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ABCB1 genetics and function determine penetrance of antidepressants into the brain: treatment implications and open research questions

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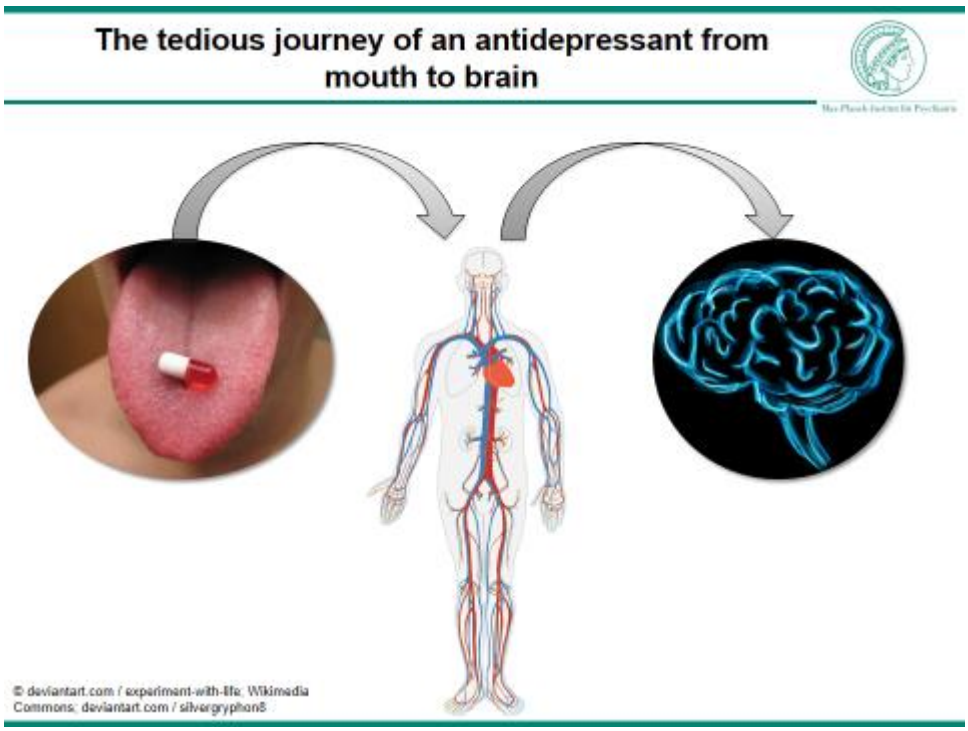
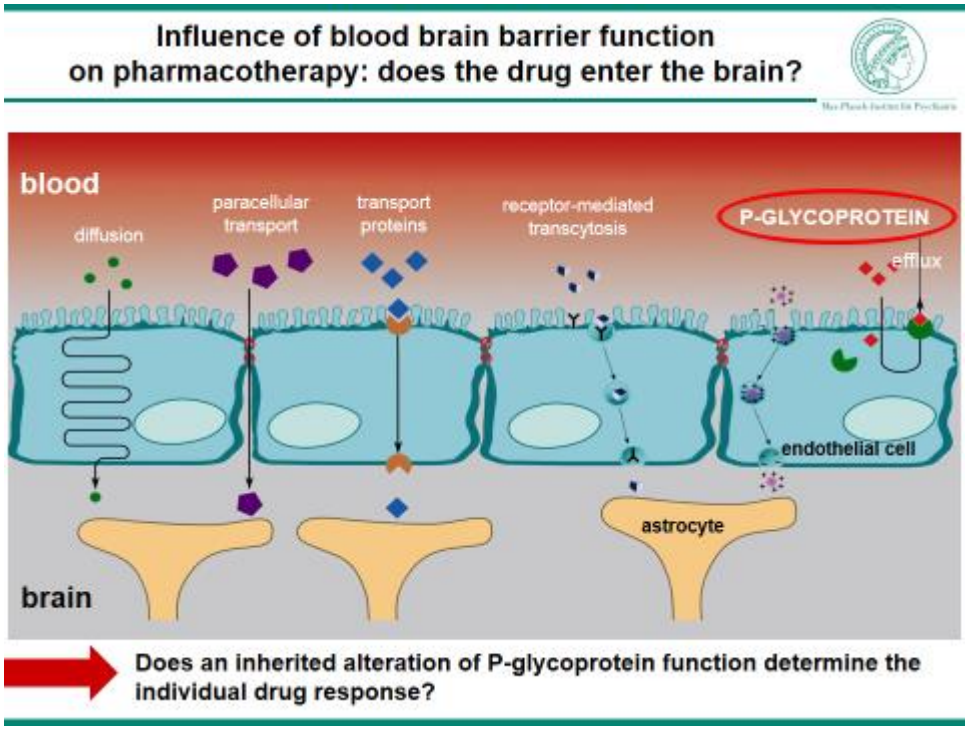
Any medication that is orally administered has quite a journey ahead: The drug has to survive enzymes and exposure to acidic milieu within the gastrointestinal tract before the difficult passage from gut to blood circulation is achieved. Then, the drug has to withstand catabolizing enzymes in the liver while attempting to do what it is supposed to do at target tissues. The psychotropic drugs that have to act in the brain have to overcome a further hurdle: the P-glycoprotein, a polyprotein that is covalently bonded to carbohydrate moieties.



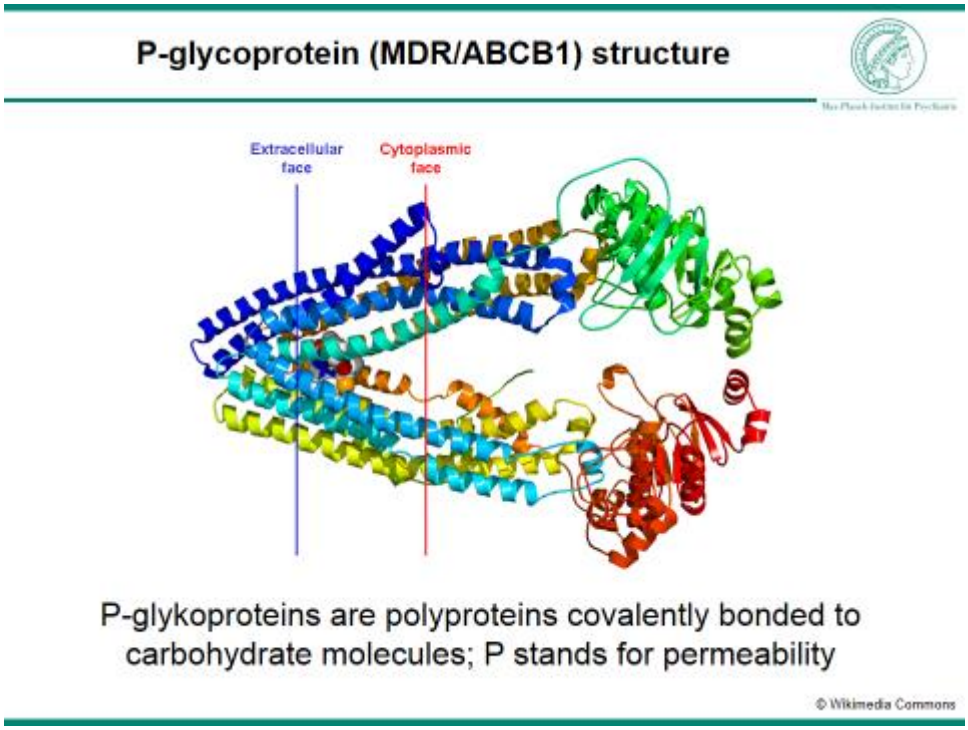


This molecule was discovered in the year 1976 by Juliano and Ling in drug resistant hamster ovary cells and is in the focus of our symposium today.

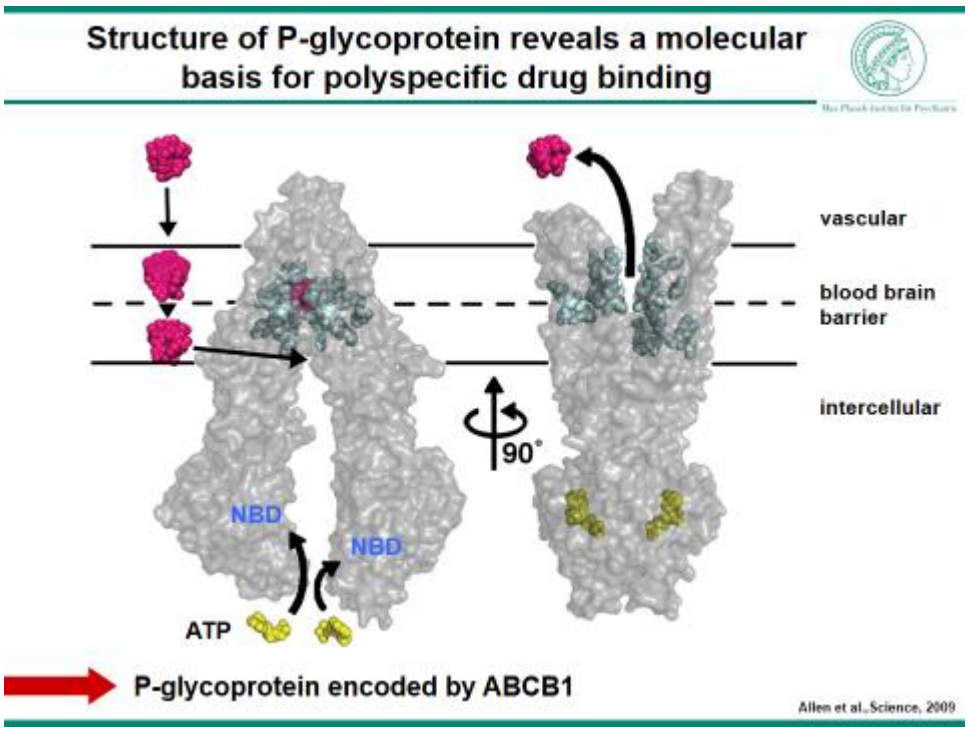
P-glycoprotein is an extensively studied molecule as it protects the brain cells from exposure to molecules that can be harmful. It is an ATP-binding cassette (ABC) transporter that extrudes toxins and xenobiotics from cells. This is not only true for its function as a barrier within the blood brain interface but also for many other cells in the body. However, its primary locations are within the blood brain barrier and the intestine and therefore P-glycoprotein seems to work specifically there. However, the capacity of the P-glycoprotein as extrusion pump in the gut is limited. Therefore, the effect upon overall drug absorption from intestinal lumen into epithelial cells is quantitatively not very important. Only if a very small dose is given, or if the dissolution and diffusion rates of the administered drug are very slow it is possible that the P-glycoprotein transport activity does not get saturated and may influence pharmacokinetics.

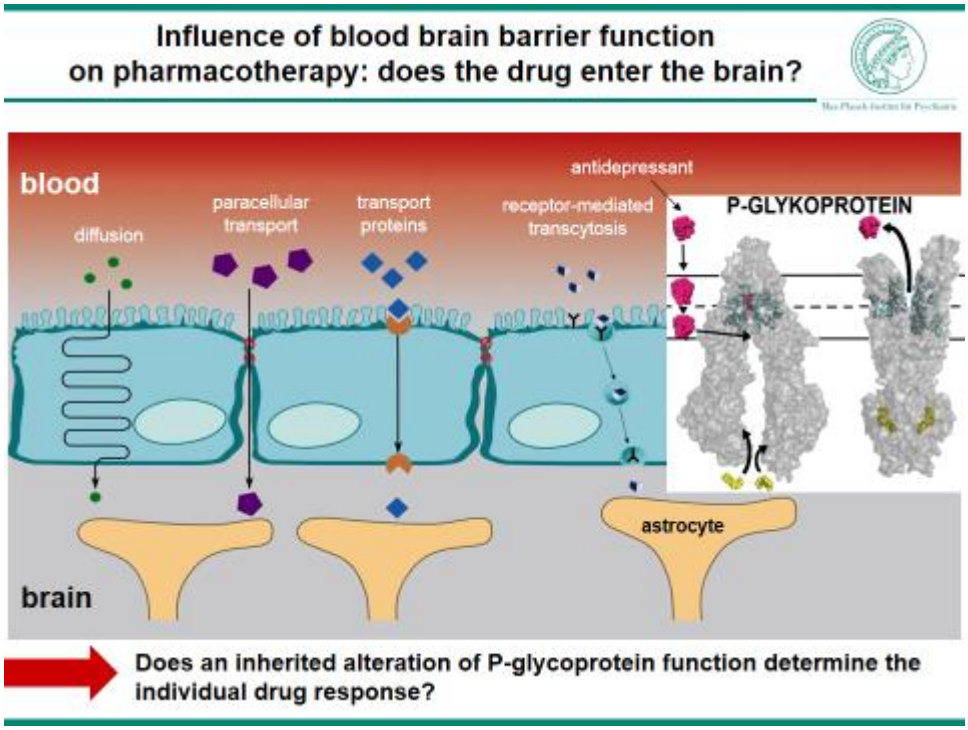
In fact, the relevance of P-glycoprotein as peripheral factor that influences pharmacokinetics is likely to be much less important than cytochrome P450 isoenzymes whose effects on antidepressant drug metabolization can be monitored by plasma drug level concentrations. In our hands, therapeutic drug monitoring has advantages over cytochrome P450 genotyping, because under real-world conditions clinicians prescribe routinely more than one drug creating variable effects on cytochrome P450. Such a direct approach, e.g. measuring drug levels, is not possible in brain tissue and in so far, any attempt to quantify

the penetrance of antidepressants by measuring electric brain activity, e.g. by recording the sleep-electroencephalogram has not yet produced viable results.



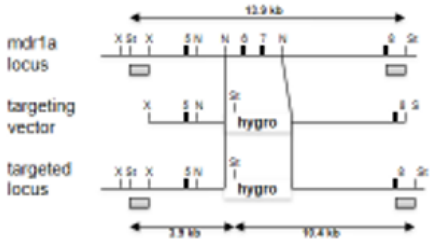
Let us have a closer look at this protein that acts at cell walls at peripheral tissues as well as at the blood brain barrier. The left N-terminal half of the protein has six transmembranal domains next to a cytoplasmatic domain and a C-terminus, coloured in red.



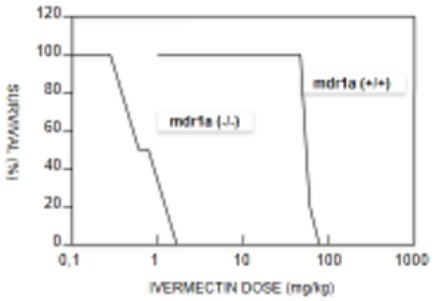


If an antidepressant that is a P-glycoprotein substrate meaning that it recognizes the binding site of this molecule enters the protein, a minor structural change occurs that allows recruiting of ATP which provides the energy for dimerization of the nucleotide binding domains located in both cytoplasmatic stretches. This major structural change prompts a drastic lowering of binding of the drug at the substrate binding site in understanding whether the predescribed drug is entering brain tissue which is followed by excretion of the substrate back into the small vessels which cross the brain at a length of about 400 miles.
With this in mind it is unsurprising that the function of the P-glycoprotein in the blood brain barrier became a subject of high interest also for clinicians.

Disruption of the gene encoding P-glycoprotein increases brain toxicity of drugs



Part of the structure of the mdr1 gene where exons 6 and 7 were targeted resulting in mdr1a-deficient mice



Ivermectin – a broad-spectrum antiparasitic agent, that is well tolerated in normal mice. Toxicity was determined by checking survival during 14 days of ivermectin administration

Mice with deleted mdr1a (ABCB1) are 50 – 100 times more sensitive to ivermectin

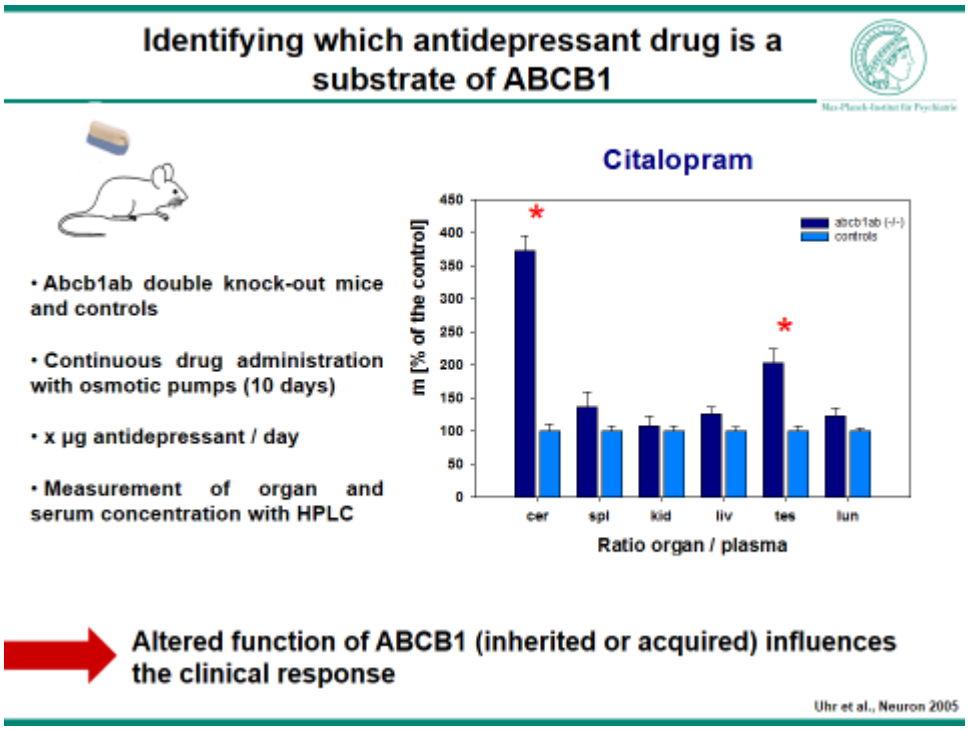
Schinkel et al, Cell; 1994

A classic example how important the “guardian molecule” P-glycoprotein can be was first demonstrated by the Dutch group of Schinkel 20 years ago. They deleted two exons in the mdr1a-gene that resulted in viable transgenic mice where mdr1a was deleted. The mouse has two genes that encode P-glycoprotein, and mdr1a is one of them. Mdr1 and ABCB1 are principally conveying the same genetic information. These transgenic mice unable to synthesize P-glycoprotein, received ivermectin an antiparasitic agent with considerable brain toxicity. The survival rate of these mice not having a functional P-glycoprotein was much lower. They already die at 100 times lower dosages of ivermectin when compared with controls. Some dogs also die from ivermectin, because races such as Collies and Bobtails very frequently have an ABCB1 deficit.

Currently, the function of P-glycoprotein is determined by many laboratories via in vitro studies that are extrapolated to the in vivo situation, which has limitations.

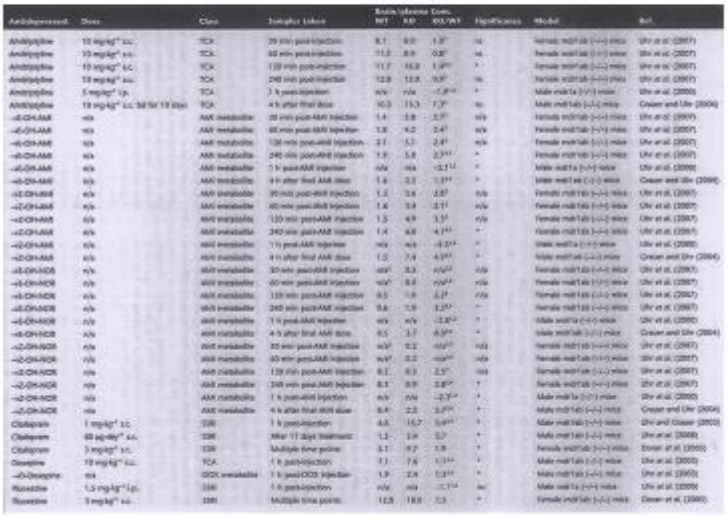
In my overview, I will share with you how we combined clinical data, DNA sequencing and studies with transgenic mice before we were able to develop a gene test with considerable potential for clinical routine.

Because patients treated with antidepressants differ so dramatically in their response to both, rather specific drugs such as citalopram, but also to unspecific drugs such as amitriptyline we were curious if these drugs are differentially transported across their blood brain barrier. We also wanted to know if functional variations of P-glycoprotein account for the clinically observed differences. To begin with we used mice where both genes, ABCB1a and ABCB1b that correspond to the human gene encoding P-glycoprotein were genetically deleted.



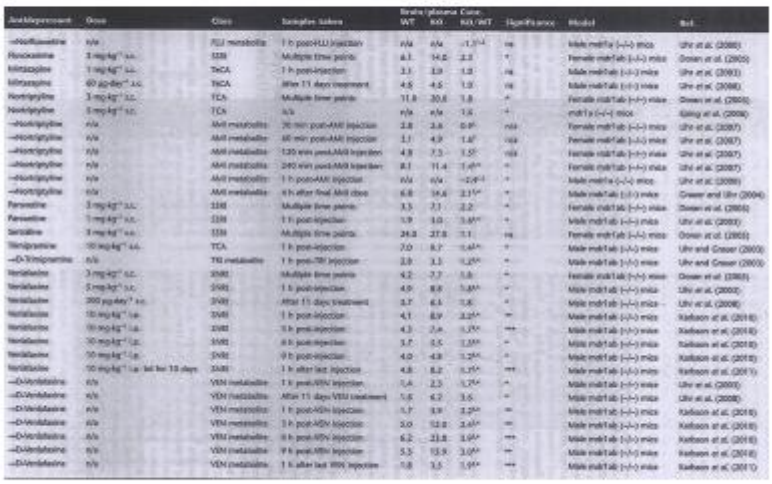
Uhr and colleagues administered many different antidepressant drugs via osmotic pumps and after sacrifice they measured the concentrations of antidepressants and their metabolites in various tissues. That allowed them to determine whether a given drug is a substrate of the P-glycoprotein or not. His laboratory at the Max Planck Institute of Psychiatry as well as many other excellent laboratories were able to provide a long list of antidepressant drugs that are substrates of P-glycoprotein or not.

In vivo studies on P-GP interaction with antidepressants using abcb1ab knock-out mice I



O'Brien, Dinan et al; British Journal of Pharmacology, 2012

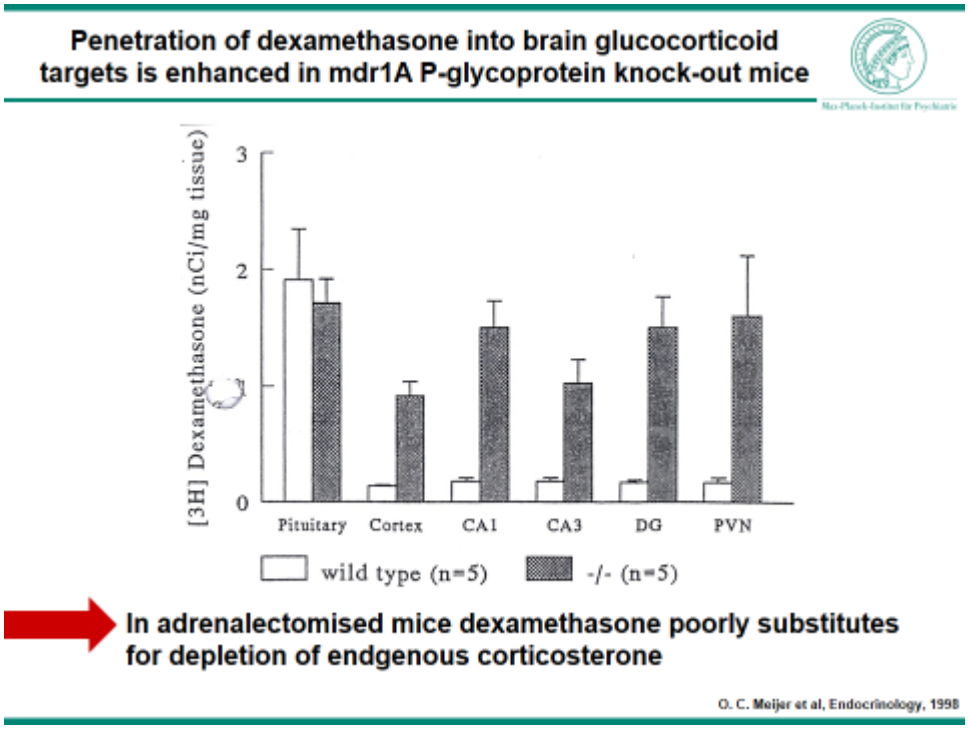
In vivo studies on P-GP interaction with antidepressants using abcb1ab knock-out mice II



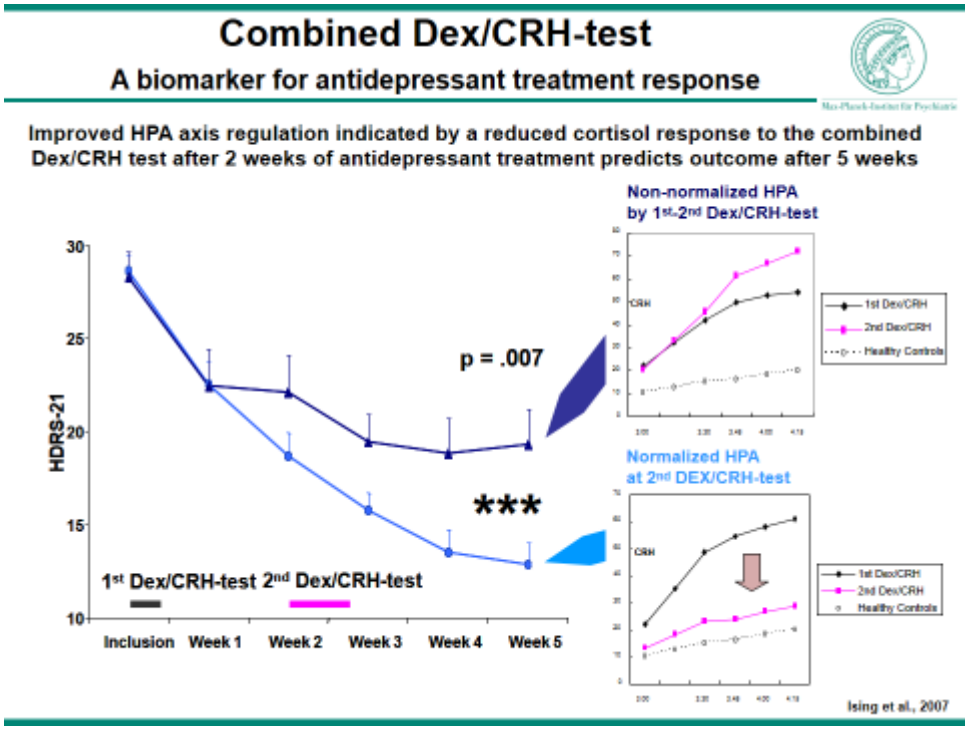
O'Brien, Dinan et al; British Journal of Pharmacology, 2012

It is very unlikely that patients have DNA sequence variations that lead to a massive functional deficit like in the knockout mice. To investigate the code of minor DNA variants in humans, a thorough analysis of the human ABCB1 gene was conducted. We showed that the ABCB1 gene in humans may carry variants which most likely determine the function of the encoded P-glycoprotein.

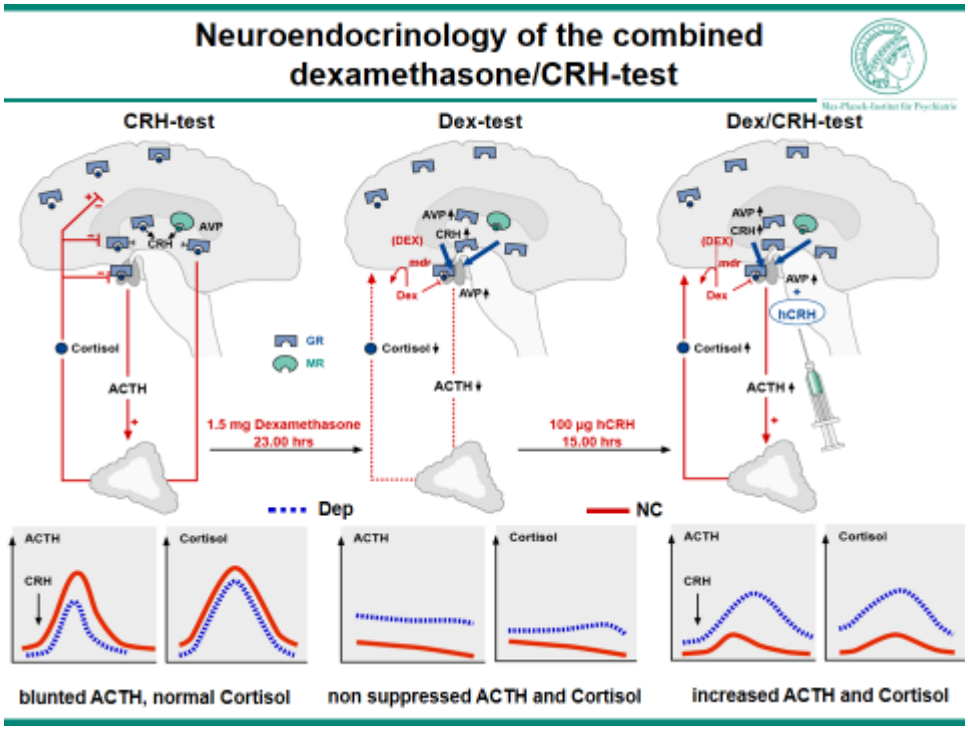
This has many implications for psychiatric research not only for psychopharmacology. P-glycoprotein is not specialized on antidepressants. On the contrary, a whole host of other molecules exist that are mostly synthetic, but also naturally occurring P-glycoprotein substrates exist and I will give you one example from neuroendocrinology of the stress hormone system that shows some of the implications.



Ronald de Kloet and coworkers demonstrated an example in rats where the adrenals that produce corticosterone were removed. Under that condition the whole brain is totally deprived from the naturally occurring corticosterone that is normally produced by the adrenals. When the corticosterone deficit is attempted to be compensated pharmacologically by the synthetic and powerful corticosteroid dexamethasone little retention occurs. This is surprising because the glucocorticoid receptor binds dexamethasone with high affinity. In contrast to brain located glucocorticoid receptors the retention of dexamethasone at pituitary glucocorticoid receptors was very high. In order to interrogate whether P-glycoprotein accounts for the phenomenon he repeated the experiment using transgenic mice where mdr1a was deleted. He demonstrated that in the absence of P-glycoprotein which can no longer be synthesized, the penetration of dexamethasone from the circulation into the brain is enhanced.



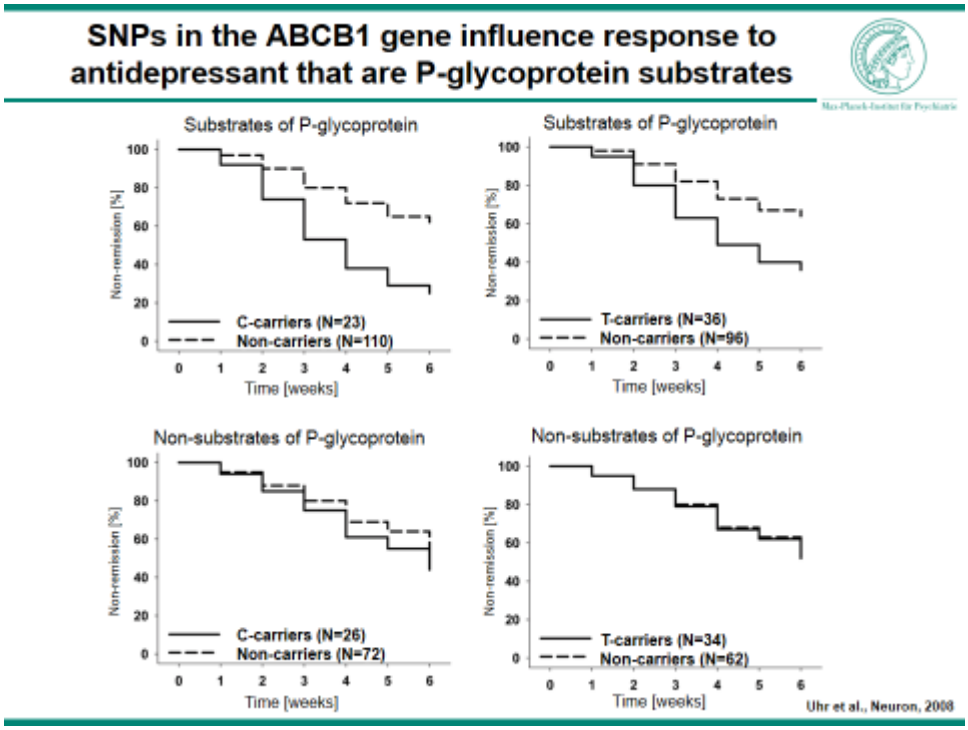
Why am I telling you this? Most of you know that we believe in the combined dexamethasone/CRH-test as a biomarker which is useful in predicting antidepressant treatment outcome. In fact, numerous studies found that many patients with depression have some sort of stress hormone hyperactivity, in this case stress hormone response to CRH increased in dexamethasone pretreated patients. Among those patients where this phenomenon disappears during treatment, the outcome is much better than among those where the abnormality persists.



If a low dose of dexamethasone is given this synthetic corticosteroid primarily binds at the pituitary. As a consequence central glucocorticoid receptors are deprived from their natural ligand. Because dexamethasone is not capable to enter the brain it cannot compensate for the corticosteroid deficiency in the brain. As a result, both neuropeptides, CRH and vasopressin that stimulate the peripheral stress hormone system are elevated in the brain. When under such conditions CRH is injected a synergy with increased vasopressin occurs, overriding the ACTH suppressive effect of dexamethasone at the pituitary.

Once central corticosteroid receptor function is reinstated by antidepressants this phenomenon disappears.

While we can only speculate how the P-glycoprotein may interact with neuroendocrine function tests among depressives, we set off to study whether or not P-glycoprotein function has clinical implications in the individual patient under treatment.



A sample of 443 inpatients with depression was treated with a variety of antidepressants from which we had determined the P-glycoprotein substrate status using - as explained before - transgenic mice where P-glycoprotein genes were deleted. We found that when comparing the particular genotype of rs2032583, C-carriers had a much higher remission rate but only when treated with a P-glycoprotein substrate (left on the figure above).

Altogether, 95 single nucleotide polymorphisms in the ABCB1 gene were investigated and we wondered whether there is an association of certain DNA sequence variations with the time needed until remission occurred among patients treated with different antidepressants that were either P-glycoprotein substrates or non-substrates.

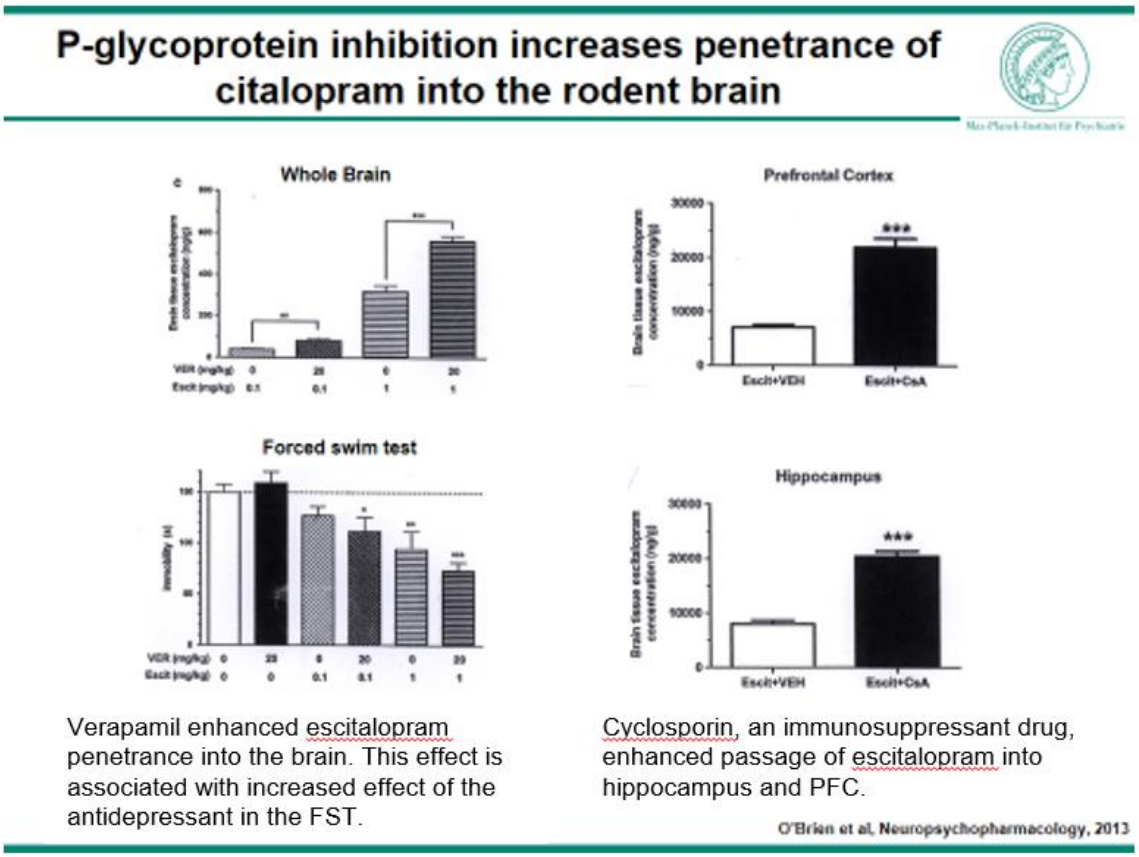
Of the 95 SNPs in the ABCB1 gene, 74 were polymorphic. All polymorphic SNP's were tested for genotypic association with the clinical condition of remission after 4, 5 or 6 weeks. Patients carrying the rare C-allele at the SNP 2032583 or the T allele at the SNP rs2235015 were tagged as C/T carriers (right on the figure above).

As shown in a survival analysis we have good reason to believe that our study supports the view that ABCB1 genotyping, especially the rs2032583 genotype is clinically useful. Many clinical studies have tested this hypothesis and not all reports confirmed our initial finding. Therefore, additional studies were conducted which will be reported by Barbara Breitenstein in this symposium. Her

presentation will include a meta-analysis that supports the notion that it is about time to integrate the ABCB1 gene test into clinical routine.

The ABCB1 gene test has already been successfully introduced into clinical routine at the hospital and at the outpatient clinic of the Max Planck Institute of Psychiatry. If a patient does not respond to a given treatment one of the causes might be an unfavorable ABCB1 genotype. What can be done? One option is to switch to a non-substrate such as mirtazapine, which may be too sedative for many patients and in a substantial number of patients resulting an increased body weight. The alternative would be to increase the dosage in order to achieve saturation of the P-glycoprotein transporter. The downside of that consequence is in many cases an increase in side effects from peripheral actions (of many SSRIs). That calls for particular attention among elderly patients where more recent drugs such as escitalopram may enhance QT prolongation in the electrocardiogram which is explicitly underscored as an important issue by Regulatory Agencies. Continuous prolongation of the QT-time reflects disturbed polarization and may lead to life-threatening cardiac arrhythmias. In such case the clinician would keep the doses too low, which might prevent treatment efficacy that by itself has negative effects on the cardiovascular system.

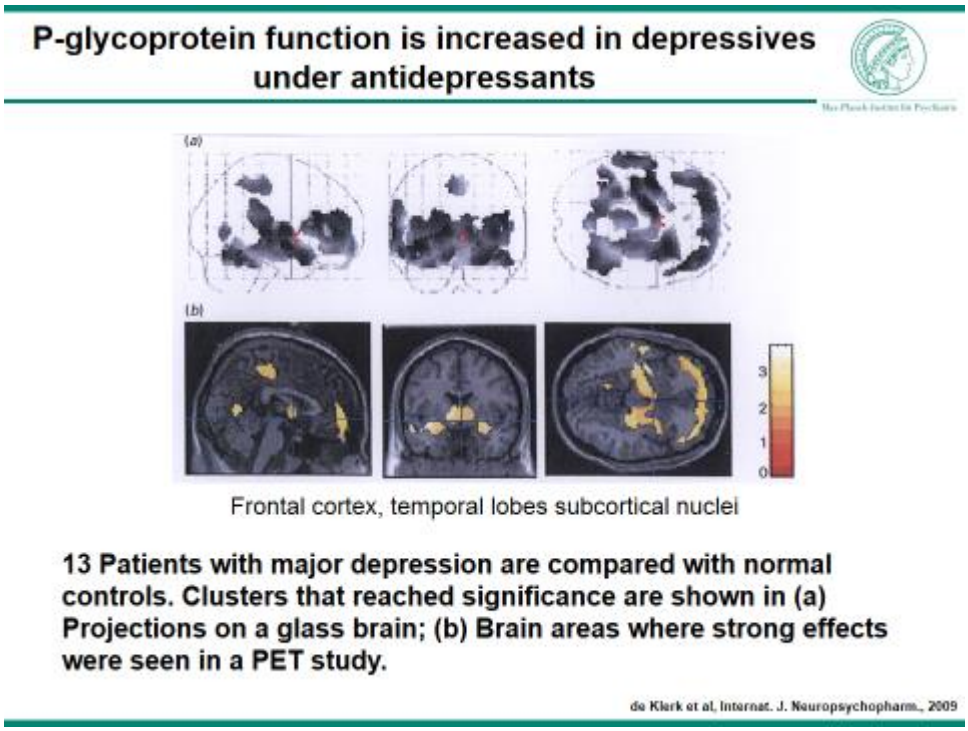
As we cannot manipulate the genotype one option would be the utilization of drugs that interfere with P-glycoprotein. Several studies using animals support at least in the idea that patients with the non-favorable ABCB1 genotype would eventually benefit from drugs that lower the efficacy of P-glycoprotein as an extrusion pump:



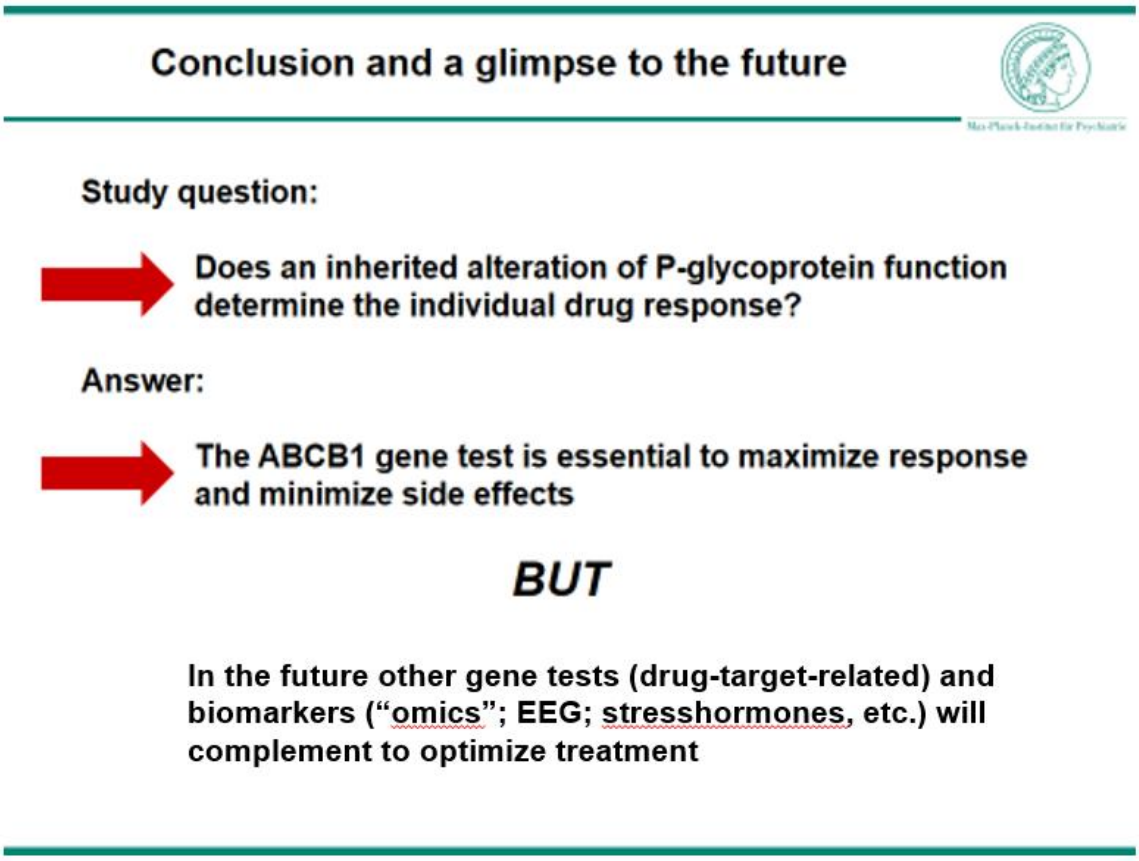
Colleagues in Ireland co-administered cyclosporine an immunosuppressant known to interfere with P-glycoprotein and found that inhibition of this transporter is associated with increased concentrations of an SSRI in the rodent brain. Another intervention which might become a potential augmentation of antidepressants that are P-glycoprotein substrates is verapamil, a calcium channel blocker. This compound decreases the function of P-glycoprotein and in rodents the co-administration of verapamil not only increased the drug concentration in the whole brain. It had also pharmacodynamic effects as the verapamil-induced increase of escitalopram import into the brain was associated with decreased immobility in the forced swim test, a behavioral assay for activation.

We know that our DNA is not a quiet place. Studies showed that stress and antidepressants have effects on P-glycoprotein on their own. Therefore, in a patient that has responded to a given drug once, may not respond to the same drug at a later depressive episode. We do not know why this is happening, a different genotype can be ruled out. The possibilities range from a shift in the causal underlying mechanism in general, or, more specifically, by antidepressant-induced changes in the way genes are activated. A role of antidepressant-induced epigenetic modifications is quite possible. Such a

mechanism could theoretically account for antidepressant-induced activation of P-glycoprotein and subsequent treatment resistance. Unfortunately, such a phenomenon occurs frequently under chemotherapy, where patients have initially responded well to their treatment but fail to do so in a second treatment course. Increased ABCB1 or mdr1 gene activity is often the cause that results in increased P-glycoprotein expression and therefore in increased difficulty to overcome the cell membrane.



Such a possibility was recently also suggested by a Dutch group that used positron emission tomography and showed that ^{11}C-verapamil uptake in prefrontal cortex and temporal lobes was decreased in depressed patients under long term treatment with antidepressants when compared with controls. It is not clear whether decreased uptake of verapamil is secondary to the state of depression or the long-term administration of antidepressants. The recent finding that venlafaxine induced P-glycoprotein in colorectal adenocarcinoma cells (caco-2) may hint toward an effect of antidepressant on P-glycoprotein. However, study results on P-glycoprotein expression in epithelial carcinoma cells may eventually tell us nothing about antidepressant effects on cells in the blood brain barrier. Most importantly, the implications briefly outlined here call for careful assessment in human patients and we have learned it the hard way that extrapolation from mice to men has limitations.



In all, I believe the whole field is very exciting and holds promise that gene tests and biomarkers can help us to better serve our patients in the near future.