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AAPS PharmSci Publishes 'Pharmacogenetics-Pharmacogenomics 2000' – Sets Stage for 2001.

Pharmacogenetics and pharmacogenomics promise profound changes in drug discovery, development, and therapy. Disease susceptibility and drug response are strongly influenced by genetic factors. Typically, multiple genes are involved in determining drug response. Therefore knowing the genotype of patients could help in optimizing therapy of the individual patient. Rather than adhering to the principle of 'one drug fits all', we move to the concept 'the right drug for the right patient at the right dosage and time'.

In the Pharmacogenetics-Pharmacogenomics 2000 Theme Issue, Mancinelli et al. provide a review of the current status of the field, its promises and limitations. McElroy et al. report on an extensive study of CYP2D6 polymorphisms to assess the metabolic ability of individual patients. Numerous drugs depend on CYP2D6 metabolism for inactivation and excretion, and 2D6 mutations can profoundly enhance drug exposure and thus toxicity. Veenstra et al. assessed the potential cost-effectiveness of pharmacogenomic strategies to assist in the design of clinical trials, and provide a guide for health care providers making reimbursement decisions. The authors concluded that that pharmacogenomics offers great potential to improve patients health in a cost-effective manner. However, careful evaluations are needed on a case-by-case basis before pharmacogenomic-based therapeutics can be successfully applied. Kulkarni et al. and Zhang et al. investigated the effects of genetic disorders, i.e., cystic fibrosis and diabetes, respectively, on drug disposition. A CF-knockout mouse model showed altered pharmacokinetics -similar to those seen in CF patients – for acetaminophen and indocyanin green. On the other hand, diabetes-associated hyperglycemia causes glycation of proteins at reactive amino groups, which can adversely affect protein function, including that of drug transporters. Zhang et al. developed a rapid method for analyzing protein glycation. Lastly, Yan et al. have developed a relational, interactive database of membrane transporters and ion exchangers with multiple links to pertinent information, such as substrates, sequences, chromosomal location, polymorphisms, etc. The database will remain open for modifications to keep it up-to-date.

The Pharmacogenetics-Pharmacogenomics 2000 Theme Issue is also posted at and linked from the Web site for the new AAPS Focus Group in Pharmacogenetics-Pharmacogenomics (available from **http://www.aapspharmaceutica.com**). Contributions for the 2001 Theme Issue are invited.

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Review

Pharmacogenetics and pharmacogenomics deal with the genetic basis underlying variable drug response in individual patients. The traditional pharmacogenetic approach relies on studying sequence variations in candidate genes suspected of affecting drug response. On the other hand, pharmacogenomic studies encompass the sum of all genes, i.e., the genome. Numerous genes may play a role in drug response and toxicity, introducing a daunting level of complexity into the search for candidate genes. The high speed and specificity associated with newly emerging genomic technologies enable the search for relevant genes and their variants to include the entire genome. These new technologies have essentially spawned a new discipline, termed pharmacogenomics, which seeks to identify the variant genes affecting the response to drugs in individual patients. Moreover, pharmacogenomic analysis can identify disease susceptibility genes
representing potential new drug targets. All of this will lead to novel approaches in drug discovery, an individualized application of drug therapy, and new insights into disease prevention. Current concepts in drug therapy often attempt treatment of large patient populations as groups, irrespective of the potential for individual, genetically-based differences in drug response. In contrast, pharmacogenomics may help focus effective therapy on smaller patient subpopulations which although demonstrating the same disease phenotype are characterized by distinct genetic profiles. Whether and to what extent this individual, genetics-based approach to medicine results in improved, economically feasible therapy remain to be seen. To exploit these opportunities in genetic medicine, novel technologies will be needed, legal and ethical questions must be clarified, health care professionals must be educated, and the public must be informed about the implications of genetic testing in drug therapy and disease management.

Original Research Articles

The emerging application of pharmacogenomics in the clinical trial setting requires careful comparison with more traditional phenotyping methodologies, particularly in the drug metabolism area where phenotyping is used extensively. The research objectives of this study were 1) to assess the utility of *cytochrome P450 2D6 (CYP2D6)* genotyping as an alternative to traditional phenotyping as a predictor of poor metabolizer status; 2) to identify issues for consideration when implementing *CYP2D6* genotyping in clinical trials; and 3) to outline the advantages and disadvantages of *CYP2D6* genotyping compared with phenotyping. DNA samples obtained from 558 previously phenotyped individuals were blindly genotyped at the*CYP2D6* locus, and the genotype-phenotype correlation was then determined. The *CYP2D6*genotyping methodology successfully predicted all but 1 of the 46 poor metabolizer subjects, and it was determined that this 1 individual had a novel (presumably inactive) mutation within the coding region. In addition, we identified 2 subjects with *CYP2D6* genotypes indicative of poor metabolizers who had extensive metabolizer phenotypes as determined by dextromethorphan/dextrorphan ratios. This finding suggests that traditional phenotyping methods do not always offer 100% specificity. Our results suggest that *CYP2D6* genotyping is a valid alternative to traditional phenotyping in a clinical trial setting, and in some cases may be better. We also discuss some of the issues and considerations related to the use of genotyping in clinical trials and medical practice.

The use of pharmacogenomics to individualize drug therapy offers the potential to improve drug effectiveness, reduce adverse side effects, and provide cost-effective pharmaceutical care. However, the combinations of disease, drug, and genetic test characteristics that will provide clinically useful and economically feasible therapeutic interventions have not been clearly elucidated. The purpose of this paper was to develop a framework for evaluating the potential cost-effectiveness of pharmacogenomic strategies that will help scientists better understand the strategic implications of their research, assist in the design of clinical trials, and provide a guide for health care providers making reimbursement decisions. We reviewed concepts of cost-effectiveness analysis and pharmacogenomics and identified 5 primary characteristics that will enhance the cost-effectiveness of pharmacogenomics: 1) there are severe clinical or economic consequence that are avoided through the use of pharmacogenomics, 2) monitoring drug response using current methods is

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difficult, 3) a well-established association between genotype and clinical phenotype exists, 4) there is a rapid and relatively inexpensive genetic test, and 5) the variant gene is relatively common. We used this framework to evaluate several examples of pharmacogenomics. We found that pharmacogenomics offers great potential to improve patients' health in a cost-effective manner. However, pharmacogenomics will not be applied to all currently marketed drugs, and careful evaluations are needed on a case-by-case basis before investing resources in research and development of pharmacogenomic-based therapeutics and making reimbursement decisions.

Human Membrane Transporter Database: A Web-Accessible Relational Database for Drug Transport Studies and Pharmacogenomics

Qing Yan, Wolfgang Sadee

The human genome contains numerous genes that encode membrane transporters and related proteins. For drug discovery, development, and targeting, one needs to know which transporters play a role in drug disposition and effects. Moreover, genetic polymorphisms in human membrane transporters may contribute to interindividual differences in the response to drugs. Pharmacogenetics, and, on a genome-wide basis, pharmacogenomics, address the effect of genetic variants on an individual's response to drugs and xenobiotics. However, our knowledge of the relevant transporters is limited at present. To facilitate the study of drug transporters on a broad scale, including the use of microarray technology, we have constructed a human membrane transporter database (HMTD). Even though it is still largely incomplete, the database contains information on more than 250 human membrane transporters, such as sequence, gene family, structure, function, substrate, tissue distribution, and genetic disorders associated with transporter polymorphisms. Readers are invited to submit additional data. Implemented as a relational database, HMTD supports complex biological queries. Accessible through a Web browser user interface via Common Gateway Interface (CGI) and Java Database Connection (JDBC) (http://128.218.190.183.81/transporter/), HMTD also provides useful links and references, allowing interactive searching and downloading of data. Taking advantage of the features of an electronic journal, this paper serves as an interactive tutorial for using the database, which we expect to develop into a research tool.

Disposition of Acetaminophen and Indocyanine Green in Cystic Fibrosis-Knockout Mice

Swarupa G. Kulkarni, Anita A. Pegram, Philip C. Smith

Drug treatment poses a therapeutic challenge in cystic fibrosis (CF) because the disposition of a number of drugs is altered in CF. Enhanced clearance of acetaminophen (APAP) and indocyanine green (ICG) have previously been reported in CF patients. The objective of the current study was to investigate if the CF-knockout mouse model ($cftr^{tm1UNC}$) shows altered pharmacokinetics similar to those seen in CF patients using the 2 model compounds APAP and ICG. Clearance (CL/F) of APAP and renal (CL_R) and formation (CL_f) clearance of acetaminophen glucuronide (AG) and acetaminophen sulfate (AS) were determined in CF-knockout mice following administration of APAP (50 mg/kg, intraperitoneal). CL_R of AS was 19.5 and 12.9 (mL/min per kg) and CL_f of AS was 10.4 and 6.7 mL/min per kg for homozygous and heterozygous males, respectively, which was significantly different between groups. CL_R of AG was 6.3 and 4.8 mL/min per kg and CL_f of AG was 9.6 and 8.9 mL/min per kg for homozygous and heterozygous males, respectively, although not reaching statistical significance. No significant differences were noted in either Cl_R or CL_f of AG and AS in female CF mice. Plasma concentrations of ICG (10 mg/kg, intravenous) were determined over 0 to 15 minutes. Homozygous females showed a higher apparent volume of distribution (96 mL/kg) relative to heterozygous females (72 mL/kg). Similar to CF patients, a trend toward a lower C_{max} was noted in homozygous male and female mice. However, contrary to human data, no significant differences in CL of ICG were noted. These results suggest that the CF-knockout mice have potential as a model for studying altered drug disposition in CF patients.

Human Proton/Oligopeptide Transporter (POT) Genes: Identification of Putative Human Genes Using Bioinformatics

Christopher W. Botka, Thomas W. Wittig, Richard C. Graul, Carsten Uhd Nielsen, Kazutaka Higaki, Gordon L. Amidon, and Wolfgang Sadée

The proton-dependent oligopeptide transporters (POT) gene family currently consists of ~70 cloned cDNAs derived from diverse organisms. In mammals, two genes encoding peptide transporters, *PepT1* and *PepT2* have been cloned in several species including humans, in addition to a rat histidine/peptide transporter (*rPHT1*). Because the *Candida elegans* genome contains five putative POT genes, we searched the available protein and nucleic acid databases for additional mammalian/human POT genes, using iterative BLAST runs and the human expressed sequence tags (EST) database. The apparent human orthologue of *rPHT1* (expression largely confined to rat brain and retina) was represented by numerous ESTs originating from many tissues. Assembly of these ESTs resulted in a contiguous sequence covering ~95% of the suspected coding region. The contig sequences and analyses revealed the presence of several possible splice variants of *hPHT1*. A second closely related human EST-contig displayed high identity to a recently cloned mouse cDNA encoding cyclic adenosine monophosphate (cAMP)-inducible 1 protein (gi:4580995). This contig served to identify a PAC clone containing deduced exons and introns of the likely human orthologue (termed *hPHT2*). Northern analyses with EST clones indicated that *hPHT1* is primarily expressed in skeletal muscle and spleen, whereas *hPHT2* is found in spleen, placenta, lung, leukocytes, and heart. These results suggest considerable complexity of the human POT gene family, with relevance to the absorption and distribution of cephalosporins and other peptoid drugs.

Determination of Membrane Protein Glycation in Diabetic Tissue

Eric Zhang, Peter Swaan

Diabetes-associated hyperglycemia causes glycation of proteins at reactive amino groups, which can adversely affect protein function. Although the effects of glycation on soluble proteins are well characterized, there is no information regarding membrane-associated proteins, mainly because of the lack of reproducible methods to determine protein glycation in vivo. The current study was conducted to establish such a method and to compare the glycation levels of membrane-associated proteins derived from normal and diabetic tissue. We present a detailed sample preparation protocol based on the borohydride-periodate assay, modified to allow manipulation of animal tissue. Assay noise associated with extraction protocols and nonproteinaceous buffer components was eliminated by the using 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) as a membrane detergent, applying desalting columns, and including a protein precipitation step. The glycation level of membrane proteins from diabetic rats is elevated to 4.89 nmol/mg protein (standard deviation [SD] 0.48) compared with normoglycemic control tissue (2.23 nmol/mg protein, SD 0.64). This result is consistent with and correlated to the total glycated hemoglobin levels in diabetic and normoglycemic rats. Using <100 µg protein, the described methods allow further study of protein glycation effects on the function of individual transporter proteins and the role of these modifications in diabetes.

Call for Papers- Pharmacogenetics-Pharmacogenomics 2001

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AAPS invites authors to submit papers to a Theme Issue of its new electronic journal, *AAPS PharmSci*, on the general topic of Pharmacogenitics - Pharmacogenomics.. Manuscripts are invited from the broad range of topics including:

1) Genetic basis of variability in drug response;
2) Polymorphisms of receptors, enzymes, and transporters;
3) Phenotyping tissues for drug effects;
4) Detection of polymorphisms;
5) Genome-wide mapping of single nucleotide polymorphisms in drug trials;
6) Genes conferring disease susceptibility and/or sensitivity to drug therapy;
7) DNA microarray technology;
8) Genomics, proteomics, cellomics;
9) Cloning genes relevant to drug effects;
10) Clinical drug studies involving pharmacogenetics - pharmacogenomics;
11) Pharmacokinetics/Pharmacodynamics and genetic variations;
12) Pharmacogenomics in drug discovery and development;
13) Genomics of microorganisms and viruses related to drug treatment;
14) Incidence of adverse drug effects in relation to genetic variationsl;
15) Pharmacoepidemiology;
16) Pharmacoeconomics.

Theme Issues will be separately advertised to target audiences, to enhance the impact of papers submitted under a common umbrella. Papers will be accepted by e-mail attachment or by disk to the AAPS Editorial office (see Instructions to Authors online at http://www.pharmsci.org) Manuscripts will undergo an expedited review by two experts to determine suitability for publication. Manuscripts will be published immediately following acceptance in this theme issue volume of ***AAPS PharmSci***; the paper will also appear in the regular monthly issue of ***AAPS PharmSci*** as an alternative entry point for our readers. Email pharmsci-edoffice@aaps.org or call 703-248-4762 for details.