Pharmacogenetics and Pharmacogenomics

The historical perspective

The individual differences in response to administered substance (medicament) were observed throughout the whole history of humankind. Pythagoras is thought to be one of the first to observe pharmaco/nutrigenetic interaction as he found out that only some people will get sick after eating fava beans, but others will not. It took approximately 2400 years to elucidate the basis of this observation, the defect of glucose-6-phosphate dehydrogenase enzyme, that causes hemolytic anemia (2) but only after exposure to fresh flava beans or antimalaric drug primachine (but also to acetylsalicylic acid, phenacetine or sulphonamides).

Sir A.E. Garrod in 1902 foresees that the effects of diet and other diseases can "mask" some inherited errors of metabolism and introduces the term of "chemical individuality", where specific reactions to drugs are due to inborn differences - in this way he envisions the formulation of pharmacogenomics and nutrigenomics more than 50 years before their real establishment.

Approximately at the same time William Bateson re-discovers the works of Mendel and in fact establishes the term genetics. Moreover, it was probably Bateson who applied genetics to inborn errors of metabgolism described by Garrod. Only in 50's of the last century the field of pharmacogenetics was established as such with three (nowadays classical) major examples of pharmacogenetic interactions.

One of them was the above mentioned hemolytic anemia after primachine treatment only in predisposed individuals, then elongated apnoe after succinylcholine due to the modified kinetics of butyrylcholinesterase (3) and a well known example of polymorphism that causes fast of slow acetylation of antituberculotic drug isoniazide (but only during the 90's the causal mutations of the N-acetyltransferase 2 gene were elucidated) (4, 5). The term pharmacogenetics was used for the first time by Friedrich Vogel in 1959 (6). During the second half of 20th century both genetics and pharmacology are extensively studied. Huge amount of synthetic drugs has been introduced into everyday clinical medicine. As the newly developed drugs are getting more and more efficient, the unique response to drug administration is getting more and more important. This response can be either extremely beneficial or a life threatening side effect. In 1997 the term pharmacogenomics emerged (7) and approximately from that time on, the amount of publications with pharmacogenetic and pharmacogenomic themes is rising exponentially. Even though the terms pharmacogenetics and pharmacogenomics do not mean the same, they are sometimes used as equals. Pharmacogenetics deals with the effect of individual gene variants on the action of a given drug (as in those above mentioned examples of pharmacogenetic interactions), while pharmacogenomics investigates the relationship of drug effect on the level of whole genome, transcriptome.

Methods of pharmacogenomics

Pharmacogenomic methods are based on recent technological advances that brought the possibility of highly parallel analysis of genetic information of human as well as model organisms. Bioinformatics methods help with mathematical and statistical apparatus that enables the overview and analysis of resulting multidimensional biological data and

sometimes bioinformatics methods are the main tool of investigation for example in in silico analyses (computer analyses) of genomic data in publicly available databases.

The global mapping of single nucleotide polymorphisms (SNP) on the whole genome level after the finished sequencing quest is one of the promising directions of today. These efforts are based on fact that out of the total predicted 11 - 15 million of genetic polymorphisms in humans the SNPs account for more than 90% of them. In publicly available database dbSNP (http://www.ncbi.nlm.nih.gov/SNP/index.html) there are nowadays (build 125 from 29.9.2005) over 5 million of identified SNPs and their number is growing on day to day basis. SNPs exist in the human genome into the blocks - haplotypes - that are usually inherited together. One of current opinions on human genomic variability states that chromosomes are mainly built out of short segments that during the short evolution history of the humankind were subjected to minimal amount of recombination changes and therefore these segments can be characterized just by few common haplotypes in majority of people. For those regions with high linkage disequilibrium and low diversity of haplotypes (haplotype blocks) it is enough to identify only few "tags" (sometimes just single SNP) that represent the given haplotype. It can be presumed that when the project of haplotype mapping of the human genome (project HapMap http://www.hapmap.org/) will be finished, the databases dealing with pharmacogenetics as PharmGKB (pharmcogenetics and pharmacogenomics knowledge base, www.pharmgkb.org) will be used for analysis and identification of the risk haplotypes for specific drug or drug class. So it probably will not be necessary to know the whole "SNP constellation" for a given individual to predict and hopefully prevent adverse side effects through pharmacogenetic and nutrigenetic interactions (8). Nowadays it is possible to analyze a few thousand SNPs at the same time using a SNP chip. For example Affymetrix company distributes chip for detection of 100000 SNPs at the same time that requires just a few hundreds of nanograms of DNA and this company will soon introduce a new chip for detection of 500000 SNPs (www.affymetrix.com).

Amplichip

At the end of the last year Affymetrix announced "Affymetrix GeneChip System 3000Dx" approved from FDA for in vitro diagnostic purposes. It's the first time ever that such approval was given to a system for DNA/RNA microarray chip analysis. Last September the joint product of Affymetrix and Roche based on the above mentioned system - AmpliChip CYP450 Test - acquired the CE certification ("Conformité Européene") that enables its use in clinical diagnostics in EU countries.

The first AmpliChip system in the central Europe was installed this year (15.6.2005) at the Institute of Clinical Biochemistry and Laboratory Diagnostics of the First Medical Faculty of Charles University and General Teaching Hospital.

DNA (or RNA) chips are tools that enable to test several tens of thousands of genes at the same time in one sample. On the area of the chip itself (one squared inch) the short segments of known sequence of single-stranded DNA (oligonucleotide probes) are extremely densely packed. Following the principle of complementarity of bases, the fluorescently labeled DNA from the analyzed sample specifically binds to the oligonucleotide probes. After the laser detection of the signal acquired from the probes that hybridized with sample DNA, the analytical software generates a database of results for further analysis.

The above mentioned AmpliChip CYP450 Test is focused on two genes that are most important for the metabolism of up to 25% of all commonly administered drugs, the genes are: gene coding for cytochrome P450 (CYP) 2D6 and CYP2C19 gene. 15000 probes placed on a chip enable to distinguish 29 different polymorphisms, duplications and deletions of CYP2D6 gene, 2 polymorphisms of CYP2C19 gene and based on that information directly predict the type of drug metabolism - from the slow to "ultra fast". Through this chip the research gave to clinical medicine one of the first pharmacogenetic tools that should help to prevent potential side effects of pharmacotherapy for the predisposed individuals, but mainly aims to become technological milestone on the path of individualized therapy based on genetic background of the patient. The limiting factor of massive use of this diagnostic tool is the relatively high price (350-400 USD per chip, per one analysis) and mainly the price of the system of Affymetrix company (approx. 100.000 USD).

Roche Amplichip
http://www.roche-diagnostics.com/products_services/amplichip_cyp450.html

Affymetrix
www.affymetrix.com

The crucial information that enabled the possibility of parallel analysis of expression of thousands of genes at the same time (in some microorganisms of all existing genes) was the verification of dynamic nature of the genome. There is no mechanistic relationship in between the DNA sequence and gene expression, many regulation processes are involved as well as the changes in all the steps of realization of genetic information (posttranscriptional, posttranslational, RNA dependent regulation of expression, and others). Detailed description of those mechanisms is beyond the scope of this article, but in summary on the cellular level

the signals coming from both the outside and inside environment are integrated and analyzed and only based on that information the time and space specific form of answer is being chosen, this answer includes among others also the setting of expression levels of given set of genes. After just turning off one gene as it is in knock-out mice models or just by adding one signal in the form of a drug into the media of cellular culture, the adaptive shift can be detected in expression of not one, but hundreds up to thousands of genes. On the transcriptome level the cRNA and cDNA express chips (microarrays) with high density of probes are being used, so for humans, mice and rats the chips with all annotated genes as well as expressed sequence tags are available (see chapter Genes on chips). Analogically, proteomics (see chapter Proteomics) studies the expression on the level of proteins utilizing modern technologies based on mass spectrometry, and metabolomics studies the expression on the metabolite level. The discussion is still whether these techniques will be implemented into practical routine life. The biological material needed for the analyses can be isolated from blood or urine, saliva, breast milk, or from biopsies from the tissues where the relevant genes are expressed. If the "expression signatures" specific for e.g. side effects of a drug in a commonly used biological material will not be found, the importance of those methods apart from genotyping will remain in experimental area.

Pharmacogenetic interactions

Majority of original pharmacogenetic observations dealt with situations where there were radical differences in the drug concentrations in blood or in drug excretion via urine and these could be explained using simple mendelian inheritance. These polymorphisms can be explained by the changes in pharmacokinetics where due to the defect in the molecule of a given transporter that metabolizes the enzyme or other factor of absorption, distribution, interaction with target structure and finally modification with excretion, resulting in high or low concentrations of the drug in the organism. The above mentioned classical example is the polymorphism in N-acetyltransferase 2 (NAT2) gene, then polymorphisms in the cytochrome P450 2D6 (CYP2D6) (9) gene or in thiopurin S-methyltransferase (TPMT) (10). The latter enzyme is involved in metabolism of thiopurines, e.g. of azathioprine (used as an immunosuppressive agent in transplant recipients and in treatment of acute lymphoblastic leukemia). TPMT became one of the first genes with commercially available genetic test (http://www.prometheuslabs.com). The warning considering the possible side effects in patients with a genetical variant coding for low activity of TPMT was approved by FDA and is present on the package.

Of course, there is a possibility of a more complicated situation in case the genetic polymorphism influences the pharmacodynamic processes or is dependent on interaction of more genes. Then even if there is an adequate dosage of the medicament, its efficacy is dependent on factors like expression level of the gene in target tissue. The gene expression can be systematically lower or higher in different ethnic groups (e.g. BiDiL has been introduced recently as a drug aimed at heart failure treatment specifically in African Americans) or it can depend on the developmental stage (newborns, adolescents, adults). The latter possibility might be the reason why paroxetin, an antidepressant from the group of selective serotonin reuptake inhibitors (SSRI) causes suicidal behavior in patients younger than 18 years, though no such effect can be seen in paroxetin-treated adults (11).

Heterogeneity as a complicating factor

The research of pharmacogenetic interactions has to deal with similar problems as the analysis of genetic component of complex diseases, i.e. traits with significant genetic and environmental components (the latter including diet and drugs). One of such hindrances is **incomplete penetrance** (non-appearance of the trait in spite of the presence of the underlying genetic variant), **genetic heterogeneity** of the analyzed population, **phenocopy** (appearance of the trait in spite of absence of the underlying genetic variant) etc (14). As stated by Thorton-Wells et al. (15), there are two basic types of genetic heterogeneity. The first is the **allelic heterogeneity**, i.e. the association between the given trait (disease, reaction to medication) and two or more alleles of a single gene - > 1000 mutations of CFTR gene leading to cystic fibrosis or multiple alleles of cytochrome P450 may serve as paradigmatic examples. The second is the trait heterogenetiy, when a vague defintion of a disease comprises several genetically distinct conditions, e.g. in autism or hypertension. These factors have to be naturally reflected in the use of new statistical models and approaches as most of the so far used methods are based on mathematical apparatus calculating with simple monogenic ingeritance. Thus, various variant of clusteirng, principal component or Bazesian methods are being developed to deal with the abovementioned matters.

The examples of pharmacogenetic models

The detailed functional genomics analysis of genetic polymorphisms in the frame of complex realtions of pharmacogenetic interactions sometimes is ethically and practically impossible in the human subjects. So, different parts of the research have to be performed in silico (computer-based), in vitro (cell culture) and in vivo (experimental, mostly mammalian models) and only then the results are validated in human. The simplest physical model are the cells expressing particular variants of human genes introduced bz transcfection of cDNA. These studies aim at resolution of the question how individual polymorphisms influence the expression and function of the coded protein (e.g. receptor). In spite of undeniable successes of this approach, the relevance of the outputs of such studies is complicated by several issues. First and foremost, the most common models are cell lines easily amenable to the transfection-mediated expression of the gene and protein, but these line lack numerous characteristics of the original tissue. Moreover, the cell culture studies take the cells out of their natural context of the particular tissue, organ and organism, formed adaptive and regulatory networks and signals, that all together shape the relevant gene expression both in health and disease situtation as well as in response to medication. Thus a great importance is attributed to experimental animal model use, currently the most utilized ones being mouse and rat. The traditional role of the latter two models was further supported by the sequencing of their genomes showing greater evolutionary relatedness of human to these models than expected (16). Under standard environmental conditions, it is feasible to follow on the organismal level the effects of such changes like transgenic expression of a gene of interest or a gene "knock-out" and more recently, a gene "knock-down" with complete or partial silencing of the gene expression.

One particular example of the animal model suitable for the pharmacogenetic and nutrigenetic research is the polydactylous rat strain (PD/Cub). This highly inbred strain has been kept at the Institute of Biology and Medical Genetics of First Faculty of Medicine, Charles University since 1969 (17). The spontaneous mutation of the Lx gene gives rise to the polydactyly-luxate syndrome. The effect of the Lx gene is modified by the genetic background, but also by its interaction with various teratogens (bromdeoxyuridine, thalidomide, retinoic acid). Even this pharmacogenetic interaction is further affected by the genetic background (18-21). This strain is therefore a unique model for analyses of

morphogenetic processes as well as pharmacogenetics/genomics of teratogenesis. In 1993, this strain was found to display high levels of triglycerides (22) and more detailed studies lead to establishment of PD/Cub as model of metabolic syndrome (because of the simultaneous presentation of hypertriglyceridemia, hyperinsulinemia, increased indices of central (visceral) obesity, high concentrations of non-esterified fatty acids and substantial insulin resistance of peripheral tissues (23)). Only recently it has been revealed that PD/Cub possesses unique pharmacogenomic profile in response to several classes of transcription-modulating drugs (24, 25). The administration of fenofibrate, a hypolipidemic drug acting mainly on nuclear receptor PPAR? (peroxisome proliferators-activated receptor alpha), lead surprisingly to deterioration of glucose tolerance together with high concentrations of insulin. Isotretinoin, a drug used to treat acne acting on another couple of nuclear receptors RAR and RXR, induced a substantial increase in triglycerides in PD/Cub, corresponding to one of the often described side effects of isotretinoin administration in humans. This side effect is though not universal, suggesting genetic predisposition is necessary for its manifestation and so, the polydactylous strain could make a useful model for a more detailed study of this phenomenon.

Figure: Male polydactylous (PD/Cub) rat.

Following example of use of experimental models in pharmacogenetics involves yet another class of agonists of nuclear receptor, this time acting on PPAR? (e.g. thiazolidinediones pioglitazone and rosiglitazone) (26). Spontaneously hypertensive rat (SHR), major rodent model of human primary hypertension, did not respond to administration of pioglitazone by improvement of its glucose tolerance. It was eventually found that mutant allele of the gene coding for fatty acid translocase (Cd36/Fat) was responsible. This was proven by the derivation of transgenic and congenic animals carrying wild-type allele of Cd36 on SHR

genetic background - all these responded to pioglitazone by improvement of glucose metabolism in peripheral tissues, in contrast to SHR. Again, particular form of effect of the mutant Cd36 (expressivity) depends on the genetic background it operates on. In congenic strain BN.SHR(Il6-Cd36), where Cd36 of SHR origin is introgressed within a small segment of chromosome 4 into the genetic background of normotensive and normolipidemic Brown Norway strain, we have observed non-responsiveness of glucose tolerance to rosiglitazone administration, accompanied by lack of increase of adiposity, which was observed both in control Brown Norway rat and in the pioglitazone-treated SHR (28). Because of the relatively frequent type 2 diabetes in human carriers of CD36 mutation, the abovementioned pharmacogenetic interaction may have clinically relevant implications for pharmacotherapy of such patients.

Pharmacogenomics in drug development

Besides the possible applications of pharmacogenetics and pharmacogenomics for understanding interactions and optimization of the therapy of currently used drugs, the attention of drug manufacturers is shifting towards the use of new technologies in development and control of new drugs. One of the more obvious approaches is scanning the available DNA sequences of human and model organisms for characteristic motifs common to genes, for which there are large libraries of test chemicals available ("drug-able targets"). These groups include e.g. nuclear receptors or kinases. As stated by A.Roses, before this approach was available, there were about 500 such target structures and it is presumed there are about 5000 more, hundreds of which may become relevant in therapy of human diseases (29). The only phase of this research that is not amenable to automation and robotization, is DNA sample acquisition with as detailed and accurate characteristics of subjects (cases and controls) as possible, moreover, the widely-based prospective cohorts with such detailed annotation are missing. Another field open for future pharmacogenomic applications is the primary phase of drug clinical trials. If the tested compounds are effective in a subset of subjects and overall the effect is ambivalent in respect to placebo, the continuation of the trial becomes financially very demanding and the whole project is often stopped at this stage. Profiling could identify the genetic commonalities in the groups that respond to the therapeutic action of the drug and if proven in the subsequent phases of the trial, the condition of testing positive for particular allele in a predictive genetic test would become mandatory for prescribing the drug. There are many reasons why there is no example of a drug with clear-cut specifications on the side of the patient's genetic constitution that would guarantee both efficacy and safety. The reverse side of the coin is identification of a genetic marker (SNP, haplotype, allele, transcription profile) associated with the side effect of the drug administration. 4% of 11500 people treated with tranilast (drug preventing restenosis) developed hyperbilirubinemia in Phase III. Large-scale genetic testing eventually found that this reaction is strongly associated to an allele of the gene coding for UDP-glucuronyltransferase 1A1 (30). In people carrying single allele with 7 repeats tranilast elicited mild hyperbilirubinemia, in those with two alleles with 7 repeats severe hyperbilirubinemia developed, while carriers of two alleles with 6 repeats were protected from this side-effect. The tranilast development was eventually stopped at this point. Using the 100,000 SNP array, only 10-20 cases compared to 3,000 controls would suffice to exact identification of the genomic region. Of course more complicated interactions (more genes, impact of other factors) render the resolution to be more complex. The transition from the globally administered drugs "for the particular disease" to drugs "for the particular patient" within the paradigm of personalized medicine will therefore be only gradual, relatively slow-moving.

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Further reading

1. The Pharmacogenetics And Pharmacogenomics Knowledge Base - www.pharmgkb.org
2. The Pharmacogenomics Journal - www.nature.com/tpj/index.html
3. Pharmacogenetics and Genomics - www.jpharmacogenetics.com/pt/re/pharmgen/home.htm