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AJG Series: Molecular Biology for Clinicians Pharmacogenetics and Genomics for Clinicians

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Abstract

Pharmacogenetics is an evolving field that provides the link between an individual's genetic code and drug metabolism and drug response. This field offers the great promise of individualized medication selection and optimized dosage to maximize treatment response and to minimize adverse side effects. As our understanding of the role of the effects of genetic variants on drug metabolism and body drug processing grows, so does our ability to educate and inform our patients about expected treatment response to the medications being prescribed to them. This brief review will provide an overview of genetics, pharmacogenetics, and current and future examples of genetic variants predicting drug response in gastrointestinal disease, and the limitations and the promise of this exciting and developing field.

OVERVIEW OF PHARMACOGENETICS

Most health-care providers and patients will agree that drugs do not affect everyone in the same way. The effect that a drug may have on an individual may vary with respect to clinical efficacy (the intended response) or experience of side or adverse effects (an unintended response). This variability in treatment effect at times can be explained by differences in dosing, administration (e.g., taken on an empty stomach or after eating), or physiology or metabolism (e.g., weight). Many times, in the absence of these factors being accounted for, there is increasing recognition that there may be a genetic basis for differences in drug effects. As the costs of sequencing DNA continue to decrease and awareness of the role of genetics in predicting drug response increases, the number of clinical applications for genetic testing in gastrointestinal (GI) disease is bound to increase in the near future.

The terms “pharmacogenetics” and “pharmacogenomics” are frequently used interchangeably, but a distinction between the two terms exists. Pharmacogenetics is the field of study that examines the relationship between individual genetic variants and drug effects. In contrast, pharmacogenomics is the field of study that examines the relationship between a number or collection of genetic variants and drug effects. At present, the scale of our current understanding of genetic effects on drug effects is still generally at the single gene level, but great promise lies in elucidating the roles of genetic combinations and their effects. To explain the current

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status of pharmacogenetics and its future in GI disease, this brief review will provide an overview of genetic terms and concepts, pharmacogenetic terms and concepts, examples of known genetic variants that affect treatment response in GI disease, and will conclude with the future directions and challenges that exist in this field.

GENOMICS AND GENETICS PRINCIPLES

Before discussing known genes and their variants that are known to affect human pharmacokinetics and pharmacodynamics, a brief overview of genetics follows to provide a contextual background. The human genome is the entire collection of DNA in a human being. Although in other organisms genetic information can be provided by RNA as well (e.g., viruses), DNA is the genetic template for humans. The human genome consists of 46 chromosomes—44 autosomal chromosomes and 2 sex-linked chromosomes. Within these chromosomes, there are ~3 billion base pairs with an estimated 30,000 coding genes in the genome. Within each gene, several coding exons and non-coding introns exist. The size of a gene is usually determined by the intron length, and the range of gene sizes is 1 – 100 kb, with the average human gene being 27 kb in length.

Throughout the genome and between individuals, there are thousands of genetic variations. The genetic variants are also referred to as polymorphisms, if the minor allele frequency is present in more than 1% of the general population. These polymorphisms are referred to as “mutations” if rare (present in <1% of the general population). These polymorphisms may be functional (resulting in a measurable change in protein quantity or function) or more often, non-functional. There are many forms of genetic polymorphisms, such as single nucleotide polymorphisms (SNPs), VNTRs (variable number tandem repeats), or RFLPs (restriction fragment length polymorphisms). SNPs, in contrast to other variants, such as VNTRs (which are 30–40 bp long and thus could have 30–40 alleles), will only have two alleles—the major allele and the minor allele. Gene names, when cited, are italicized. The standardized notation for SNPs is either (i) amino acid position, wild-type amino acid greater than substituted amino acid (e.g., 399R>Q for an arginine to glutamine substitution at the amino acid position 399), or (ii) nucleotide position, wild-type nucleotide greater than substituted nucleotide (e.g., 1,298A>C for cytosine replacing an adenine nucleotide at the nucleotide position 1,298) (1). These genetic differences are thought to be the basis for individual differences as well as for disease development (e.g., Crohn's disease, Hirschprung's disease). These clinical characteristics are referred to as phenotype.

How are these genetic differences detected on an individual basis? Traditionally, methods for sequencing DNA were quite labor intensive requiring direct sequencing by cleaving DNA base pair by base pair. However, the most cost- and time-efficient methods for genotyping polymorphisms relevant in pharmacogenetics typically are PCR-based modalities that require the selection of a sense and anti-sense primer, which flanks the polymorphism of interest. Furthermore, with the creation of high-throughput modalities, sequencing of thousands of genetic markers spanning the genome or a segment of the genome is now feasible in a matter of days and weeks rather than years. These significant advances in sequencing technologies have made genotyping of a single gene to a thousand genes more time efficient, and therefore, cost efficient. The lower genotyping costs provide the future promise that pharmacogenetic genotyping and therefore, individualized treatment selection will be available for all patients.

PHARMACOGENETICS: THE LINK BETWEEN GENETICS AND DRUG RESPONSE

In contrast to genetics, which focuses on disease development and susceptibility, pharmacogenetics focuses on the link between genes and drug response. By definition, only

functional genetic variants are of interest in pharmacogenetics. However, these functional genetic variations or alterations need not lie in the coding exons to be functional, but may also lie in the non-coding, regulatory regions of the genome. Genes that encode proteins that function as transporters responsible for drug absorption and metabolism, drug receptors, or enzymes, in the metabolic and catabolic pathways are of the greatest relevance in pharmacogenetics. These genetic alterations have the potential to affect drug pharmacodynamics (the relationship between drug concentrations and effect) or pharmacokinetics (the relationship between drug dose and drug concentrations). Factors related to pharmacodynamics—which focus on drug effects—tend to be disease specific, whereas those related to pharmacokinetics—which focus on drug concentrations—are more generalizable across drugs and diseases.

Identification of the relevant polymorphisms that affect drug response is not necessarily an easy task, as many proteins may be involved in the trafficking and processing of drugs, and the number of polymorphisms in the genome is high. Not counting VNTRs or RFLPs, there are approximately 8–10 million SNPs in the human genome with ~1 SNP every 1,000 base pairs. Thus, if the average gene is 27 kb, there will be ~27 SNPs per gene. Elucidating which SNPs are functional and which are not requires a combination of clinical and laboratory work to determine the physiological consequence and clinical relevance of each polymorphism.

PHARMACOGENETIC APPLICATIONS IN GI DISEASES

This field promises clinicians and patients the future ability to allow individualized, patient-specific selection of drugs and drug dosing, which maximizes treatment response and minimizes adverse effects. However, at present, within the field of gastroenterology and hepatology, pharmacogenetics currently only has one established clinical application, that of thiopurine-S-methyltransferase (TPMT) genetic testing for azathioprine and 6-mercaptopurine (6-MP) dosing in inflammatory bowel disease. In addition to TPMT testing, there are a number of known genetic polymorphisms linked to drug response that have yet to reach prime time. A summary of GI diseases, drugs, genes, protein products, clinical effects, and alleles is outlined in Table 1.

PRESENT DAY PHARMACOGENETICS: TPMT TESTING IN INFLAMMATORY BOWEL DISEASE

As the only clinically used test at present, TPMT testing in inflammatory bowel disease is a good example of how clinical decision making can be guided by genetic testing. Azathioprine and 6-MP are related immunomodulating agents used in corticosteroid-dependent patients with either ulcerative colitis or Crohn's disease. Azathioprine is a pro-drug that becomes 6-MP non-enzymatically. 6-MP can then be converted into 6-methyl-mercaptopurine by TPMT, into 6-thiouric acid by xanthine oxidase, or into 6-thioguanine nucleotides by other enzymes. The enzymatic activity level of TPMT is one factor that determines how quickly 6-MP is broken down, and thus how long 6-MP is present and available in the body, and the type and quantity of metabolites present after administration. Specifically, low TPMT activity results in increased production of cytotoxic thioguanines.

The *TPMT* gene is 34 kb long and contains 10 exons. This gene is located on chromosome 6 at location 6p22.3 (Figure 1). More than 20 mutant alleles have been reported to be responsible for TPMT activity deficiency. The genotype of an individual determines side effects from immunomodulators. An individual who is homozygous wild type will have high TPMT activity, whereas an individual who is heterozygous will have intermediate TPMT activity, and an individual who is homozygous variant will have low TPMT activity. Those with low TPMT activity are at the greatest risk for myelosuppression. As such, the FDA (Food and Drug

Administration) recommends that the *TPMT* genotype should be evaluated before administration of azathioprine or 6-MP. Those with low *TPMT* activity levels should not receive these agents. Those who are homozygous wild type should receive an initial dose of 2–2.5 mg/kg of azathioprine or 1–1.5 mg/kg of 6-MP but should still have their blood counts monitored. Those with intermediate activity should receive an attenuated initial dose of 1–1.5 mg/kg of azathioprine or 0.75 mg/kg of 6-MP (2,3). In summary, *TPMT* genetic testing is a successful example of how pharmacogenetics can positively affect disease management outcome in a GI disease.

NEAR-FUTURE PHARMACOGENETICS: AMPLICHIP CYP450

Proton pump inhibitors (PPIs) are used frequently to treat acid reflux, peptic ulcers, and functional dyspepsia, and are used in combination with antibiotics to treat *Helicobacter pylori* infections. In contrast to drugs, such as azathioprine or 6-MP, which have significant adverse event profiles, less thought regarding dosing has generally been given to the generally safe PPIs. Nonetheless, the pharmacokinetics of this drug class is influenced by genetics, and may have relevance in assisting health-care providers in selecting the optimal dose for their specific patients. Cytochrome P450, subfamily IIC, polypeptide 19 (CYP2C19) in the liver is the main enzyme responsible for PPI drug elimination (4). Various alleles (Table 1) have been reported with a gene-dose effect that influences rates of healing for endoscopically-proven esophagitis as well as rates of *H. pylori* eradication. For example, cure rates were 73, 69, and 57% at 4 weeks for poor metabolizers, heterozygotes, and extensive (wild-type) metabolizers, and 100, 95, and 77% at 8 weeks in Japan (5). Similarly, the extensive, wild-type metabolizers showed 20% lower cure rates in the treatment of *H. pylori* (6). More recently, a microarray-based AmpliChip CYP450 test was made commercially available by Roche (Nutley, NJ). Because of the ease of testing, this test may become incorporated in routine practice, particularly in Asia where these genetic variants are common. However, the cost effectiveness of such testing still remains to be determined, and additional validation of the clinical yield for this chip is merited before incorporation in standard clinical practice.

LIMITATIONS AND CHALLENGES OF PHARMACOGENOMIC DATA

Ideally, genetic variants would allow for a clear interpretation of its results with actionable clinical implications or consequences. This tends to occur when a genetic variant has a “strong” effect. However, many genetic variants exist in the genome, and many will likely have weaker genetic effects with less clear clinical implications. For example, a specific genetic variant may be associated with a “30% increased risk of pancreatitis,” but in the right clinical setting, may not preclude a health-care provider from prescribing such a medication if the potential benefits are high. Furthermore, racial differences in genotype and allele frequencies are common, and thus, the interpretation of genetic data will need to be implemented in a race-conscious manner. Some patients have relatively homogeneous ethnicities but many do not, and greater knowledge and understanding of the race-specific genotype frequencies may be useful to the ordering clinician. In addition, gene–environment interactions may also be the rule rather than the exception, and interpretation of some genetic data may not be feasible without a clear understanding of the environmental contributors to disease and response to therapy.

THE FUTURE PROMISE OF PHARMACOGENETICS

The combination of genetics and pharmacology is exciting with respect to maximizing treatment outcomes and/or minimizing adverse treatment effects. At present, the promise of the field is great but has yet to anywhere reach its full potential. Although the sequence of the human genome is known, the clinical significance of each variation in the DNA code is not yet known, let alone the effect of a combination of variations. Much of these limitations will be overcome as sequencing technologies facilitate the ease of genotyping, bringing down the

cost of genotyping further and allowing for greater research into the field. The current status of the field is evaluating a single polymorphism in a single gene for a handful of diseases. However, the greater promise lies in evaluating multiple polymorphisms in multiple genes—potentially those that lie in the same drug metabolic pathway—spanning the entire genome. Thus, in the not-so-distant future, genotyping 20–30 functional SNPs in several genes may allow for greater accuracy in recommending specific drug dosing for patients.

In the more distant future, pharmacogenetics will likely go beyond genotyping 20–30 functional SNPs in an individual to genotyping 100–300,000 select SNPs using high-throughput genotyping technologies, which are currently available (albeit these platforms are still relatively expensive) by analyzing haplotypes, which are alleles that tend to be inherited together because their link has not been disrupted by recombination. The advantage that haplotypes offer is that they allow the genotyping of one allele in a haplotype as a proxy marker for the other alleles in the haplotype, thus decreasing the need to genotype all polymorphisms in the haplotype. The HapMap Project is a multi-country, collaborative effort to catalog the SNP genetic variants and their haplotype combinations of four populations with African, Asian, and European ancestry (<http://www.hapmap.org>). The project is identifying “tag” SNPs that uniquely identify these haplotypes, with an estimated 300–600,000 tag SNPs to be identified. This figure is much smaller than the estimated 10 million SNPs that one would need to genotype across the genome. Thus, it is conceivable that patients will be carrying medical information “chips” with their haplotype data to allow optimal selection of drug dosing for any drug with a pharmacogenetic basis.

CONCLUSIONS

Although there has been much hype about pharmacogenetics in medical circles and in the general public promising “individualized medicine,” pharmacogenetics at present has limited applications, particularly in the field of clinical gastroenterology. Furthermore, pharmacogenomics—the field that evaluates multiple genetic effects on drug response—offers great promise in the future, but at present, has sparse concrete applications in gastroenterology. Nonetheless, TPMT gene testing for azathioprine and 6-MP dosing in inflammatory bowel disease management clearly illustrates how genetic testing can positively affect patient care. It is the hope that as the understanding of science regarding the human genome grows, the clinical implications of our genetic variants will soon be uncovered and will significantly change clinical practice and patient management of GI disorders in the 21st century.

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Figure 1.

Thiopurine-S-methyltransferase (TPMT) gene and the *3A allele. The *TPMT* gene consists of 10 coding exons. The *3A allele is the most common functional variant allele in Caucasians and results in significantly decreased TPMT enzyme activity. The *3A allele consists of two non-synonymous coding single-nucleotide polymorphisms that result in alterations of encoded amino acids.

Table 1
Summary of known genetic variants with pharmacogenetic effects in gastrointestinal tract

Disease	Drug(s) or drug class	Gene	Protein products	Clinical drug effect	Polymorphisms/alleles	Reference(s)
Colorectal cancer	5-Fluorouracil, capecitabine	DPYD	Dihydropyrimidine dehydrogenase	Low activity → ↓ response ↑ toxicity	*2A	Salonga <i>et al.</i> (7); Milano <i>et al.</i> (8); Wei <i>et al.</i> (9)
	Irinotecan	TYMS	Thymidylate synthase	Carriers → ↓ response	TSER repeats	Popat <i>et al.</i> (10)
	Cisplatin, carboplatin, oxaliplatin	UGT1A1 XRCC1	Uridine diphosphate-glucuronyl transferase X-ray cross-complementing group 1 protein	Carriers of 1 or 2 alleles → ↑ diarrhea ↑ myelosuppression Carriers of 1 or 2 alleles → ↓ response to 5-FU/ platinum regimens	399R>Q	Ando <i>et al.</i> (11); Iyer <i>et al.</i> (12)
Functional dyspepsia	Proton pump inhibitors, prokinetics, spasmolytics, and tricyclic antidepressants	GNβ3	G protein, β3 subunit	CC genotype → ↑ response	825C>T	Stoehlmacher <i>et al.</i> (13)
Gastroesophageal reflux disease or <i>Helicobacter pylori</i> infection	Proton pump inhibitors	CYP2C19	Cytochrome P450, subfamily IIC, polypeptide 19	Homozygous wild type → slow healing of esophagitis, ↓ cure rates of <i>Helicobacter pylori</i>	*2, *3, *4, *5	Kawamura <i>et al.</i> (5); Chong and Ensom (6); Furuta <i>et al.</i> (15); Schwab <i>et al.</i> (16)
Inflammatory bowel disease	Azathioprine	TPMT	Thiopurine-S-methyltransferase	High, intermediate, low metabolizers predicts dosing	*2, *3A, *3B	Colombel <i>et al.</i> (17); Wang and Weinsilboun (18)
Irritable bowel syndrome	6-Mercaptopurine, methotrexate	MTHFR	Methylene tetrahydrofolate reductase	CC patients experience ↑ side effects	1298A>C	Urano <i>et al.</i> (19)
Liver transplantation	Alosetron	SLC6A4	Serotonin transporter (solute carrier family 6, member 4)	Patients with diarrhea and LL homozygotes predict response to alosetron	5-HTT LPR: long (L) and short (S) allele	Camilleri <i>et al.</i> (20)
	Tacrolimus	CYP3A4	Cytochrome P450, subfamily 3A, polypeptide 4	Carriers require ↑ tacrolimus dosing	*1B	Amirani <i>et al.</i> (21)
		CYP3A5	Cytochrome P450, subfamily 3A, polypeptide 5	Carriers require ↑ tacrolimus dosing	*3, *6	Zhao <i>et al.</i> (22)