

Pharmacogenomics of Sex Difference in Chemotherapeutic Toxicity

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Abstract: Except for few cases, chemotherapeutic toxicity is in general influenced by multiple genetic factors and non-genetic factors including age, sex and drug-drug interactions. The manifestations of adverse drug reactions differ between men and women. In particular, women experience greater toxicity from certain chemotherapeutic drugs than men. Sex-related factors are likely to play an increasing role in drug development and therapeutic decision-making in the future. The sex-selective toxicity could be attributed to sex-related differences in pharmacokinetic and pharmacodynamic properties of these drugs. Systematic pharmacogenomic investigation into sex difference in chemotherapeutic toxicity potentially presents an opportunity to assess the effects of multiple genetic factors or gene networks on sex-related differences in the toxicity of anti-cancer drugs. A thorough understanding of the interactions between sex, drug and gene will provide valuable insights in assessing the susceptibility of an individual to chemotherapy-induced toxicity, predicting the sex-related effects for any anticancer drug and ultimately achieving the goal of personalized cancer therapy.

Key Words: Sex difference, pharmacogenomics, chemotherapeutic toxicity, personalized drug therapy.

INTRODUCTION

Except for few examples where single genetic variations have pronounced effects, adverse drug reactions (ADRs) are in general influenced by multiple genetic factors and non-genetic or environmental factors [1]. Sex has been considered as an environmental factor that could interact with drug properties and genetic factors [2]. Just like one size does not fit all, one treatment does not fit both men and women [3]. There are differences between males and females in drug response and incidence of ADRs. However, according to 1977 guideline of the Food and Drug Administration (FDA), women were excluded from clinical drug trials due to the difficulty to analyze sex-related data and potential occurrence of birth defects resulting from fetal exposure to certain drugs [4]. Therefore, information about the role of sex in treatment-related toxicity had historically been limited, which became a growing concern through the following decades [4-6].

In 1993, FDA revised its 1977 guideline to include women in all stages of drug development and to analyze the resulting data for sex difference. Since then, sex difference in ADRs has attracted considerable attention. According to numerous publications describing the difference in treatment-related toxicity, the female sex is a risk factor for ADRs (for reviews, see [5, 7, 8] and the 10 June, 2005 issue of Science). Sex difference is of particular concern for drugs that have narrow therapeutic windows and must be given at optimal doses. Examples of such drugs are cancer chemotherapeutic drugs, which are associated with the highest

incidence of ADRs compared with other drugs [6]. These drugs are generally toxic; and are often given at doses near those that produce toxicity [9]. Emerging data suggest that women receiving 5-fluorouracil (5-FU)-based chemotherapy for colorectal carcinoma, or combinatory therapy consisting of doxorubicin for small cell lung carcinoma (SCLC), experience more frequent and more severe toxicity than men [10, 11].

Sex difference in chemotherapeutic toxicity could be attributed to multiple factors, including differences in pharmacokinetics and pharmacodynamics of the drug as well as age, sex, drug-drug interactions and immunological factors [8, 12]. There is a growing body of knowledge on sex-related differences in pharmacokinetics of many drugs [13-17]. The potential sources for this phenomenon may exist in each of the four pharmacokinetic processes, *i.e.*, absorption, distribution, metabolism and excretion (ADME), which may all have sex-related variability [18]. Sex-related expression and function of gene(s) involved in the pharmacokinetics of drugs, *e.g.*, those for drug-metabolizing enzymes and drug-transport systems, may result in the differential treatment-related toxicity between women and men. There is also sex difference in pharmacodynamics involving drug targets and the downstream signaling events, although mainly observed for cardiac and psychotropic drugs [7]. Obviously, many genes, which form complex functional networks, collectively and/or cooperatively contribute to the sex-selective toxicity to anticancer drugs. Therefore, intricate interplays exist between sex factors, gene networks and drug response phenotypes (Fig. 1). A better understanding of these interplays with respect to chemotherapeutic toxicity will be of paramount importance for identifying factors that predispose the patients to chemotherapeutic toxicity, predicting the sex-related effects for any anticancer drugs and ultimately achieving the goal of personalized cancer therapy.

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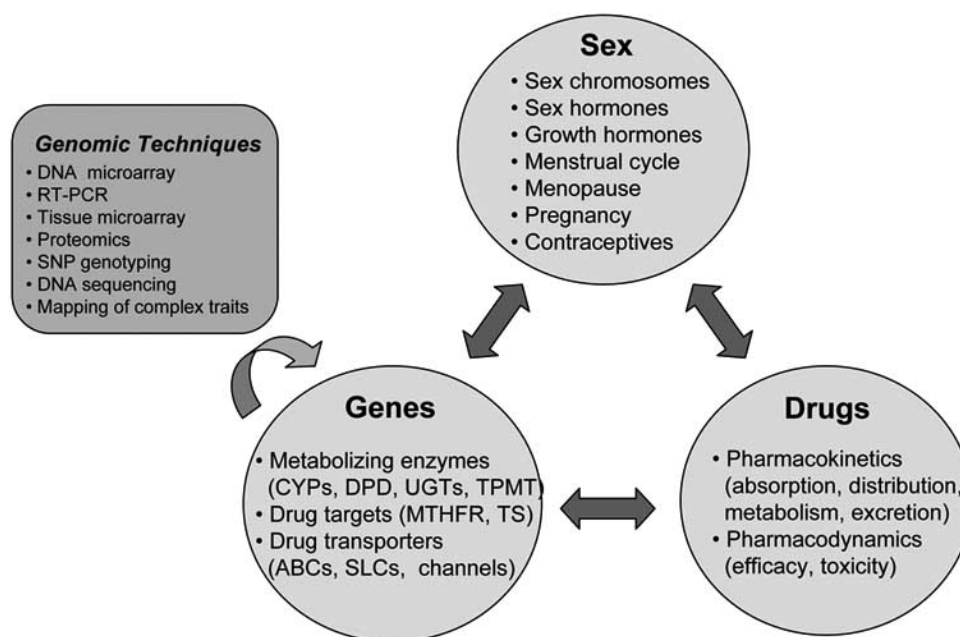


Fig. (1). Sex-gene, sex-drug and gene-drug interactions. There are intricate interplays between sex factors, drug response phenotypes such as pharmacokinetic and pharmacodynamic parameters, and genetic factors which are important for pharmacology and can be systematically studied using genomic approaches. A good understanding of these interplays with respect to drug efficacy and adverse drug reactions will be of paramount importance for identifying factors that predispose the patients to chemotherapeutic toxicity, predicting the sex-related effects for any anticancer drugs and ultimately achieving the goal of personalized cancer therapy. CYPs, cytochromes P450; DPD, dihydropyrimidine dehydrogenase; UGTs, UDP-glucuronosyltransferases; MTHFR, methylenetetrahydrofolate reductase; TS, thymidylate synthase; ABCs, ATP-binding cassette transporters; SLCs, solute carrier transporters.

Recent advances in pharmacogenomics permit the use of a global approach to revealing complex gene networks (at DNA, RNA and protein levels) involved in anticancer drug toxicity. Due to the toxic characteristics of the majority of chemotherapeutic drugs and the severity of clinical outcomes of cancer, cancer therapy appears to be one of the most appropriate areas for the application of pharmacogenomics and has already begun to be benefited from these studies [9]. Large amounts of data on the association between genetic variations and drug efficacy/toxicity have been generated and stored into databases [19]. Several recent reviews described the potential of using pharmacogenomic data for individualization of cancer chemotherapy [9, 20-22]. To provide insights into sex difference in chemotherapeutic toxicity, pharmacogenomics on the gene-drug interaction must incorporate the added dimension presented by the sex difference in gene expression and function as well as the sex differences in toxicity to anticancer drugs (sex-gene and sex-drug interactions, (Fig. 1). Pharmacogenomics of sex difference in chemo-toxicity will investigate whether the levels of expression of gene networks varies between women and men and whether they can be regulated by the sex-related factors such as sex hormones, menopause, menstruation and pregnancy. Meanwhile, pharmacogenomic researchers focusing on the sex difference must realize that many efforts have already been made thus far with respect to the interactions between sex and drug pharmacokinetics/toxicity, as well as the interactions between the sex and gene expression. The purpose of this review is to 1) highlight several findings on sex-gene, sex-drug and gene-drug interactions; 2) discuss how to integrate pharmacogenomics into studies on sex difference that is relevant to the toxicity of chemotherapeutic

drugs; and 3) provide recommendations for future directions in this research area.

SEX-DRUG INTERACTION: SEX DIFFERENCE IN TOXICITY AND PHARMACOKINETICS OF CHEMOTHERAPY

Women and men respond to drugs differently. Sex-drug interaction is to study the role of sex-related factors on effects of currently used drugs or sex-specific manifestation of drug efficacy and ADRs. Sex differences in chemotherapeutic toxicity have been mostly documented for 5-FU. 5-FU is the most commonly used chemotherapeutic agent for the treatment of patients with colorectal cancer. The common ADRs associated with 5-FU are gastrointestinal (GI) and hematologic toxicities and mucositis [23]. A meta-analysis of the toxicity profiles on 1,093 women and 1,355 men from 12 treatment arms of clinical trials found a significantly greater incidence of stomatitis, leucopenia, alopecia, nausea, emesis, and diarrhea among women in comparison with men [10]. Further examination of the incidence of severe (grade ≥ 3) toxicities showed that women experience more severe toxicities than men, where stomatitis and leucopenia showed the largest difference. In this study, the response and survival rates to the treatment were not different for men and women [10]. Consistently, other studies that included sex analysis for 5-FU toxicity have shown the similar differences between the two sexes [10, 23-27]. These results raise the question of whether the recommended dose of 5-FU-based chemotherapy for women should be lower than that for men [10].

Sex differences in drug toxicity have also been reported for other drugs used to treat other types of cancer. SCLC accounts for ~20% of all lung cancers [28]. All chemotherapeutic drugs used to treat SCLC are associated with some forms of toxicity for almost all patients. Therefore, patients are required to give informed consent after receiving information about the risks and benefits of the therapy. However, there is little information available on the sex-related toxicity, as data from clinical trials have been generalized and applied to both sexes [11]. Singh *et al.* performed a sex-based retrospective analysis of four SCLC trials conducted by the Clinical Trials Group of the National Cancer Institute of Canada between 1987 and 1999, including 648 male and 358 female patients that received a chemotherapy consisting of cyclophosphamide-doxorubicin-vincristine (CAV) followed by etoposide-cisplatin (EP) treatment [11]. Women experienced significantly more frequent and more severe stomatitis, vomiting and hematologic toxicity than men during the SCLC chemotherapy. The differences identified were highly significant and remained significant after the adjustments on potentially confounding covariates, such as age, performance status, and pretreatment lactate dehydrogenase (LDH) values [11]. Other groups reported increased rates of nausea and vomiting in women from cisplatin-based therapy [29, 30]. Moreover, women showed poorer maintenance of complete protection from vomiting and nausea provided by antiemetic treatment during chemotherapy [30, 31]. The mechanism that leads to the sex-selective toxicity is unknown, but is likely multi-factorial. One possibility is the decreased drug clearance in females. For the SCLC treatment, it was suggested that the differences in toxicity might be related to reduced hepatic clearance of etoposide or doxorubicin in women [11], which was reported previously [32, 33].

Chemotherapeutic drugs show a wide interindividual variability in pharmacokinetics, which results in unpredictable toxicity in patients [18]. Any factors that change the pharmacokinetics may easily cause toxicity due to their narrow therapeutic windows. Sex is one of the factors that contribute to the interindividual differences in pharmacokinetics [34]. Sex difference in pharmacokinetics has been reported for numerous drugs, possibly because sex is easily identified and pharmacokinetics parameters are measurable. In a study conducted by FDA of 300 new drugs reviewed from 1994 and 2000, 20% of those that included sex analysis showed pharmacokinetic differences between men and women [3, 7]. Four major factors may contribute to the pharmacokinetic variability in sex differences – absorption, distribution, metabolism and excretion [16]. With respect to absorption, there is little evidence of sex difference [7]. The fact that females weigh less and have a higher percent body fat than males may affect drug distribution and potentially increase toxicity, but this alone can not adequately account for observed differences in toxicity [7]. The most frequently observed sex-related pharmacokinetic difference is in drug clearance, which is a direct quantitative measure of body's ability for drug elimination (including both metabolism and excretion). Existing evidence indicates that the clearance values of many chemotherapeutic drugs, *e.g.*, 5-FU [35] and doxorubicin [32], are lower in women compared with men. In addition, significant sex-dependent clinical pharmacoki-

netic behavior has been described for other chemotherapeutic drugs, such as methotrexate [36, 37], topotecan [38, 39], gemcitabine [40] and epirubicin [41], where drug clearance was also reduced in women as compared to men. Thus, sex-related differences in drug clearance may play an important role in the differences in clinically relevant toxicity.

GENE-DRUG INTERACTION: PHARMACOGENOMICS

Gene-drug interaction is to study the association between variations of genes (at DNA, RNA and protein levels) and drug efficacy/toxicity. This is the definition for pharmacogenomics. Identifying genetic factors which may predispose a patient to chemotherapeutic toxicity is essential for achieving the goal of personalized cancer therapy. After more than 50 years of pharmacogenetic and pharmacogenomic research, it has been well-known that genetic variances in some genes, especially in genes encoding drug-metabolizing enzymes, can at least partly explain the inter-individual variability in observed toxicity of some anticancer drugs. Recent findings on gene-drug interactions include those between the genetic polymorphisms in genes encoding dihydropyrimidine dehydrogenase (DPD), thiopurine methyltransferase (TPMT), UDP-glucuronosyl transferase 1A1 (UGT1A1), methylenetetrahydrofolate reductase (MTHFR), and the toxicity to 5-FU, 6-mercaptopurine (6-MP), irinotecan and methotrexate, respectively [42, 43]. However, these drug-related genes usually do not function alone. Here, we use 5-FU as an example to illustrate how gene networks influence chemotherapeutic toxicity.

5-FU, a pyrimidine antagonist, is a prodrug that requires intracellular activation to produce cytotoxic nucleotides (Fig. (2)). Several enzymes of the pyrimidine metabolic pathway are involved in the anabolic activation, including orotate phosphoribosyl transferase (OPRT), uridine phosphorylase (UP), uridine monophosphate kinase (UMP5K), thymidine phosphorylase (TP) and thymidine kinase (TK) [44, 45]. The anti-tumor activity of 5-FU is due to the inhibition of thymidylate synthase (TS) by 5-FU metabolites and the incorporation of 5-FU metabolites into RNA and DNA. In fact, only a small part of the administered 5-FU is activated. More than 80% of the administered dose is catabolized into 5,6-dihydrofluorouracil (DHFU) by DPD and eliminated in the liver. DHFU can be further degraded by dihydropyrimidinase (DHP/DPYS) and β -ureidopropionase (UBP1). Genetic polymorphisms in genes encoding these enzymes, either alone or in combination, may have significant influence on 5-FU efficacy and toxicity. Polymorphisms have been described for UMP5K, OPRT and the target enzyme TS and have been correlated with chemosensitivity of cancer cells to 5-FU [44]. Expression levels of TS, TP, TK, and DPD have been associated with 5-FU sensitivity and resistance of cancer cells [44]. DPD is one of the most important factors that determine 5-FU toxicity, and genetic variation in DPD has been associated with 5-FU toxicity. Low levels of DPD or inherited DPD deficiency can result in dramatic reduction in 5-FU clearance, causing severe toxicity [44, 46]. However, the mechanisms for 5-FU toxicity is multifactorial, because DPD has been found intact in patients who suffered from severe 5-FU toxicity [24, 47]. Deficiency in DHP/DPYS, the

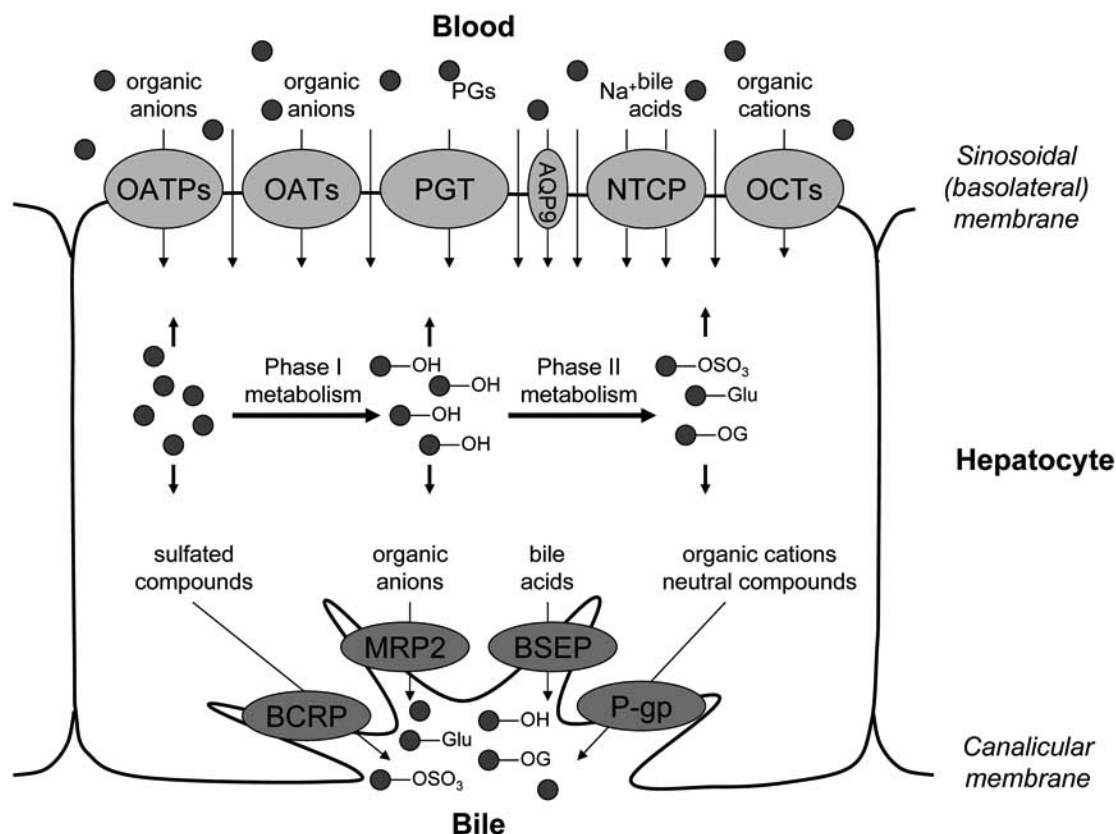


Fig. (3). Transport and metabolism of drugs in the liver. OATP, organic anion transporting polypeptide; OAT, organic anion transporter; PGT, prostaglandin transporter; NCTP, sodium-dependent bile acid transporter; OCT, organic cation transporter; BCRP, breast cancer resistance protein; MRP, multidrug resistance protein; BSEP, bile salt export pump; Pgp, p-glycoprotein.

dependent manner, such as cytochrome P450 enzymes CYP1A2, CYP2B6, CYP2E1, CYP3A4 and UDP-glycosyltransferases (UGTs) [7]. The different CYP450 expression level between two sexes may influence the rate of clearance for any given substrate drug. CYP3A4 is the most abundant cytochrome P450 isozyme in the human liver, accounting for ~ 30% of total hepatic CYP activities [59]. A 1.4 times greater expression was found in women compared with men [51]. Sex differences in drug clearance have been demonstrated for a variety of CYP3A4-metabolized drugs [60, 61], such as doxorubicin and etoposide. However, drug metabolism through CYP3A4 alone does not adequately explain the observed interindividual variability in drug clearance or toxicity. Drug transport must be considered together with drug metabolism [13, 62, 63].

Sex Difference in Hepatic Transporters

Two major superfamilies of membrane transporter proteins that influence pharmacokinetics of drugs are the ATP-binding cassette (ABC) transporters and the solute carrier (SLC) transporters. The majority of sex-related studies on hepatic transporters have been focused on Pgp encoded by the *ABCB1* gene. It belongs to the ABC transport family [64]. In cancer cells, it mediates energy-dependent drug efflux and plays a major role in multidrug resistance [64]. It is significantly expressed in normal tissues as well, including the liver, at the canalicular pole of the hepatocyte, responsible for biliary drug excretion. When Pgp is blocked or genetically reduced in hepatocytes, intracellular drug level is

increased, which results in reduced drug elimination and increased toxicity [65, 66]. Pgp shares common substrates with CYP3A4, such as doxorubicin and etoposide. Pgp and CYP3A4 may act in concert to increase drug clearance from the liver [62]. In one study, Pgp activity was found to be 2.4-fold lower in the liver of females (opposite to that for CYP3A4) [67], but in another study this enzyme was not differentially expressed between males and females [51]. Pgp and CYP3A4 expression have also been compared in male and female proximal small intestines, the major site of drug absorption, but no sex difference was found [68]. This is in agreement with the report describing lack of sex difference in the oral clearance of some CYP3A4 or Pgp substrates [13]. In addition, research has shown that mRNA and protein expression of Pgp can be induced by female sex hormones [69]. However, estrogens mediate post transcriptional down-regulation of Pgp expression in Pgp-transfected, estrogen receptor alpha (ER- α)-positive human breast cancer cells, but not in ER- α -negative cancer cells [70]. The synthetic progestins for oral contraceptives and hormone replacement therapy show inhibitory effect on Pgp activity [71].

Sex-dependent expression and activity has also been reported for human breast cancer resistance protein (BCRP/ABCG2), another ABC family transporter important for multidrug resistance of cancer [52]. Similar to Pgp, BCRP/ABCG2 is also present at the canalicular membrane (Fig. (3)), and mediates biliary excretion of drugs [52]. Drug clearance and hepatobiliary excretion for substrate drugs of

BCRP/ABCG2 were significantly lower in wildtype female mice than those in wildtype male mice, but no significant sex difference observed in these parameters in *ABCG2*^{-/-} mice. Expression study of murine ABCG2 in several pharmacokinetically important tissues showed that only the hepatic ABCG2 expression was higher in male mice compared with female mice. Analysis of human ABCG2 showed that the hepatic expression in women was lower than men [52]. This study indicates that sex difference in liver ABCG2 expression causes the sex difference observed in the pharmacokinetics of ABCG2 substrates, which is in agreement with the sex difference observed in the pharmacokinetics of several chemotherapeutic drugs known to be ABCG2 substrate, such as topotecan, methotrexate, doxorubicin and epirubicin [52]. In addition, recent data showed that estrogen could also downregulate ABCG2 expression [72].

It has been shown that male rat liver has higher protein and mRNA levels of aquaporin-9 (AQP9) than females [73]. AQP9 is a water channel membrane protein, which is permeable to anticancer drugs including 5-FU [74]. In female liver the expression of AQP9 was mostly confined to perivascular hepatocytes, whereas in male liver it showed a more homogeneous hepatocyte expression. The AQP9 expression is confined to the sinusoidal membrane of hepatocyte, the site of drug uptake from the blood. Therefore, it is of interest to know if AQP9 plays a role in mediating 5-FU uptake from blood into the hepatocytes. It will also be highly interesting to determine whether the sex-dependent expression can be found in human liver, and whether lower expression of AQP9 in females contributes to reduced clearance of 5-FU.

Genome-Wide Study of Sex Difference in Gene Expression

Recent progresses in genome sequencing, microarray and proteomic technologies have provided powerful tools to identify genes that are differentially expressed in each sex in a variety of tissues and to reveal mechanisms of sex-specific transcriptional regulation [49]. In a recent study, the expression pattern of 13,977 mouse genes has been surveyed in male and female hypothalamus, kidney, liver and reproductive tissues using DNA microarrays [75]. Extensive differential gene expression was observed not only in the reproductive tissues, but also in the liver and kidney. A majority of the genes differentially expressed are involved in drug and steroid metabolism such as CYP450s and in osmotic regulation [75]. Microarray analysis has also been used to study the gene expression difference between two sexes in multiple somatic tissues of 334 mice and revealed significant differences in the liver, adipose, muscle and brain [76]. These mice were derived from an intercross between two inbred mouse strains, which allow analysis of the genetic control of sexually dimorphic gene expression. Microarray analysis of 23,574 transcripts revealed sexual dimorphism in liver, adipose, muscle and brain in an extent much greater than previously recognized. Another large-scale gene expression study using microarray was conducted in the livers from male and female mice, which were either wildtype or with the transcription factor, signal transducer and activator of transcription (STAT5b) inactivated [77]. Extensive sex differences in hepatic expression were observed. The sex-dependent genes

were dependent on STAT5b activity, suggesting that STAT5b is essential for sex-dependent liver gene expression in mice. Delongchamp *et al.* reported a genome-wide study of sex differences in the gene expression of human liver samples from nine males and nine females [78]. However, this study failed to identify any specific gene with sex difference because the observed sex difference in gene expression was too small to satisfy the statistical criteria, probably due to the low sensitivity of the type of microarray used (filter array) and the small sample size. Such comprehensive investigation of sex-related gene expression has been rarely performed in human tissues, possibly due to the difficulty in obtaining normal tissue samples.

Sex-Gene Interaction: Sex Difference in Genetic Basis of Quantitative Traits

Although there are some well-known examples of single genetic variation having pronounced effects on drug toxicity, in general, variable drug pharmacokinetics and toxicity appear to be genetically complex traits or quantitative traits [1, 79]. Many quantitative traits show sex-specific architecture [2]. Many complex human diseases such as diabetes, systemic lupus, depression, asthma and cardiovascular diseases display sex-related differences [2]. Sex could be considered an environmental factor. Gene-sex interaction is one of the challenges for successful mapping of quantitative traits. In fact, sex-gene interaction is most often ignored by geneticists, simply because little is known about how sex factors influence the expression of these traits and how to incorporate sex-related factors into genetic models [80]. Recently, it has been suggested that sex-gene interactions may lead to different effects of the same genetic variations in men and women [2]. Genetic determinants of some complex traits such as morning serum cortisol level and whole blood serotonin level have been shown to be different for men and women [80, 81]. Kurina *et al.* performed a genome-wide screen using linkage and association methods to search for the quantitative-trait locus (QTL) in a large pedigree of a founder population [81]. Results of the sex-partitioned analysis indicated that significant association with an allele on chromosome 11p is restricted to females. Females homozygous for the allele had an 89% increase in morning cortisol levels compared with female noncarriers. A study on the whole blood serotonin level showed a similar selective architecture in the founder population [80]. In another study, Weiss *et al.* evaluated sex-specific heritability and genome-wide linkage for 17 quantitative traits that are associated with complex human diseases in a founder population. Of the 17 traits that they surveyed, 11 were shown to be sexually dimorphic, and 12 showed evidence of sex difference in heritability or linkage, suggesting that sex-gene interaction is a relatively general phenomenon. Therefore, integrating sex into the model of gene-drug interactions may improve the detection of susceptibility loci in genome-wide screens for determinants of drug efficacy and toxicity.

FUTURE DIRECTIONS

Up to now sex-related data are sparse for the majority of drug-related hepatic SLC transporters such as organic anion transporters (OATs), organic cation transporters (OCTs),

organic anion transporting polypeptides (OATPs) and ABC transporters other than Pgp and ABCG2 [7] (Fig. (3)). Significant research on sex difference has been conducted in animals, but there has been very limited research in humans. Compounded with this issue, the differences found in animals are not always consistent with those reported in humans [34]. Therefore, the findings obtained from animals need to be confirmed in humans if they are to provide information in understanding clinically relevant differences in toxicity.

There are several potential problems in demonstrating sex-related differences in gene expression and in their implications. First of all, gene expression may show a large inter-individual variability. Sex may not be the only factor determining the difference. Other contributing factors may include pharmacological or dietary induction, stimulation, or inhibition of gene expression as well as genetic polymorphisms. Therefore, evaluation of the role of sex in gene expression requires a sufficient sample size with enough statistical power and an optimal study design that excludes confounders such as age, race, smoking habits and pharmacological treatment history. Secondly, it has been shown that metabolizing enzymes and transporters exhibit multiplicity, *i.e.*, there is a degree of substrate overlapping with many of the enzymes and transporters, causing problems in predicting toxicological effects. Thirdly, expression at mRNA and protein levels may not always correlate well with gene activity. Sex-related gene expression levels may not necessarily indicate differences in gene functions. Therefore, to correlate the findings in sex-dependent transporter gene expression with pharmacokinetic/pharmacodynamic parameters including clinical drug toxicity, it is necessary to conduct appropriate functional studies to identify substrates for specific transporters. The use of specifically validated substrate as probes is also important. In addition, more studies are needed to clarify the interactions between drug toxicity and specific sex-related factors such as menopause, pregnancy, menstruation and the use of hormonal steroid contraceptives and hormone replacement therapy in drug toxicity.

Until now, the majority of the analysis on sex-drug interaction has focused on individual gene candidates. Few genome-wide screens have been conducted for sex-specific gene effects. As pharmacogenomics is increasingly being employed to identify the entire gene networks that are relevant to the pharmacologic phenotype of a given drug, it will provide a considerable incentive to invest in the area of sex-related toxicity and treatment efficacy, *i.e.*, to comprehensively identify the interactions between sex, genes and drugs. However, the huge number of comparisons involved when interactions among genes, drugs and sex are considered together calls for high quality experimental design and data interpretation. Learning how genes or gene networks, as well as sex-related factors contribute to the chemotherapeutic toxicity will be important for predicting sex-related effects on pharmacokinetics and toxicity of chemotherapeutic drugs and for developing treatments that are equally safe for both men and women.

ABBREVIATIONS

FDA	=	The Food and Drug Administration
NCI	=	The National Cancer Institute

ADRs	=	Adverse Drug Reactions
5-FU	=	5-Fluorouracil
DPD	=	Dihydropyrimidine Dehydrogenase
SCLC	=	Small-Cell Lung Cancer
Pgp	=	P-glycoprotein
ABC	=	ATP-binding Cassette
SLCs	=	Solute Carriers

REFERENCES

- [1] Need A.C., Motulsky A.G., Goldstein D.B.: *Priorities and standards in pharmacogenetic research*. Nat. Genet. 37(7), 671-681, (2005).
- [2] Weiss L.A., Pan L., Abney M., Ober C.: *The sex-specific genetic architecture of quantitative traits in humans*. Nat. Genet. 38(2), 218-222, (2006).
- [3] Bren L.: *Does sex make a difference?* FDA Consumer magazine. 39(4), (2005).
- [4] Merkatz R.B., Temple R., Subel S., Feiden K., Kessler D.A.: *Women in clinical trials of new drugs. A change in Food and Drug Administration policy. The Working Group on Women in Clinical Trials* N. Engl. J. Med. 329(4), 292-296, (1993).
- [5] Tran C., Knowles S.R., Liu B.A., Shear N.H.: *Gender differences in adverse drug reactions*. J. Clin. Pharmacol. 38(11), 1003-1009, (1998).
- [6] Fattinger K., Roos M., Vergeres P., Holenstein C., Kind B., Masche U., Stocker D.N., Braunschweig S., *et al.*: *Epidemiology of drug exposure and adverse drug reactions in two swiss departments of internal medicine*. Br. J. Clin. Pharmacol. 49(2), 158-167, (2000).
- [7] Anderson G.D.: *Sex and racial differences in pharmacological response: where is the evidence? Pharmacogenetics, pharmacokinetics, and pharmacodynamics*. J. Womens Health (Larchmt). 14(1), 19-29, (2005).
- [8] Rademaker M.: *Do women have more adverse drug reactions?* Am. J. Clin. Dermatol. 2(6), 349-351, (2001).
- [9] Cheok M.H., Evans W.E.: *Acute lymphoblastic leukaemia: a model for the pharmacogenomics of cancer therapy*. Nat. Rev. Cancer 6(117-129), (2006).
- [10] Sloan J.A., Goldberg R.M., Sargent D.J., Vargas-Chanes D., Nair S., Cha S.S., Novotny P.J., Poon M.A., *et al.*: *Women experience greater toxicity with fluorouracil-based chemotherapy for colorectal cancer*. J. Clin. Oncol. 20(6), 1491-1498, (2002).
- [11] Singh S., Parulekar W., Murray N., Feld R., Evans W.K., Tu D., Shepherd F.A.: *Influence of sex on toxicity and treatment outcome in small-cell lung cancer*. J. Clin. Oncol. 23(4), 850-856, (2005).

- [12] Drici M.D., Clement N.: *Is gender a risk factor for adverse drug reactions? The example of drug-induced long QT syndrome*. *Drug Saf.* 24(8), 575-585, (2001).
- [13] Cummins C.L., Wu C.Y., Benet L.Z.: *Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein*. *Clin. Pharmacol. Ther.* 72(5), 474-489, (2002).
- [14] Meibohm B., Beierle I., Derendorf H.: *How important are gender differences in pharmacokinetics*. *Clin. Pharmacokinet.* 41(5), 329-342, (2002).
- [15] Schwartz J.B.: *The influence of sex on pharmacokinetics*. *Clin. Pharmacokinet.* 42(2), 107-121, (2003).
- [16] Gandhi M., Aweeka F., Greenblatt R.M., Blaschke T.F.: *Sex differences in pharmacokinetics and pharmacodynamics*. *Annu. Rev. Pharmacol. Toxicol.* 44, 499-523, (2004).
- [17] Tanaka E.: *Gender-related differences in pharmacokinetics and their clinical significance*. *J. Clin. Pharm Ther.* 24(339-346), (1999).
- [18] Undevia S.D., Gomez-Abuin G., Ratain M.J.: *Pharmacokinetic variability of anticancer agents*. *Nat. Rev. Cancer* 5(6), 447-458, (2005).
- [19] Gurwitz D., Lunshof J.E., Altman R.B.: *A call for the creation of personalized medicine databases*. *Nat Rev Drug Discov.* 5(1), 23-26, (2006).
- [20] Ulrich C.M., Robien K., McLeod H.L.: *Cancer pharmacogenetics: polymorphisms, pathways and beyond*. *Nat. Rev. Cancer* 3(12), 912-920, (2003).
- [21] Efferth T., Volm M.: *Pharmacogenetics for individualized cancer chemotherapy*. *Pharmacol. Ther.* 107(2), 155-176, (2005).
- [22] Sadee W., Dai Z.: *Pharmacogenetics/genomics and personalized medicine*. *Hum Mol Genet.* 14 Spec No. 2, R207-214, (2005).
- [23] Tsalic M., Bar-Sela G., Beny A., Visel B., Haim N.: *Severe toxicity related to the 5-fluorouracil/leucovorin combination (the Mayo Clinic regimen): a prospective study in colorectal cancer patients*. *Am. J. Clin. Oncol.* 26(1), 103-106, (2003).
- [24] Milano G., Etienne M.C., Pierrefite V., Barberi-Heyob M., Deporte-Fety R., Renee N.: *Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity*. *Br. J. Cancer* 79(3-4), 627-630, (1999).
- [25] Chansky K., Benedetti J., Macdonald J.S.: *Differences in toxicity between men and women treated with 5-fluorouracil therapy for colorectal carcinoma*. *Cancer.* 103(6), 1165-1171, (2005).
- [26] Sloan J.A., Loprinzi C.L., Novotny P.J., Okuno S., Nair S., Barton D.L.: *Sex differences in fluorouracil-induced stomatitis*. *J. Clin. Oncol.* 18(2), 412-420, (2000).
- [27] Stein B.N., Petrelli N.J., Douglass H.O., Driscoll D.L., Arcangeli G., Meropol N.J.: *Age and sex are independent predictors of 5-fluorouracil toxicity. Analysis of a large scale phase III trial*. *Cancer* 75(1), 11-17, (1995).
- [28] Payne S.: *'Smoke like a man, die like a man?': a review of the relationship between gender, sex and lung cancer*. *Soc. Sci. Med.* 53(8), 1067-1080, (2001).
- [29] Liaw C.C., Wang C.H., Chang H.K., Liao C.T., Yeh K.Y., Huang J.S., Lin Y.C.: *Gender discrepancy observed between chemotherapy-induced emesis and hiccups*. *Support Care Cancer.* 9(6), 435-441, (2001).
- [30] Liaw C.C., Chang H.K., Liao C.T., Huang J.S., Lin Y.C., Chen J.S.: *Reduced maintenance of complete protection from emesis for women during chemotherapy cycles*. *Am. J. Clin. Oncol.* 26(1), 12-15, (2003).
- [31] Hesketh P.J., Gandara D.R., Hesketh A.M., Facada A., Perez E.A., Webber L.M.: *Dose-ranging evaluation of the antiemetic efficacy of intravenous dolasetron in patients receiving chemotherapy with doxorubicin or cyclophosphamide*. *Support Care Cancer* 4(2), 141-146, (1996).
- [32] Dobbs N.A., Twelves C.J., Gillies H., James C.A., Harper P.G., Rubens R.D.: *Gender affects doxorubicin pharmacokinetics in patients with normal liver biochemistry*. *Cancer Chemother. Pharmacol.* 36(6), 473-476, (1995).
- [33] Kaul S., Srinivas N.R., Mummaneni V., Igwemezie L.N., Barbhaya R.H.: *Effects of gender, age, and race on the pharmacokinetics of etoposide after intravenous administration of etoposide phosphate in cancer patients*. *Semin Oncol.* 23(6 Suppl 13), 23-29, (1996).
- [34] Morris M.E., Lee H.J., Predko L.M.: *Gender differences in the membrane transport of endogenous and exogenous compounds*. *Pharmacol. Rev.* 55(2), 229-240, (2003).
- [35] Milano G., Etienne M.C., Cassuto-Viguiere E., Thyss A., Santini J., Frenay M., Renee N., Schneider M., et al.: *Influence of sex and age on fluorouracil clearance*. *J. Clin. Oncol.* 10(7), 1171-1175, (1992).
- [36] Godfrey C., Sweeney K., Miller K., Hamilton R., Kremer J.: *The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis*. *Br. J. Clin. Pharmacol.* 46(4), 369-376, (1998).
- [37] Wall A.M., Gajjar A., Link A., Mahmoud H., Pui C.H., Relling M.V.: *Individualized methotrexate dosing in children with relapsed acute lymphoblastic leukemia*. *Leukemia* 14(2), 221-225, (2000).
- [38] Gallo J.M., Laub P.B., Rowinsky E.K., Grochow L.B., Baker S.D.: *Population pharmacokinetic model for topotecan derived from phase I clinical trials*. *J. Clin. Oncol.* 18(12), 2459-2467, (2000).
- [39] Loos W.J., Gelderblom H.J., Verweij J., Brouwer E., de Jonge M.J., Sparreboom A.: *Gender-dependent pharmacokinetics of topotecan in adult patients*. *Anticancer Drugs* 11(9), 673-680, (2000).

- [40] Grochow L.B. *Individualized dosing of anticancer drugs and the role of therapeutic monitoring*, Lippincott, Williams and Wilkins, Baltimore, MD, (1998).
- [41] Wade J.R., Kelman A.W., Kerr D.J., Robert J., Whiting B.: *Variability in the pharmacokinetics of epirubicin: a population analysis*. *Cancer Chemother. Pharmacol.* 29(5), 391-395, (1992).
- [42] Nagasubramanian R., Innocenti F., Ratain M.J.: *Pharmacogenetics in cancer treatment*. *Annu. Rev. Med.* 54(437-452), (2003).
- [43] Innocenti F., Ratain M.J.: *Update on pharmacogenetics in cancer chemotherapy*. *Eur. J. Cancer* 38(5), 639-644, (2002).
- [44] Maring J.G., Groen H.J., Wachters F.M., Uges D.R., de Vries E.G.: *Genetic factors influencing pyrimidine-antagonist chemotherapy*. *Pharmacogenomics J.* 5(4), 226-243, (2005).
- [45] Pinedo H.M., Peters G.F.: *Fluorouracil: biochemistry and pharmacology*. *J. Clin. Oncol.* 6(10), 1653-1664, (1988).
- [46] van Kuilenburg A.B., Meinsma R., Zonnenberg B.A., Zoetekouw L., Baas F., Matsuda K., Tamaki N., van Gennip A.H. *Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity*. *Clin. Cancer Res.* 9(12), 4363-4367, (2003).
- [47] van Kuilenburg A.B., Haasjes J., Richel D.J., Zoetekouw L., Van Lenthe H., De Abreu R.A., Maring J.G., Vreken P., et al.: *Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene*. *Clin. Cancer Res.* 6(12), 4705-4712, (2000).
- [48] Pratt S., Shepard R.L., Kandasamy R.A., Johnston P.A., Perry W., 3rd, Dantzig A.H.: *The multidrug resistance protein 5 (ABCC5) confers resistance to 5-fluorouracil and transports its monophosphorylated metabolites*. *Mol. Cancer Ther.* 4(5), 855-863, (2005).
- [49] Rinn J.L., Snyder M.: *Sexual dimorphism in mammalian gene expression*. *Trends Genet.* 21(5), 298-305, (2005).
- [50] Dhir R.N., Dworakowski W., Thangavel C., Shapiro B.H.: *Sexually dimorphic regulation of hepatic isoforms of human cytochrome p450 by growth hormone*. *J. Pharmacol. Exp. Ther.* 316(1), 87-94, (2006).
- [51] Wolbold R., Klein K., Burk O., Nussler A.K., Neuhaus P., Eichelbaum M., Schwab M., Zanger U.M.: *Sex is a major determinant of CYP3A4 expression in human liver*. *Hepatology* 38(4), 978-988, (2003).
- [52] Merino G., van Herwaarden A.E., Wagenaar E., Jonker J.W., Schinkel A.H.: *Sex-dependent expression and activity of the ATP-binding cassette transporter breast cancer resistance protein (BCRP/ABCG2) in liver*. *Mol. Pharmacol.* 67(5), 1765-1771, (2005).
- [53] Simon F.R., Fortune J., Iwahashi M., Qadri I., Sutherland E.: *Multihormonal regulation of hepatic sinusoidal Ntcp gene expression*. *Am J Physiol Gastrointest Liver Physiol.* 287(4), G782-794, (2004).
- [54] Simon F.R., Fortune J., Iwahashi M., Sutherland E.: *Sexual dimorphic expression of ADH in rat liver: importance of the hypothalamic-pituitary-liver axis*. *Am J. Physiol. Gastrointest Liver Physiol.* 283(3), G646-655, (2002).
- [55] Rost D., Kopplow K., Gehrke S., Mueller S., Friess H., Ittrich C., Mayer D., Stiehl A.: *Gender-specific expression of liver organic anion transporters in rat*. *Eur. J. Clin. Invest.* 35(10), 635-643, (2005).
- [56] DeLeve L.D. *Liver function and hepatotoxicity in cancer*, B.C. Decker Inc., Hamilton, Ontario, (2000).
- [57] Zhang L., Brett C.M., Giacomini K.M.: *Role of organic cation transporters in drug absorption and elimination*. *Annu. Rev. Pharmacol. Toxicol.* 38(431-460), (1998).
- [58] Kato R., Yamazoe Y.: *Sex-specific cytochrome P450 as a cause of sex- and species-related differences in drug toxicity*. *Toxicol Lett.* 64-65 Spec No. 661-667, (1992).
- [59] Shimada T., Yamazaki H., Mimura M., Inui Y., Guengerich F.P.: *Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians*. *J. Pharmacol. Exp. Ther.* 270(1), 414-423, (1994).
- [60] Anderson G.D.: *Sex differences in drug metabolism: cytochrome P-450 and uridine diphosphate glucuronosyltransferase*. *J. Gen. Specif. Med.* 5(1), 25-33, (2002).
- [61] Hunt C.M., Westerkam W.R., Stave G.M.: *Effect of age and gender on the activity of human hepatic CYP3A*. *Biochem. Pharmacol.* 44(2), 275-283, (1992).
- [62] Davis M.: *Gender differences in p-glycoprotein: drug toxicity and response*. *J. Clin. Oncol.* 23(26), 6439-6440, (2005).
- [63] Tucker G.T., Houston J.B., Huang S.M.: *Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential-toward a consensus*. *Clin. Pharmacol. Ther.* 70(2), 103-114, (2001).
- [64] Huang Y., Sadee W.: *Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells*. *Cancer Lett.* 239(2), 168-182, (2006).
- [65] van Asperen J., van Tellingen O., Beijnen J.H.: *The role of mdr1a P-glycoprotein in the biliary and intestinal secretion of doxorubicin and vinblastine in mice*. *Drug Metab. Dispos.* 28(3), 264-267, (2000).
- [66] van Asperen J., van Tellingen O., Tijssen F., Schinkel A.H., Beijnen J.H.: *Increased accumulation of doxorubicin and doxorubicinol in cardiac tissue of*

- mice lacking *mdr1a* P-glycoprotein. *Br. J. Cancer* 79(1), 108-113, (1999).
- [67] Schuetz E.G., Furuya K.N., Schuetz J.D.: *Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms*. *J. Pharmacol. Exp. Ther.* 275(2), 1011-1018, (1995).
- [68] Paine M.F., Ludington S.S., Chen M.L., Stewart P.W., Huang S.M., Watkins P.B.: *Do men and women differ in proximal small intestinal CYP3A or P-glycoprotein expression?* *Drug Metab. Dispos.* 33(3), 426-433, (2005).
- [69] Kim W.Y., Benet L.Z.: *P-glycoprotein (P-gp/MDR1)-mediated efflux of sex-steroid hormones and modulation of P-gp expression in vitro*. *Pharm. Res.* 21(7), 1284-1293, (2004).
- [70] Mutoh K., Tsukahara S., Mitsuhashi J., Katayama K., Sugimoto Y.: *Estrogen-mediated post transcriptional down-regulation of P-glycoprotein in MDR1-transduced human breast cancer cells*. *Cancer Sci.* 97(11), 1198-1204, (2006).
- [71] Frohlich M., Albermann N., Sauer A., Walter-Sack I., Haefeli W.E., Weiss J.: *In vitro and ex vivo evidence for modulation of P-glycoprotein activity by progestins*. *Biochem. Pharmacol.* 68(12), 2409-2416, (2004).
- [72] Imai Y., Ishikawa E., Asada S., Sugimoto Y.: *Estrogen-mediated post transcriptional down-regulation of breast cancer resistance protein/ABCG2*. *Cancer Res.* 65(2), 596-604, (2005).
- [73] Nicchia G.P., Frigeri A., Nico B., Ribatti D., Svelto M.: *Tissue distribution and membrane localization of aquaporin-9 water channel: evidence for sex-linked differences in liver*. *J. Histochem. Cytochem.* 49(12), 1547-1556, (2001).
- [74] Tsukaguchi H., Shayakul C., Berger U.V., Mackenzie B., Devidas S., Guggino W.B., van Hoek A.N., Hediger M.A.: *Molecular characterization of a broad selectivity neutral solute channel*. *J. Biol. Chem.* 273(38), 24737-24743, (1998).
- [75] Rinn J.L., Rozowsky J.S., Laurenzi I.J., Petersen P.H., Zou K., Zhong W., Gerstein M., Snyder M.: *Major molecular differences between mammalian sexes are involved in drug metabolism and renal function*. *Dev. Cell* 6(6), 791-800, (2004).
- [76] Yang X., Schadt E.E., Wang S., Wang H., Arnold A.P., Ingram-Drake L., Drake T.A., Lusis A.J.: *Tissue-specific expression and regulation of sexually dimorphic genes in mice*. *Genome Res.* 16(8), 995-1004, (2006).
- [77] Clodfelter K.H., Holloway M.G., Hodor P., Park S.H., Ray W.J., Waxman D.J.: *Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis*. *Mol. Endocrinol.* 20(6), 1333-1351, (2006).
- [78] Delongchamp R.R., Velasco C., Dial S., Harris A.J.: *Genome-wide estimation of gender differences in the gene expression of human livers: statistical design and analysis*. *BMC Bioinformatics* 6 (Suppl2), S13, (2005).
- [79] Goldstein D.B.: *The genetics of human drug response*. *Philos Trans. R. Soc. Lond. B. Biol. Sci.* 360(1460), 1571-1572, (2005).
- [80] Weiss L.A., Abney M., Cook E.H., Jr., Ober C.: *Sex-specific genetic architecture of whole blood serotonin levels*. *Am. J. Hum. Genet.* 76(1), 33-41, (2005).
- [81] Kurina L.M., Weiss L.A., Graves S.W., Parry R., Williams G.H., Abney M., Ober C.: *Sex differences in the genetic basis of morning serum cortisol levels: genome-wide screen identifies two novel loci specific to women*. *J. Clin. Endocrinol. Metab.* 90(8), 4747-4752, (2005).