Pharmacogenetics of breast cancer therapies

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Abstract

Treatment decisions for breast cancer patients are currently based on a small number of crude predictive markers, despite the known complexity and heterogeneity of the disease. The field of pharmacogenetics can increase the precision with which therapeutic decisions are made. Discovering associations between genetic variation and treatment response will allow clinicians to tailor therapies to most effectively treat that specific tumor in that patient. In this review we outline two genes with potential clinical relevance in breast cancer treatment: A common polymorphism in the gene encoding Fc fragment of IgG low affinity IIIa receptor (FCGR3A; gene: FCGR3A) may substantially influence a patient's likelihood of responding to trastuzumab. The other gene that will be discussed in the review is cytochrome P450 2D6 (CYP2D6; gene: CYP2D6), which has many genetic variants that impair the bioactivation and effectiveness of tamoxifen therapy.

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Introduction

Breast cancer is associated with an enormous amount of morbidity and mortality worldwide. According to estimates by the World Health Organization International Agency for Research on Cancer approximately 1.29 million women were diagnosed, and over 400,000 women died, with breast cancer in 2008 worldwide.1 Treatment for breast cancer is constantly evolving as new technologies, therapies, and strategies are discovered. The individualization of breast cancer treatment promises to increase effectiveness and decrease drug related toxicities while mitigating health care costs.

Breast cancer is not a single disease, but a spectrum of related disease states. The genetic influences of breast cancer etiology are being explored by researchers in order to unlock the mysteries of effective cancer therapy. The first major breakthroughs were the identification of the estrogen (ER) and progesterone receptors (PRs).

Differentiating breast cancers, just on the basis of ER and PR status vastly improves treatment efficacy.2 ER-positive (ER+) tumors are dependent on estrogen signaling for their growth and replication. They are effectively treated by antiestrogen therapy with either tamoxifen or an aromatase inhibitor. ER-negative (ER−) tumors, on the other hand, are not estrogen dependent and will not respond to antiestrogens but are far more responsive to first line chemotherapeutic combinations such as paclitaxel followed by 5-FU, doxorubicin, and cyclophosphamide (T/FAC).3 Expression of the PR is highly correlated with ER expression. Although the effect of PR status on treatment seems to be less important than that of ER, low PR expression is a poor prognostic indicator in general.4

The other well characterized tumor marker is human epidermal growth factor receptor 2 (HER2/neu). HER2 has been identified as a marker of poor prognosis and increased cancer aggressiveness.5 The treatment of HER2+ tumors has changed significantly with the inclusion of trastuzumab, a monoclonal antibody with specificity for the HER2/neu receptor. Trastuzumab and HER2 will each be discussed in more detail later in this review.

With the advent of gene expression analysis, new tumor markers are being identified and validated. One tool that is finding mainstream acceptance is the OncotypeDX™ test. OncotypeDX™ is a prognostic tool to classify a node negative, ER+ breast cancer patient’s recurrence risk6 and likely benefit from chemotherapy.7 The assay employs a 16 gene algorithm, with five internal standard controls, to calculate a recurrence score on a scale of 0–100.8 OncotypeDX™ is one example of the use of genetic information to guide treatment decisions in breast cancer therapy. Assays which employ different genes to make predictions for other breast cancer populations exist. For a more complete review on genes, markers, and assays recommended by the American Society for Clinical Oncology (ASCO) see Harris et al. 2007.9

The field of pharmacogenetics investigates the link between a patient’s genetic blueprint and their response to drug therapy. The substitution of a nucleotide, called a single nucleotide polymorphism (SNP), can have a significant downstream impact upon the disposition of and/or response to a drug. SNPs that alter drug response are often found in the coding region of DNA and they can cause amino acid substitutions in the translated protein (a nonsynonymous SNP). The resulting variant protein can have
altered biological behavior relative to the wild type protein. It has also been demonstrated that a SNP in a coding region that does not change the amino acid sequence, a synonymous SNP, can impact protein folding kinetics and create an improperly folded and functionally different protein.\textsuperscript{10} Non-coding SNPs in the upstream promoter region of a gene can have a substantial influence by affecting the extent of DNA transcription and subsequent protein expression. Identifying SNPs that considerably alter the function or expression of proteins involved in the pharmacokinetics or pharmacodynamics of drugs and their ultimate effect on efficacy and safety is the aim of pharmacogenetics.

This review will look more closely at two genes with potential pharmacogenetic relevance; at different stages of development. The first gene encodes the Fc fragment of immunoglobulin G (IgG) low affinity IIIa receptor (FCGR3A; gene: FCGR3A). A nonsynonymous SNP in this gene encodes a variant protein which responds differently to trastuzumab. Evidence is accumulating that this could be a clinically relevant polymorphism; however, improved outcomes have not been reproduced in multiple patient cohorts. The other gene is cytochrome P450 2D6 (CYP2D6; gene: CYP2D6). The evidence supports a clinically relevant association between CYP2D6 polymorphisms and tamoxifen outcome. At this time the clinical benefit of genotyping patients before tamoxifen treatment is unknown.

**Trastuzumab and FCGR3A**

Trastuzumab is approved for use in HER2 overexpressing metastatic breast cancer. It is used as a first line treatment in combination with paclitaxel, or as a single agent in the second line treatment of patients who have failed prior chemotherapy. Approximately 20\% of breast tumors overexpress the HER2 receptor, however, only 25–30\% of these tumors respond to trastuzumab therapy.\textsuperscript{11} It would be extremely beneficial to find markers that can predict which patients are more likely to respond to trastuzumab treatment.

Fragment Fc gamma receptors enable immune effector cells, such as macrophages, natural killer cells, and neutrophils, to bind to the Fc portion of IgG antibodies that are attached to invading antigens.\textsuperscript{12} This binding triggers the effector cell to kill the unwelcome invader in a process termed antibody-dependent cell-mediated cytotoxicity (ADCC). The receptor subtypes are differentially expressed on effector cells; macrophages express activating FCGR2A (CD32) and FCGR3A (CD16), while natural killer cells express only the CD16.

A nonsynonymous SNP of FCGR3A causes an amino acid substitution at position 158 (rs396991; current genome build (36.3)) maps SNP to R166H. A common nonsynonymous SNP in the gene encoding fragment of Fc gamma receptor IIIa (FCGR3A).\textsuperscript{15,16} This SNP affects the function of the protein. Non-coding SNPs in the upstream promoter region of a gene can have a substantial influence by affecting the extent of DNA transcription and subsequent protein expression. Identifying SNPs that considerably alter the function or expression of proteins involved in the pharmacokinetics or pharmacodynamics of drugs and their ultimate effect on efficacy and safety is the aim of pharmacogenetics.

**Anti-CD20 rituximab** was the first monoclonal antibody in which the impact of FCGR3A and FCGR2A SNPs were clinically investigated. Two separate studies in lymphoma patients demonstrated improved survival for FCGR3A V/V patients.\textsuperscript{25} Only one of those studies found an independent improvement in survival for FCGR2A H/H patients.\textsuperscript{21} A third lymphoma trial, by Kim et al.,\textsuperscript{24} included study and control groups treated with the same combination regimen aside from the administration of rituximab to the study cohort but not the control group. Significantly improved response was seen in patients with the FCGR3A V/V genotype, compared to F carriers, only in the study cohort; indicating that the SNP specifically influences rituximab treatment as opposed to disease prognosis.

One published breast cancer clinical trial has evaluated genetic associations between FCGR3A F158V genotype and clinical outcomes to trastuzumab-based therapy.\textsuperscript{25} Trastuzumab and paclitaxel treated patients with the V/V genotype achieved higher rates of objective response (Fig. 1) and had prolonged progression free survivals when compared to F/F patients. This survival benefit was not seen in a control cohort of HER2– patients treated with only paclitaxel. A correlation between ADCC in vitro activation and response to trastuzumab treatment was also reported; however, the ADCC measure was normalized for the prevalence of effector cells, thus demonstrating ADCC activation efficiency, not true ADCC response.

Overall, these studies support a potential association between an FCGR3A SNP and effectiveness of monoclonal antibody therapy that is treatment related, not prognostic. While the preponderance of evidence supports a clinically relevant association in rituximab and lymphoma treatment, these findings cannot necessarily be extended to trastuzumab and breast cancer, particularly in light of the lack of association observed by other FCGR3A pharmacogenetic monoclonal antibody cancer therapy studies.\textsuperscript{26,27}

One issue that is yet to be fully addressed is the impact of FCGR2A H131R. Significant treatment effects of this polymorphism alone\textsuperscript{28} or in combination with FCGR3A F158V\textsuperscript{24,25} were reported before it was determined that the two SNPs are in linkage disequilibrium.\textsuperscript{29} Results of these previous studies are now inconclusive as to the true impact of the FCGR2A SNP on treatment outcomes.\textsuperscript{30}

Aside from answering the outstanding pharmacological questions, there is a long way to go before genotyping the FCGR3A and FCGR2A SNPs can be recommended for patients receiving trastuzumab. Data from larger HER2+ breast cancer patient cohorts must replicate the findings of the above mentioned studies of limited sample size,\textsuperscript{31,25} followed by a prospective study investigating the clinical utility of such a genetic test before this test is used to guide therapy decisions.
Tamoxifen and CYP2D6

Most malignant breast tissue is ER+ (75%) and its growth is stimulated by estradiol. Selective estrogen receptor modulators (SERMs) inhibit estrogen binding to ERs in breast tissue, reducing or eliminating estrogen-driven proliferation of ER+ tumors and are therefore effective therapies for the treatment of breast cancer.31 Antiestrogen therapy, including SERMs or aromatase inhibitors, is recommended for ER+, but not for ER− breast cancers.32 Tamoxifen, a SERM, is the most widely used antiestrogen therapy for pre- and postmenopausal women with metastatic breast cancer, adjuvant treatment of primary breast cancer, and as a chemopreventive agent for women with a high risk of developing breast cancer.

The metabolism of tamoxifen is complex, undergoing Phase I and II reactions. It is oxidized to N-desmethyltamoxifen and 4-hydroxytamoxifen by CYP3A4/5 and CYP2D6, respectively, and both primary metabolites are converted to endoxifen.33 Endoxifen formation from N-desmethyltamoxifen is almost exclusively catalyzed by CYP2D6 and from 4-hydroxytamoxifen by CYP3A4/5.3,31 The antiestrogenic activities of endoxifen and 4-hydroxytamoxifen are comparable and are considerably greater than those of tamoxifen.34–37 The ~5–10 fold higher plasma concentrations of endoxifen compared with 4-hydroxytamoxifen suggest endoxifen is largely responsible for the in vivo activity of tamoxifen.3,34,38

The CYP2D6 gene (CYP2D6) on chromosome 22 is subject to several genetic polymorphisms that affect its enzyme activity.32,33 Impaired oxidative metabolism of CYP2D6 substrates is inherited in a Mendelian (monogenic) fashion and is an autosomal recessive trait.40 Six to 10% of Caucasians have two null CYP2D6 alleles (poor metaboliser [PM] alleles, e.g. CYP2D6*4) and are deficient in CYP2D6 enzymatic activity. In contrast, individuals with one or more wild type alleles (extensive metaboliser [EM] alleles) have extensive metabolism of CYP2D6 substrates. A third class of alleles includes polymorphisms that reduce enzyme activity (intermediate metaboliser [IM] alleles) while a fourth class includes gene duplication/multiplication of normal activity alleles (ultra-rapid metaboliser [UM] alleles).

Several pharmacokinetic studies have demonstrated that women who are poor metabolisers of CYP2D6, either by genotype (PM/PM) or induced by a CYP2D6 inhibitor like some of the selective serotonin reuptake inhibitors (SSRIs, e.g. paroxetine or fluoxetine) which are often co-prescribed to alleviate hot flashes, have lower endoxifen plasma concentrations than patients with normal CYP2D6 metabolism.3,34,38,41 These findings confirm a role for CYP2D6 in the formation of endoxifen.

The impact of CYP2D6 activity on tamoxifen pharmacokinetics has also been shown to translate into an effect on clinical benefit from tamoxifen therapy. One study used data from the North Central Cancer Treatment Group (NCCTG) adjuvant breast cancer trial (89–30–52) in which U.S. based post-menopausal women with ER+ breast cancer received adjuvant tamoxifen.42 Analysis adjusting for tumor size and nodal status demonstrated patients with a PM/PM genotype tended to have worse relapse- and disease-free survival. Two subsequent studies have confirmed the effect of CYP2D6 genotype on tamoxifen treatment outcomes. In a retrospective study of German women diagnosed with primary invasive breast cancer who received adjuvant tamoxifen monotherapy, adjusted analysis for tumor size and nodal status showed that patients with decreased CYP2D6 activity (PM/PM, IM/IM, IM/PM, or EM/PM) had shorter relapse- and event-free survival rates, but not overall survival, than patients with normal metabolism (IM/EM, or EM/EM).43 In a second study, from The Netherlands, patients with the PM/PM genotype had increased breast cancer mortality, but not all-cause and all-cancer mortalities, compared with EM/EM patients.44 The findings are consistent with women with a PM/PM genotype having inefficient conversion of tamoxifen to its active metabolite, endoxifen, and as a consequence being at increased risk of tamoxifen therapeutic failure.

Goetz and colleagues investigated whether potent inhibitors of CYP2D6 (eg SSRIs) affect the efficacy of tamoxifen using the NCCTG 89–30–52 trial data.42,43 Adjusted analysis for tumor size and nodal status found that patients with decreased CYP2D6 metabolism (EM/PM, PM/PM, or EM/EM taking a potent or moderate CYP2D6 inhibitor) had shorter times to breast cancer recurrence, relapse-, and disease-free survivals, but not overall survival, compared with EM/EM patients not taking a CYP2D6 inhibitor. Together the findings of all four studies confirm CYP2D6 plays a role in the activation of tamoxifen and suggest that patients with decreased CYP2D6 metabolism, as a result of genetic and/or environmental factors, are less likely to derive clinical benefit from adjuvant tamoxifen therapy than patients with normal CYP2D6 metabolism.

The findings of three independent tamoxifen pharmacogenetic studies are in conflict with those mentioned thus far.44–48 Wegman et al. demonstrated that in postmenopausal women with ER+ and ER− tumors treated with adjuvant tamoxifen, those with EM/PM or PM/PM genotypes had a similar distant recurrence-free survival to EM/EM patients.48 Considering ER+ tumors only, patients with EM/PM and PM/PM genotypes who received tamoxifen had a reduced risk of recurrence compared to those who did not receive tamoxifen, suggesting tamoxifen is activated in patients with PM alleles and efficacy is not dependent on CYP2D6 activity. The risk of recurrence for EM/EM patients with ER+ tumors was the same between the tamoxifen and no-tamoxifen groups, also suggesting CYP2D6 does not play a role in the activation of tamoxifen.

In a second study of a U.S. based population of primary invasive breast cancer patients treated with adjuvant tamoxifen, analysis adjusting for patient clinicopathological characteristics, demonstrated that patients with EM/PM or PM/PM genotypes had similar progression-free and overall survivals to EM/EM patients.46 In the third study, Wegman and colleagues evaluated the genetic association in an independent, large cohort of postmenopausal women with breast cancer treated with tamoxifen.47 In contrast to previous studies,42,43,45 the Kaplan–Meier estimates demonstrated that patients with a PM/PM genotype had a significantly better prognosis than patients with the EM/PM or EM/EM genotypes. Together these studies suggest CYP2D6 activity is not necessary for tamoxifen bioactivation and efficacy.

Inconsistent relationships are common for genetic association studies and there is considerable heterogeneity among the tamoxifen pharmacogenetic studies. For instance, different PM and IM alleles were genotyped, different genotypes were grouped for analysis, many studies did not consider co-prescribed inhibitors of CYP2D6, the dose of tamoxifen and duration of therapy varied, as did the coadministered chemotherapies or radiotherapies, patient menopausal and tumor hormonal statuses. These factors make it difficult to compare the findings across the studies. Also, the effect size appears to be relatively small (HR ~2), and because of the low frequency of the PM/PM genotype only a limited number of patients with decreased CYP2D6 activity were included in each study. Hence many of the studies may have been underpowered to show a significant difference in efficacy to tamoxifen therapy among genotypes. Moreover, all of the studies are retrospective, which may have introduced various biases.

There is enough evidence to indicate that CYP2D6 inhibitors such as fluoxetine and paroxetine should not be co-prescribed with tamoxifen and the current recommendation is that breast cancer patients receive venlafaxine, which does not affect CYP2D6 activity, to alleviate hot flashes. Adjuvant tamoxifen is the drug of choice for pre- and postmenopausal women but recent research suggests aromatase inhibitors are superior to tamoxifen in the postmenopausal setting.49 Postmenopausal women with a PM/PM genotype might respond better to an aromatase inhibitor...
than tamoxifen but there is insufficient evidence at present to support changing standard clinical practice to genotype-guided therapy. Ideally, the results of a large prospective, randomized clinical trial that compares outcomes between genotype-guided and standard of care dosing of tamoxifen would be the best study upon which to make a decision about whether to change adjuvant antiestrogen prescribing practice, however such a study is unlikely to ever be performed for this off-patent medication. The results of other large, retrospective tamoxifen pharmacogenetic trials, including those that compared tamoxifen therapy to alternative hormone therapies such as aromatase inhibitors will clarify the prognostic and predictive relevance of CYP2D6 testing.

Conclusion

This review covered two genes that may one day be useful clinically as predictive markers for breast cancer treatment decision making. A common SNP in FCGR3A has shown promise as an important predictor of rituximab and trastuzumab effectiveness. The association demonstrated by these studies warrants additional studies to elucidate the extent to which the SNP influences ADCC and the tumor response to monoclonal antibodies. Potentially, FCGR3A genotype could be useful in distinguishing between patients who should and should not receive trastuzumab in their chemotherapy regimens to treat HER2+ breast cancer. CYP2D6 plays an essential role in the bioactivation of tamoxifen to its more active metabolites, endoxifen and 4-hydroxytamoxifen. Trial results indicate that a CYP2D6 genotype test could be valuable in predicting patient's response to tamoxifen treatment. In October 2006, the United States Food and Drug Administration’s (FDA’s) Clinical Pharmacology Subcommittee of the Advisory Committee for Pharmaceutical Science reviewed the relationship between tamoxifen and CYP2D6. The subcommittee recommended the FDA amend the package insert for tamoxifen to warn post-menopausal women of the potential heightened risk of treatment failure for patients with deficient CYP2D6 activity. However, widespread adoption of a CYP2D6 test to guide tamoxifen treatment is not sensible at present until a large, well designed study demonstrates a meaningful clinical advantage of its use.

We have only scratched the surface of the potential benefits of applying genetic information to clinical decision making. It is possible that someday a thorough genetic analysis of the tumor and patient will enable clinicians to identify the drug and dosage that will most effectively battle the patient's breast cancer, substantially reducing the health burden of breast cancer worldwide.

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References