatment with etoposide, mitoxantrone and daunorubicin, substances known as P-gp substrates were also noticed in patients with acute myeloid leukemia [27]. Moreover, this polymorphism can be used as a predictive factor for drug response in children with acute lymphoblastic leukemia, although these observations were not confirmed in adults [28].

Pharmacogenetic studies have shown that MDR1 allele frequency is varied in different population groups. Thus, MDR1 gene polymorphism is more common in Caucasian and African-American populations compared to other population groups. The genotypes studied in terms of population differences are C1236T, G2677T and C3435T. The 3 genotypes are found in 40-45% of the Caucasian population, but only in 5-10% of African-Americans. Genotype frequency in Asian population is located between the two, whereas variations in Indonesian and Mexican populations come close to the values observed in the Caucasian population [29].

There are some haplotypes that appear more frequently in specific ethnic populations. Kroetz et al. studied different population groups, analyzed 100 DNA samples from each (Caucasian, African-American, Asian, Mexican, Indonesian) and identified 64 MDR1 haplotypes, of which 33 were noticed on 3 or more chromosomes.

MDR1 gene polymorphism and availability of antiepileptic drugs

Due to their hydrophobic structure, antiepileptic drugs could be substrates for P-gp transporter. There are several in vitro studies concerning the level of these drugs in the brain. Schinkel et al. did not discover any changes in the concentration of radiolabeled phenytoin in the brain of mdr1a/1b (-/-) mice compared to the native type[30]. Another preliminary study investigated 8 AEDs, 4 classical AEDs and 4 new generation AEDs in mdr1a/1b mice (-/-) compared to the native type. All antiepileptics had the same plasma levels in the 2 groups submitted for analysis. However, the brain / plasma concentration ratio was higher for carbamazepine, lamotrigine, gabapentin and topiramat in mdr1a/1b (-/-) mice, compared to the native type, which suggests that these AEDs could be substrates for P-gp [31].

In vivo studies associated AEDs like phenytoin, carbamazepine, phenobarbital, lamotrigine and felbamat with known P-gp inhibitors such as verapamil. The conclusion was that the local inoculation of verapamil significantly increases the extracellular levels of these AEDs in the brain, suggesting that they are transported by P-gp at the blood brain barrier [32].
Inter-individual variability between the absorption and biodistribution of carbamazepine and phenytoin could contribute to inadequate control of epileptic seizures. A low carbamazepine and phenytoin plasma level was noticed in patients with intestinal overexpression of MDR1 gene, so the polymorphism in position 3435 and 2677 influences the dosage of AED used [33]. As mentioned before, inter-individual variability also depends on ethnicity. A study conducted in Turkey on 97 patients with epilepsy, both responsive and non-responsive to treatment, discovered that MDR1 gene C3435T polymorphism is not significantly related to carbamazepine resistance [34].

MDR1 gene polymorphism and development of neurological diseases

In addition to the bioavailability of specific drugs, recent studies on MDR1 polymorphism have also taken into account the natural evolution of diseases associated with the decrease of the protective function of P-gp. Thus, a remarkable number of studies demonstrated the association between SNP and susceptibility to certain diseases, such as refractory epilepsy, Parkinson’s disease, inflammatory bowel disease, colorectal and kidney cancer [35] and other diseases whose pathogenesis involves natural barriers, such as the intestinal and the blood brain barrier.

Early studies have focused on Parkinson’s disease and MDR1 gene polymorphism without a statistically significant association between G2677T and C3435T genotype and Parkinson’s disease. However, there is a higher frequency of 3435TT genotype in patients with rapid disease onset (36%) compared to those who experience the onset at an older age (22.9%) [36] and the control subjects (18.9%) [37]. The hypothesis that was put forward revealed that patients with 3435TT genotype have functional deficiencies in the blood brain barrier causing a reduced expression of P-gp, and therefore are more susceptible to the neurotoxic action of xenobiotics. This was demonstrated by a study showing that there is a 5-fold risk to develop Parkinson’s disease after patients with 3435T allele were exposed to pesticides [38].

At a later stage, research has been extended to patients with drug-refractory epilepsy. It was noticed that individuals with the 3435CC genotype compared to those with 3435TT genotype are more susceptible to develop resistance to the disease, thus showing once again the functional efficiency of the blood brain barrier around the epileptic foci[39]. The most frequently studied transport systems in the brain are P-gp and multiple drug resistance protein (MRP). Drug-refractory epilepsy is associated with an overexpression of transport protein in the brain, proven by several in vivo and in vitro studies. Epileptic foci in the temporal lobe were the most frequently studied process in terms of drug transport regulation in the brain. Tishel et al. showed a ten-fold increase in mRNA MDR1 in the removed brain tissue at 11 out of 19 patients subject to temporal lobectomy, compared to the normal brain tissue (resected during interventions for arteriovenous malformations) [40]. Dombrovski et al. discovered the overexpression of MDR1, MRP2 and MRP5 in endothelial cells isolated from temporal vessels in tissue samples taken from 5 patients with refractory epilepsy treated with temporal lobectomy [41].

An important question is whether the mechanism leading to this overexpression of efflux transporters in the brain with epilepsy is native, acquired or mixed. Native overexpression may occur as a result of a genetic predisposition or perhaps an intrinsic development of specific pathologies. The acquired type may appear in the context of frequent seizures or may be induced by AEDs used to prevent seizures. To date most studies have shown the involvement of both mechanisms.

Tests concerning the genetic hypothesis of drug resistance were performed in a large study conducted by Siddiqui et al. Genotyping was performed of 315 white patients diagnosed with chronic epilepsy (200 patients with treatment-resistant epilepsy and 115 responsive patient) and 200 patients without epilepsy. Patients with the refractory disease had a higher ratio of 3435 CC genotype than the 3435TT genotype, compared to responsive patients or the patients in the control group. There were no differences between patients with generalized forms of the disease (which is presumed genetic) and those with focal forms. Also, P-gp overexpression is restricted to tissue lesion. Positive response to pharmacological therapy and C3435T genotype association was not confirmed in other studies involving patients with similar ethnic features [42]. In a study involving a total of 400 patients with epilepsy, of which 170 patients responsive and 230 patients non-responsive to treatment, no correlation was found between C3435T polymorphism of MDR1 gene and the resistant form of the disease.

Zimprich conducted studies on patients with temporal lobe epilepsy and observed association.
of haplotype C1236T/C3435T/G2677T with a high risk of resistance to treatment [43]. As mentioned before, MDR1 gene polymorphism varies in different population groups so the data is also variable. A study conducted on 214 Chinese children with epilepsy, of which 50 were resistant to treatment and 164 were responsive, revealed no significant differences between the 2 groups in terms of 3435CC genotypes and C allele frequency in locus 3435 [44]. Similar results were recorded in the Korean population [45]. However studies by Bialecki et al. showed a correlation between MDR1 gene polymorphism and drug-resistant epilepsy. Thus 3435TT genotype is associated with a reduced expression of P-gp in the brain compared to 3435CC and 3435CT genotypes [46].

The correlation of the structure genotype and the expression of MDR1 in the brain need to be clarified in further studies, given that the studies conducted so far have been performed mostly on a small number of patients, and had conflicting results. Data resulting from studies on the availability of medication associated with polymorphisms is inconsistent, in most cases explained by heterogeneity of haplotypes in different populations and relatively low numbers of samples analyzed, and due to the fact that only a small number of mutations (especially C3435T polymorphism) were studied. Also, the economic aspects of genotyping will have to be taken into consideration, by evaluating the cost-benefit ratio.

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