Pharmacogenetics and personalized medicine: Does gender have a role?

[1]**Anthony G. Fenech** BPharm (Hons), MPhil, PhD (Nott)
[2]**Godfrey Grech** BSc, MPhil, PhD (Erasmus)

[1]Department of Clinical Pharmacology & Therapeutics,
Faculty of Medicine & Surgery, University of Malta
Email: anthony.fenech@um.edu.mt
[2]Department of Pathology, Faculty of Medicine & Surgery, University of Malta
Email: godfrey.grech@um.edu.mt

Educational aims

- To increase awareness of the contribution of gender to therapeutic outcomes
- To discuss the interaction of gender with drug pharmacogenetics
- To gain an appreciation of the genetic contribution to personalized medicine

Key words
Pharmacogenetics, gender, polymorphisms, drug response

Abstract

The study of the role of genetic polymorphisms in drug responses, is now a firmly established field of pharmacology research. It has robust applications in predicting drug effect, and therefore contributes to the process of optimum selection of drug and dose for specific patients. Since the last 10 years, the FDA as well as the EMA have set up their own pharmacogenomics advisory groups, and have flagged an increasing number of medicinal products with specific genotyping recommendations in order to reap their greatest benefit. The contribution of gender to therapeutic outcomes has long been recognised, but recent research suggests that gender influence may not only occur via well recognised hormonal pathways, but also via direct non-hormone-mediated mechanisms. This influence may confound pharmacogenetic predictors, and gender stratification may therefore be an important consideration in pharmacogenetic-based drug trials.

Introduction

As early as 1993, in the "Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs" the FDA encouraged the participation of women in clinical trials and recommended that "sponsors should collect gender-related data during research and development and should analyze the data for gender effects."[1] However a scan of the literature indicates that clinical trials carried out during drug development phases have rarely considered gender as a stratification parameter in therapeutic outcome investigations. Evidence supporting gender-specific therapeutic outcomes, mainly stems from Phase IV post-marketing studies. The gradual but expanding trend towards rationalized medicine, is however set to change the "one size fits all" paradigm to a scenario where specific patient parameters, including genetic factors and gender, form part of a treatment algorithm which is optimized on a patient-per-patient platform. This is the basis of personalized medicine.

Gender and drug response

Gender may modify therapeutic drug outcomes through pharmacokinetic, pharmacodynamic as well as pharmacogenetic mechanisms. From a pharmacokinetic aspect, gender differences in metabolic enzyme activities is a well-known phenomenon. For example, the anti-anxiety and sedative drug midazolam, as well as verapamil (a calcium channel blocker) both show higher bioavailability in females than males[2,3] due to gender specific variations in several CYP450 enzyme expression levels; while the selective α_2-adrenergic agonist azepexole shows a higher degree of venoconstriction in men than in women, despite various non-gender-specific single nucleotide polymorphisms (SNPs) in the ADRA2B gene which codes for its target, the α_{2b}-adrenergic receptor.[4]

Pharmacogenetics and gender

Polymorphic variation in the human genome is recognised to contribute to a profound effect on the therapeutic and adverse effects of several drugs. Drug outcomes may be influenced by variation in genes which code for drug receptors, metabolic enzymes, membrane transporters, signalling molecules or any other proteins which influence any aspect of drug mechanisms and actions. For example, polymorphic variability in the *CYP2D6* gene, may influence the outcomes of more than 70 drugs by modifying their rate of metabolism. Fast CYP2D6 metabolisers may not respond adequately to CYP2D6 substrates such as desipramine and nortriptyline (tricyclic antidepressants), carvedilol and metoprolol (β-blockers), clozapine (an antipsychotic), fleicainide (an anti-arrhythmic), tramadol (an opioid analgesic) and others, while slow metabolizers may develop toxic effects at conventional therapeutic doses. Codeine, is a rather special case, since CYP2D6 contributes to its metabolism to morphine, and therefore *fast* CYP2D6 metabolizers may show opioid *toxicity* with conventional doses of codeine.[5]

The influence of gender on pharmacogenetics has just started to be debated in the scientific literature. Its contribution as well as the mechanisms through which it exerts it, are complex and still not well understood.

Firstly, gender may exert effects on therapeutic outcome, in a manner which is independent of pharmacogenetic contributions. For example, multiple regression analysis of variability in S-oxazapam (an anti-anxiety benzodiazepine drug) glucoronidation, attributes about 35% of variability to presence of the D85Y genotype of the hepatically-expressed *UGT2B15* (UDP Glucuronosyltransferase 2 Family, Polypeptide B15) gene, while an additional 14% is attributed to gender. Males expresses generally higher activities than females, and presence of D85Y variant results in a low activity enzyme.[6] Similarly, the presence of the [PvuII- / XbaI+] haplotype in the *ESR1* (oestrogen receptor 1) gene contributes to a greater post-atorvastatin HDL increase in women than in men; however multiple regression analysis suggests that these genotype and gender influences operate independently of each other. Indeed, a greater post-atorvastatin HDL increase can still be identified in a non-gender stratified analysis of the *ESR1* [PvuII- / XbaI+] haplotype.[7] Torasemide, a drug used for the management of heart failure, is a substrate for CYP2C9 metabolism and the SLCO1B1 membrane transporter and plasma concentrations of the drug depend on the genotypic contributions of these genes. In addition, female gender has also been determined to be a predictor of higher plasma torasemide concentrations. Factorial ANOVA analysis has however indicated that the contributions these genes and gender, occur independently of each other,[8] therefore indicating that a separate mechanism must be in operation for the gender influence.

Secondly, gender may exert effects which operate within a pharmacogenetic framework. Since very few known pharmacogenetically-relevant gene variants are located on any of the sex chromosomes, gender-dependent pharmacogenetic differences are therefore likely to be due to male/female differences in the autosome, in transcriptional regulation or in post-transcriptional changes. More specifically, this may relate to (a) hormonal-dependent gene expression alterations, (b) gender-dependent differences in allelic frequencies of specific polymorphisms; sexually dimorphic pharmacogenetic allelic frequencies manifest as a gender-dependent skewness in the distribution of these polymorphisms within a population, and therefore may contribute to gender-biased therapy outcomes, and (c) physiological and/or psychological male/female differences which contribute to particular behavioural phenotypes, especially in the case of drugs which act on the central nervous system. Such differences may cause the same pharmacogenetic genotype to generate what may appear to be a different phenotype in males and females, but what in reality may be the result of an independent separate male/female characteristic which modifies the phenotype expression of the pharmacogene. For example, males and females may perceive pain differently, and therefore a single genotype which influences analgesic efficacy may have different influences in males and females carrying the same allele due to this psychological effect. Nortriptyline pharmacokinetics are known to be strongly influenced by the metabolic enzyme CYP2D6, and genotyping of the *CYP2D6* gene can be used to help provide clinical dose optimization.[9] However female patients carrying the same CYP2D6 genotype as their male counterparts, may require lower doses in order to attain the same clinical effect, and this is thought to be due to physiological effects of oestrogen on the brain.[10] The cyclic variations in oestrogen secretion may further compound these issues. Dorado et al., (2012)[11] later reported gender-dependent differences in CYP2C9-dependent hydroxylation of the angiotensin II receptor antagonist losartan(an antihypertensive), to its more active metabolite E3174, with females expressing a higher losartan:E3174 ratio than males, post administration of a single 25mg losartan dose. However it is not clear whether this observation could be attributed to differences in male/female frequencies of specific CYP2C9 genotypes, and whether other confounding factors such as bodyweight, were taken into consideration in this study.

Emerging data suggests that several classically recognised pharmacogenetic genotype/phenotype associations may need to be reanalysed in a gender-stratified manner, in order to understand the gender contribution, and better qualify the applicability of such associations to the field of personalized medicine.

Gender-dependent gene expression

Evidence for significant gender-dependent influences on gene expression is accumulating. Microarray-based work on murine RNA expression levels in several somatic tissues, revealed several thousand genes to exhibit sexually dimorphic expression in liver, fat and muscle, while several hundred exhibited sexually dimorphic expression in brain tissue.[12,13] Such dimorphic expression has also been shown using *in vitro* generated bovine blastocytes, where one third of RNA transcripts have been shown to be expressed at different levels in males and females.[14,15] This provides evidence to indicate that significant male/female differences in gene expression, may occur at a very early stage in development, and in the absence of hormonal influences. Such events also occur in humans.[16] The mechanisms by which the sex chromosomes impose this extensive transcriptional regulation upon

autosomal genes are yet unclear. In adult humans, gender-dependent gene expression has been detected in various tissues, such as the brain[17,18], salivary glands[19], heart[20] and liver CYP450 enzymes.[21]

The cytochrome P450 enzyme CYP1B1 is expressed at higher transcript levels in Caucasian females than males, and this effect is not due to CYP1B1 genotype. Therefore CYP1B1 substrates, such as the chemotherapeutic agents docetaxel, epirubicin, capecitabine, paclitaxel, gemcitabine and cisplatin may have higher rates of metabolism in females.[22] Moreover, this gender-dependent gene expression effect, may co-exist with other CYP1B1 polymorphisms, creating a phenotype for which the genetic contributions are difficult to dissect.

Sexually dimorphic allelic distribution

Alcohol provides an excellent example of a drug which has a gender-dependent pharmacogenetic profile. A study carried out in Korean alcoholic patients, found alcohol dependence in men to be associated with a significantly higher frequency of alleles *1/1 of the aldehyde dehydrogenase 2 gene (*ALDH2*) and *1/1 of the alcohol dehydrogenase gene (*ADH2*). However, in women, alcohol dependence was associated with a significantly higher frequency of a different allelic subset, namely *ADH2*1/1*, *ALDH2*1/2* and *ALDH2*2/2*. Moreover, the same study found the μ-opioid receptor gene (*OPRM1*) A118G allele, which is also recognised to contribute to a tendency towards alcohol dependence, to be present at a significantly higher frequency in alcohol-dependent females, than alcohol-dependent males.[23] The same research group had earlier found naltrexone, a μ-opioid receptor antagonist, to be more effective against alcohol dependence in patients who were genotyped A/G or G/G at the *OPRM1* 118 locus, than those whose genotype was A/A. This data suggests that naltrexone may be more effective in females with alcohol dependence, than males.[24]

The existence of gender-specific allelic frequencies, however, cannot always be considered to be independent of other variables. In some genes, sexual dimorphic distribution has been shown to be dependent on factors such as ethnicity. For example, the genes encoding 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and thymidylate synthase (*TYMS*) are recognised methotrexate (a drug used in the treatment of cancer and autoimmune diseases) pharmacogenes. Specifically, the *MTHFR* C677T and A1298C SNPs, and the *TYMS* 5'UTR [28bp tandem repeat]$_x$ and 3'UTR [6bp del/ins] polymorphisms are known to be important in methotrexate outcomes. Inoue et al., (2007)[25] identified a statistically significant male/female difference between the allelic frequency of the *TYMS* 3'-UTR in a Japanese population (p=0.015), but the other 3 variants did not appear to exhibit any sexual dimorphic distribution. Moreover none of the variants demonstrated sexual dimorphism in Caucasians.

Key points

- The understanding of genetically-influenced therapeutic outcomes is a critical component towards the development and application of personalized medicine.
- Gender may influence drug response, not simply through DNA sequences located on the sex chromosomes, but more commonly through gender-dependent variability present throughout the rest of the genome.
- The influence of gender on drug response, may act in isolation of other response-determining factors, but more commonly acts within a framework of pharmacogenetic determinants.
- During both preclinical and clinical drug development phases, there is an increased need to address gender as a potential variable in the assessment of therapeutic outcomes and adverse reactions.
- Prescribers should be aware of drugs which are reported to exhibit a degree of gender-dependent outcomes.

Conclusion

Pre-clinical and clinical trial design may often mask the effects of gender on drug response. The gender distribution of animals and human volunteers used is often not amenable to study such effects, and *in vitro* cell-based models are usually considered to be sex-less. The relevance of this variable is however now coming to light. Clinical studies need to be carried out in a way that makes them sensitive to detect gender-specific variations. Several commonly used drugs have been denied this opportunity. Adrenergic β_2 receptors, the product of the ADRB2 gene, demonstrate a higher ligand sensitivity in women, compared to men, and this effect may compound known functional ADRB2 gene polymorphisms. Adrenergic β_1 blockers such as metoprolol and propranolol, are sensitive to gender-dependent CYP2D6 metabolic activity. Statins also exert different therapeutic outcomes in males and females, but there is disagreement on the precise underlying mechanisms. Common drug adverse effects, such as hypokalaemia, hyponatraemia, nausea, vomiting, anti-coagulant induced bleeding and antipsychotic drug-induced weight gain are also more common in women than in men. Pharmacogenetics contributes to several adverse effects but is often unable explain the unequal gender-distribution of these reactions.[26] Clinical studies having a gender-sensitive design are required. Personalized medicine is a challenging target. A pre-requisite to its application is the unravelling and understanding of variables that contribute to inter-patient differences. Pharmacogenetics and gender are undoubtedly two major candidates.

References

1. FDA. Evaluation of Gender Differences in Clinical Investigations - Information Sheet. Guidance for Institutional Review Boards and Clinical Investigators. Available at: http://www.fda.gov/RegulatoryInformation/Guidances/ucm126552.htm. Accessed 10 May 2014.
2. Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara Jr EM, Hall SD. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. Clin Pharmacol Ther 1998:64:133–43.
3. Krecic-Shepard ME, Barnas CR, Slimko J, Jones MP, Schwartz JB. Gender-specific effects on verapamil pharmacokinetics and pharmacodynamics in humans. J Clin Pharmacol 2000:40:219–30.
4. King D, Etzel JP, Chopra S, Smith J, Cadman PE, Rao F, Funk SD, Rana BK, Schork NJ, Insel PA, O'Connor DT. Human response to alpha2-adrenergic agonist stimulation studied in an isolated vascular bed in vivo: Biphasic influence of dose, age, gender, and receptor genotype. Clin Pharmacol Ther. 2005: 77(5):388-403.
5. Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, Desmeules J. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med. 2004: 351(27):2827-31.

6. Court MH, Hao Q, Krishnaswamy S, Bekaii-Saab T, Al-Rohaimi A, von Moltke LL, Greenblatt DJ. UDP-glucuronosyltransferase (UGT) 2B15 pharmacogenetics: UGT2B15 D85Y genotype and gender are major determinants of oxazepam glucuronidation by human liver. J Pharmacol Exp Ther. 2004 Aug;310(2):656-65.
7. Kajinami K, Brousseau ME, Lamon-Fava S, Ordovas JM, Schaefer EJ. Gender-specific effects of estrogen receptor alpha gene haplotype on high-density lipoprotein cholesterol response to atorvastatin: interaction with apolipoprotein AI gene polymorphism. Atherosclerosis. 2005 Feb;178(2):331-8.
8. Werner D, Werner U, Meybaum A, Schmidt B, Umbreen S, Grosch A, Lestin HG, Graf B, Zolk O, Fromm MF. Determinants of steady-state torasemide pharmacokinetics: impact of pharmacogenetic factors, gender and angiotensin II receptor blockers. Clin Pharmacokinet. 2008;47(5):323-32.
9. Hicks JK, Swen JJ, Thorn CF, Sangkuhl K, Kharasch ED, Ellingrod VL, Skaar TC, Müller DJ, Gaedigk A, Stingl JC. Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. Clin Pharmacol Ther. 2013: 93(5):402-8.
10. Piatkov I, Jones T. Pharmacogenetics and gender association with psychotic episodes on nortriptyline lower doses: patient cases. ISRN Pharm. 2011;2011:805983. doi: 10.5402/2011/805983. Epub 2011 Jul 20.
11. Dorado P, Beltrán LJ, Machín E, Peñas-Lledó EM, Terán E, Llerena A, CEIBA FP Consortium of the Ibero-American Network of Pharmacogenetics and Pharmacogenomics RIBEF. Losartan hydroxylation phenotype in an Ecuadorian population: influence of CYP2C9 genetic polymorphism, habits and gender. Pharmacogenomics 2012: 13(15):1711-7.
12. Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ. Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Res. 2006: 16(8):995-1004.
13. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol. 2009: 76(2):215-28.
14. Bermejo-Alvarez P, Rizos D, Rath D, Lonergan P, Gutierrez-Adan A. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. Proc Natl Acad Sci USA. 2010: 107(8):3394-9.
15. Itoh Y, Arnold AP. X chromosome regulation of autosomal gene expression in bovine blastocysts. Chromosoma. 2014 May 13. [Epub ahead of print]
16. Bourc'his D, Proudhon C. Sexual dimorphism in parental imprint ontogeny and contribution to embryonic development. Mol Cell Endocrinol. 2008: 282(1-2):87-94.
17. Davies W, Wilkinson LS. It is not all hormones: alternative explanations for sexual differentiation of the brain. Brain Res. 2006 Dec 18;1126(1):36-45.
18. Trabzuni D, Ramasamy A, Imran S, Walker R, Smith C, Weale ME, Hardy J, Ryten M, North American Brain Expression Consortium. Widespread sex differences in gene expression and splicing in the adult human brain. Nat Commun. 2013: 4:2771.
19. Michael D, Soi S, Cabera-Perez J, Weller M, Alexander S, Alevizos I, Illei G, Chiorini J. Microarray analysis of sexually dimorphic gene expression in human minor salivary glands. Oral Dis. 2011: 17(7):653-61.
20. Isensee J, Witt H, Pregla R, Hetzer R, Regitz-Zagrosek V, Noppinger PR. Sexually dimorphic gene expression in the heart of mice and men. J Mol Med (Berl). 2008 Jan;86(1):61-74.
21. Thangavel C, Boopathi E, Shapiro BH. Intrinsic sexually dimorphic expression of the principal human CYP3A4 correlated with suboptimal activation of GH/glucocorticoid-dependent transcriptional pathways in men. Endocrinology. 2011: 152(12):4813-24.
22. Finnström N, Ask B, Dahl ML, Gadd M, Rane A. Intra-individual variation and sex differences in gene expression of cytochromes P450 in circulating leukocytes. Pharmacogenomics J. 2002: 2(2):111-6.
23. Kim SG. Gender differences in the genetic risk for alcohol dependence--the results of a pharmacogenetic study in Korean alcoholics. Nihon Arukoru Yakubutsu Igakkai Zasshi 2009: 44(6):680-5.
24. Kim SG, Kim CM, Choi SW, Jae YM, Lee HG, Son BK, Kim JG, Choi YS, Kim HO, Kim SY, Oslin DW. A mu opioid receptor gene polymorphism (A118G) and naltrexone treatment response in adherent Korean alcohol-dependent patients. Psychopharmacology (Berl). 2009: 201(4):611-8.
25. Inoue S, Hashiguchi M, Chiyoda T, Sunami Y, Tanaka T, Mochizuki M.Pharmacogenetic study of methylenetetrahydrofolate reductase and thymidylate synthase in Japanese and assessment of ethnic and gender differences. Pharmacogenomics. 2007 Jan;8(1):41-7.
26. Franconi F, Campesi I. Pharmacogenomics, pharmacokinetics and pharmacodynamics: interaction with biological differences between men and women. Br J Pharmacol. 2014: 171(3):580-94.