# Cancer Pharmacoethnicity: Ethnic Differences in Susceptibility to the Effects of Chemotherapy

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**Abstract** A long-term goal of pharmacogenomics research is the design of individualized therapy based on the genomic sequence of the patient, in order to maximize response and minimize adverse drug reactions. Pharmacoethnicity, or ethnic diversity in drug response or toxicity, is becoming increasingly recognized as an important factor accounting for interindividual variation in anticancer drug responsiveness. Although pharmacoethnicity is determined by genetic and nongenetic factors, there is rapidly accumulating clinical evidence about ethnic differences in the frequencies of polymorphisms within many of the important cancer drug-related genes. This article reviews the current clinical evidence for ethnic differences in anticancer drug disposition and sensitivity while highlighting the challenges, and potential solutions, to acquiring such knowledge. The discovery of "ethnic-specific genetic signatures," representing unique sets of drug susceptibility-governing polymorphisms, may be the outcome of such work. Ultimately, such understanding will further the lofty goal of individualization of chemotherapy based on a person's unique genetic make-up to improve the tolerability and effectiveness of chemotherapy for all patients.



Interethnic differences are becoming increasingly recognized as important factors accounting for interindividual variations in drug responsiveness. In the field of anticancer agents, similar doses are often prescribed to different ethnic populations without consideration of potential differences in pharmacokinetics or pharmacodynamics among populations. Pharmacoethnicity might be described as ethnic diversity in drug response or toxicity. This diversity can result in differences in the recommended safe or effective dose of a drug in different populations or avoidance of ineffective therapy (1). Determinants of pharmacoethnicity include many of the same factors that influence pharmacokinetics and pharmacodynamics, only with an emphasis on variables acting at the population level. These include strong environmental influences on bioavailability and metabolism, which can be ethnically divergent (e.g., rates of smoking, alcohol use, herbal medicine use, or local dietary habits); local practice preferences of treating health care providers (e.g., a cultural tendency to overemphasize or, alternatively, perhaps ignore certain medical conditions; or prevailing social morays that govern aggressiveness of care for patients with advanced disease); ethnic-specific drug–drug interactions (e.g., an interacting drug is approved for use in one country, but not in another); ethnic variation in a drug's targets (e.g., the prevalence of an ethnically restricted mutation in a drug's receptor, which causes particular sensitivity or resistance to that drug); and genetic polymorphisms in drug-related genes (e.g., germline inheritance of a risk genotype that causes rapid metabolism of a drug), among other factors (Fig. 1) (refs. 1, 2).

## Pharmacogenomic Pharmacoethnicity in Cancer Therapeutics

We have chosen to focus this review on the aspects of pharmacogenomic pharmacoethnicity in cancer therapeutics, providing specific examples supporting the idea that genetic differences explain at least a portion of the larger, observed pharmacoethnic differences in chemotherapeutic toxicity and response. Ethnic differences for anticancer agents have only recently begun to be realized even though they have potentially far-reaching importance given the narrow therapeutic index of many chemotherapeutics. Such recognition of differences should further the progress of cancer pharmacogenomic research as it may identify populations that are genetically enriched toward predisposition to a given susceptibility or resistance, or at least inform researchers that some discovered polymorphisms may be especially important or relatively unimportant in certain populations. With accumulating evidence about the important effects of polymorphisms in genes involved in a drug's metabolic pathway or its mechanism of action, attention has turned to the importance of also recognizing that ethnic differences in the frequencies of these polymorphisms

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### Translational Relevance

Interethnic differences are becoming increasingly recognized as important factors accounting for interindividual variations in drug responsiveness. In the field of anticancer agents, similar doses are often prescribed to different ethnic populations without consideration of potential differences in pharmacokinetics or pharmacodynamics among populations. This article reviews the subject of pharmacoethnicity (ethnic diversity in response or toxicity) for cancer drugs with a focus on how recent advances in pharmacogenomics can inform our understanding of pharmacoethnic observations. The challenges and limitations to pharmacogenomic and pharmacoethnic research are included along with suggestions of how emerging preclinical tools can complement clinical studies of pharmacoethnicity in cancer therapeutics. These topics are timely for clinicians and researchers as ethnic diversity considerations within cancer research grow and as global anticancer strategies become increasingly intertwined.

may account for differences in drug effectiveness or toxicity. Ultimately, a better understanding of the role of specific genetic markers will allow the field to move closer to the design of individualized therapy (to maximize response and minimize adverse drug reactions) on the basis of the genomic sequence of each patient, and not simply ethnicity (3).

This review will broadly use the term "ethnicity" to describe the population differences that are being discussed, although we acknowledge that there exists significant historic multiplicity among the various terms often used (ethnicity, race, ancestry, heritage, geographical origin, etc.), and we admit that the concordance between ancestry, genetic ancestry (4), geography, and self-identified race (5–9) is itself a complex subject. Because of the evolution by which the anticancer drug development community has considered issues of ethnicity and race, there has unfortunately been no uniform convention for defining race in much of the prior research to date. Some clinical studies have used "self-reported race" when providing demographic information, other projects have used more detailed information about the ancestry of both sets of grandparents, and even others have simply used geographical identity or have failed altogether to report how ethnicity was designated. More recently, cancer pharmacogenomic studies have considered genetic ancestry informative markers with reported self-identified race to determine whether this further informs pharmacoethnic research on chemotherapies (10). Deciphering genotypic ethnicity using markers for assignment in clinical studies remains infrequent. The potential lack of concordance of ethnic demographic information across studies should be considered when evaluating the current literature.

## Pharmacoethnicity and Pharmacogenomics: Chemotherapy Drug Examples

To illustrate the emerging field of cancer pharmacoethnicity pharmacogenomics, we will begin by exploring several drug-class examples of clinical evidence for ethnic differences in anticancer disposition and sensitivity, specifically highlighting pharmacogenetic research that may begin to explain the interethnic differences in response to drugs. Table 1 provides a summary of clinical pharmacoethnic findings.

## Antimetabolites

Among chemotherapeutics classified as antimetabolites, 5-fluorouracil (5-FU) is perhaps the best studied agent with regards to clinically apparent ethnic differences, likely due to world-wide use.

5-FU, and the related oral prodrug capecitabine (which is converted to 5-FU by a three-step enzymatic process; refs. 11, 12), are used commonly in the setting of advanced stage colon cancer, and ethnic disparities exist in colon cancer treatment outcomes between African Americans (who do worse) and Caucasians. It remains unclear whether, and to what degree, this ethnic difference may be due to decreased 5-FU tumor responsiveness and or decreased tolerance of therapy among African Americans (13). Several studies have attempted to address the latter question. McCollum and colleagues published a comprehensive clinical analysis of the toxicities of 3,380 colon cancer patients who received 5-FU–based adjuvant regimens (14). The hematologic toxicities of leukopenia and anemia were significantly more common in African Americans compared with Caucasians ($P < 0.006$). At the same time, the overall incidence of any toxicity was actually lower in African Americans compared with Caucasians ($P = 0.005$), including lower rates of diarrhea ($P < 0.001$), nausea and or vomiting ($P < 0.02$), and mucositis ($P < 0.001$). There was no ethnic difference in the observance of skin toxicity.

Mattison and colleagues recently suggested a possible explanation for the hematologic toxicity disparity by demonstrating that healthy African Americans, compared with Caucasians, have significantly lower peripheral blood mononuclear cell levels of dihydropyrimidine dehydrogenase (DPD) activity, the rate-limiting enzyme of 5-FU catabolism (15). The prevalence of DPD deficiency, defined as enzyme activity below the lower limit encompassed by 95% of the studied population, was 8.0% in African Americans, and only 2.8% among Caucasians. For even further comparison, a separate study of 150 healthy Japanese subjects showed only one patient (0.7%) to be DPD deficient (16). Significant research on *DPYD*, the gene encoding DPD, has revealed more than 30 single nucleotide polymorphisms (SNPs) and deletions within *DPYD*, although only a minority has been found to directly result in enzymatic alterations (17) and only one allele, *DPYD*2A*, has been prospectively linked to toxicity in a large clinical trial (18). The clinical utility of pharmacogenetic DPD testing remains an active area of research.

Alternatively, interethnic differences with the antimetabolite target enzyme thymidylate synthase (TS; gene *TYMS*) have been appreciated. Although the clinical importance of TS overexpression in various cancers is somewhat ambiguous (19–21), genetic changes regulating TS levels have been correlated with 5-FU toxicity development (22), preclinical studies have suggested that overexpression of TS confers tumor resistance to 5-FU (23, 24), and population studies show that TS regulation is ethnically divergent (25, 26). Specifically, tandem repeats in the *TYMS* enhancer region (TSER) have been shown to correlate

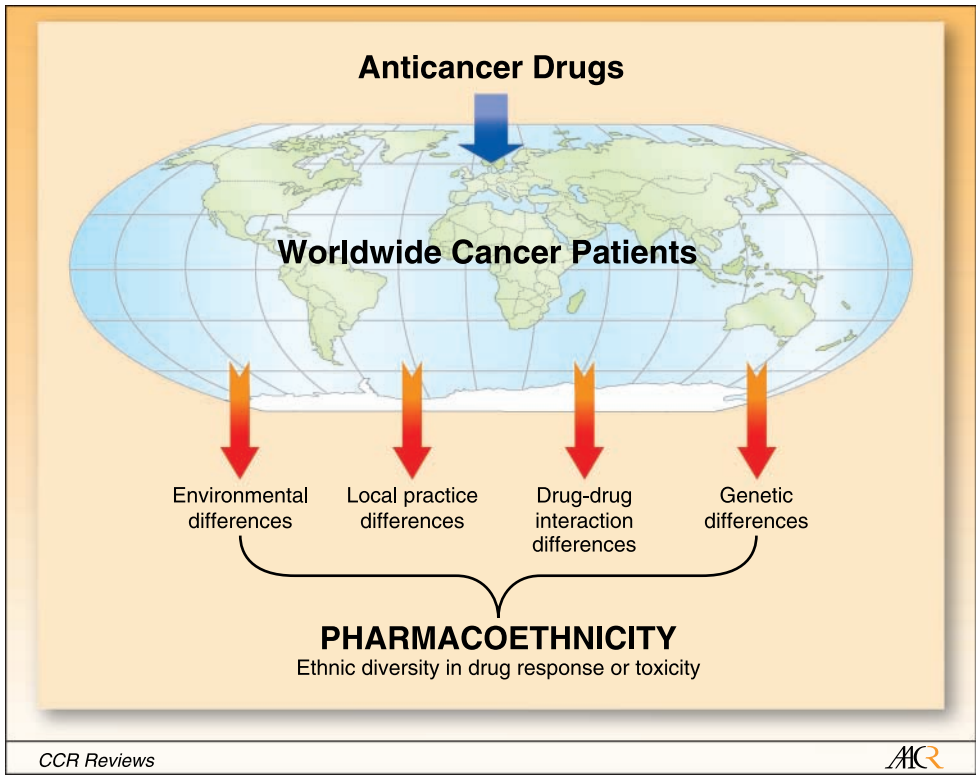


**Fig. 1.** Pharmacoethnicity, or ethnic diversity in drug response or toxicity, results from the combined interaction of many factors, principally differences in environment, local practice habit and regulatory control differences, drug–drug interaction differences, and genetic differences. Pharmacoethnicity impacts global drug development and anticancer drug efficacy because the safe, tolerable, or therapeutic doses may differ among populations on the basis of ethnic factors. The importance of studying pharmacogenetics and pharmacogenomics as factors in pharmacoethnicity is becoming increasingly apparent. See also refs. (1, 2). World map courtesy of http://english.freemap.jp/ and used with permission under the Creative Commons Attribution 3.0 License.

with TS expression, with three copies of the tandem repeat (TSER*3) resulting in approximately 2.6 times greater TS expression than two copies (TSER*2) (27). An important study by Lecomte and colleagues showed that polymorphisms in TSER were related to clinical development of 5-FU toxicity (22). In this study, the cohort of patients who were germline homozygotes for TSER*2 had an incidence of any grade 3 or 4 toxicity of 43%, compared with only 18% for TSER*2-TSER*3 patients, and 3% for TSER*3 homozygotes ($P < 0.01$). TSER genotype was not associated with 5-FU disease response or survival in this study. The increased 5-FU toxicity risk of homozygosity for TSER*2 was recently confirmed in a large prospective trial (18). The ethnic importance of the above findings lies in the fact that 67% of Chinese individuals have the TSER*3-TSER*3 genotype, compared with only 30% to 40% of Caucasians (25). This finding is important when comparing 5-FU tolerability in these two populations. The population frequency of the TSER*3 allele in Caucasian Americans (54%) is similar to that of African Americans (52%), but two rare alleles (TSER*4 and TSER*9), having as-yet-unknown importance, are found at a higher frequency in African Americans (2%) than in Caucasians (0%) (26), an interesting finding that deserves further investigation.

Although the above studies underscore the notion that germline polymorphisms in key genes might account for interethnic differences in toxicity, and potentially response, to antimetabolites including 5-FU, the interactions between these polymorphisms and the role of other as-yet-undiscovered polymorphisms remain incompletely explained.

## Anthracyclines

The most clinically devastating toxicity of anthracyclines is arguably cardiotoxicity, a manifestation known to be dose related. In 1997, the first comprehensive study was published that showed an increased risk of cardiotoxicity, independent of dose, among African Americans treated with an anthracycline (28). In this study, African American ethnicity was independently associated with a 1.7-fold greater relative risk of cardiotoxicity, which included congestive heart failure, a decline in observed cardiac function, or cardiac-related death. A second study reported similar increased risk among African Americans (29). A subsequent study showed that African Americans are less likely than Caucasians to receive the expected number of cycles of doxorubicin, and that early termination of doxorubicin was associated with both African American ethnicity and decreased survival (30). The mechanism by which African Americans may be more sensitive to cardiotoxicity is still unclear, yet two recent studies in Asian patients have reported several ethnically variant polymorphisms that influence doxorubicin pharmacokinetics (31, 32). One of these studies describes the pharmacokinetic impact of several polymorphisms in *CBR1* (carbonyl reductase

1) and *CBR3*, genes whose products catalyze the conversion of doxorubicin to doxorubicinol. Doxorubicinol is a metabolite believed to have decreased antitumor activity but that has been associated with increased cardiotoxicity in animals (31). A study of whether polymorphisms in these genes explain the enhanced toxicity susceptibility in African Americans is warranted.

## Alkylating Agents

Cyclophosphamide is a prodrug that must be converted via the cytochrome P450 (CYP) enzymes CYP3A4, CYP2B6, and CYP2C9 to its active forms (33, 34). CYP3A4 also inactivates cyclophosphamide via conversion to dechloroethylcyclophosphamide (35). The importance of the CYP enzymes in anticancer pharmacokinetics has been well described (36, 37), and ethnic differences in the activity of several CYP enzymes are known (38, 39). One salient study of breast cancer patients showed that many *CYP3A4*, *CYP3A5*, and *CYP2B6* variant polymorphisms are more prevalent in African Americans compared with Caucasians (40), leading the authors to hypothesize that certain polymorphisms that prevent the activation of cyclophosphamide could result in ethnic-specific drug exposure differences. Although some polymorphisms were clearly associated with decreased cyclophosphamide activation, ethnic differences in cyclophosphamide area-under-the-curve were not observed (40). (This negative result, however, may have been the result of the study being underpowered to show such an effect). This has nevertheless led to speculation that the ethnic differences in *CYP3A* polymorphism frequencies between African Americans and Caucasians may explain outcome differences of therapy with cyclophosphamide, which have been shown in at least one nononcology population: lupus nephritis patients (41). In that study, 95% of Caucasian patients with lupus nephritis who were treated with cyclophosphamide retained renal function on 5-years follow-up, compared with only 58% of African Americans, a difference that was independent of other confounding factors. Such findings deserve further investigation in clinical trials involving oncology patients to elucidate whether a similar signal exists and to determine the genetic explanations.

## Vinca Alkaloids

Vincristine is also a substrate for the enzyme CYP3A and is preferentially metabolized by CYP3A5 compared with CYP3A4. Since *CYP3A5* expression shows significant ethnic differences between African Americans (70% prevalence) and Caucasians (20%), Renbarger and colleagues (42) recently investigated whether African Americans would therefore have lower vincristine-associated toxicities due to more rapid vincristine clearance. In their study, a staggering 34.8% of Caucasians experienced vincristine-related neurotoxicity compared with only 4.8% of African Americans ($P = 0.007$). Caucasians experienced a higher average grade of toxicity (2.72 versus 1, $P < 0.0001$), were more likely to undergo vincristine dose-reduction (4% versus 0.1%, $P < 0.0001$), and had more doses omitted (1.2% versus 0.1%, $P < 0.01$) than African Americans. How these ethnic differences in vincristine exposure correlate to antitumor effect in the two populations was not measured, and

**Table 1.** Examples of clinical pharmacoethnic differences in chemotherapeutic susceptibilities, with implicated genes and important known functional variants shown

| Drug | Clinical findings | Genes implicated | Important variants | Mechanisms | Ethnic differences of variants |
|---|---|---|---|---|---|
| 5-fluorouracil | Hematologic toxicities more common in AA > CAU; Diarrhea, mucositis, and nausea/vomiting more common in CAU > AA | *DPYD* *TYMS* | *DPYD*2A* *TSER*2, *3* | (a) DPD deficiency; (b) three copies of *TYMS* enhancer region tandem repeat (TSER*3) result in greater TS expression (and decreased toxicity) | (a) Frequency of DPD deficiency is 8% AA, 2.8% CAU, < 1% ASN; (b) 67% of ASN are TSER*3-TSER*3 genotype, compared with 30–40% of CAU |
| Doxorubicin | Increased cardiotoxicity in AA | Possibly *CBR1, CBR3* | — | Possibly increased CBR1-CBR3 conversion of doxorubicin to more cardiotoxic doxorubicinol | — |
| Cyclophosphamide | Possible decreased efficacy in AA compared with CAU | *CYP3A4, CYP3A5, CYP2B6* | Many potentials | Decreased conversion of cyclophosphamide to more active metabolites | Many found |
| Vincristine | Neurotoxicity more common in CAU compared with AA | *CYP3A5* | — | Vincristine metabolism by CYP3A5 | *CYP3A5* expression more prevalent in AA (70%) compared with CAU (20%) |
| EGFR inhibitors | Increased antitumor efficacy | *EGFR* | Many potentials | — | East Asians have more *EGFR* mutations and are more susceptible to EGFR inhibitors |

NOTE: Individual references for the supporting evidence are cited within the text.
Abbreviations: AA, African American; CAU, Caucasian; ASN, Asian; EGFR, epidermal growth factor receptor.

**Table 2.** Pharmacogenomic challenges to the study of pharmacoethnicity in cancer therapeutics, with potential solutions and ways of advancing investigation

| Challenge | Solution |
|---|---|
| 1. Clinical trials of pharmacoethnicity require diverse populations | 1. (a) International collaborations<br>(b) Repeat trials in multiple different countries |
| 2. Chemotherapy-related effects are likely to be under multigenic control | 2. (a) Include multiple mechanism-related and metabolism-related pathways in the same model<br>(b) Use unbiased, genome-wide models |
| 3. Chemotherapy cannot be administered to healthy volunteers | 3. Incorporate cell-based models (e.g., HapMap Project) |
| 4. Potentially important polymorphisms may be generally uncommon | 4. (a) Appropriately power clinical trials<br>(b) Study ethnic populations enriched for the phenotype of interest<br>(c) Employ next generation sequencing to discover rare variants |
| 5. Genome-wide associations use multiple-SNP testing, which generates many false-positive SNPs | 5. (a) Include discovery and replication sets<br>(b) Validate findings (preclinically and clinically)<br>(c) Use rigorous statistical methodology |

ethnicity was used as a surrogate for *CYP3A5* genotype in this study, a major limitation. Still, this study of vincristine pharmacoethnicity is an intriguing example of the potential impact of ethnic variation in a CYP metabolizing enzyme among anticancer therapies.

## Epidermal Growth Factor Receptor Inhibitors

In addition to interethnic differences in the metabolism of anticancer agents, there are also potential differences in the target genes of these drugs. One particularly interesting and recent observation is that East Asian individuals, especially Asian females with lung cancer of the adenocarcinoma subtype, have much higher tumor response rates upon treatment with an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor than do other cohorts (43, 44). As one representative study showed, Asian lung cancer patients treated with an EGFR inhibitor had significantly longer median survival than individuals from all other ethnic groups (9.5 versus 5.5 months, $P = 0.01$) (ref. 45). An explanation for this is that Asians have been found to have a higher prevalence of somatic mutations (between 20–40%, compared with 6% in other populations) in exons 18 to 21 of *EGFR* in non small cell lung cancer specimens, mutations that have been associated with a clinical response to EGFR inhibitors (43, 46). However, the ethnic-specific enrichment of these mutations in Asians, and the augmented clinical response, suggest that these findings may be driven by ethnic-related germline differences in genes affecting the tumor biology and or the primary etiology of the disease. In other words, germline polymorphisms that are present at different frequencies in Asians compared with other ethnic groups may cause a predisposition to tumor-specific mutations, which then confer therapeutic susceptibility (43). This theory is bolstered by the observation that individuals of Asian descent who live outside of East Asia still show a higher prevalence of *EGFR* mutations compared with their non-Asian counterparts (47). This germline-ethnic link is receiving ongoing attention.

In related studies, Liu and colleagues (48) evaluated the question of ethnic differences in *EGFR* by examining the genotypes of 183 Caucasian, 84 African American, and 66 Asian individuals specifically for polymorphisms in the (CA)n dinucleotide repeat in intron 1 of *EGFR*, a polymorphism that has been suggested to regulate *EGFR* expression. Shorter dinucleotide repeat segments ($n = 16$) result in higher *EGFR* expression compared with longer segments ($n = 21$). In their study, the population frequency of one of the longer segment alleles ($n = 20$) was significantly higher in Asians (63%) compared with Caucasians (21%) ($P = 2 \times 10^{-18}$), and the shorter allele ($n = 16$) was the most common allele in Caucasians (43%) and African Americans (42%) but was less frequent in Asians (17%). The presence of longer segments in Asians would result in lower EGFR levels, perhaps explaining why Asians have higher response rates to standard doses of EGFR inhibitors. The exciting finding of an ethnically enhanced response to EGFR inhibitors in Asian lung cancer patients has been one of the most dramatic recent examples of the potential utility of pharmacoethnicity in oncology.

## East Asians as a Particular Susceptible Population

Although EGFR inhibitors have emerged as one recent example, there has been other, consistent historical evidence that East Asian individuals are more susceptible to the effects of some chemotherapy agents than their Western counterparts. Japanese physicians have long identified that the standard, approved doses of many agents are intolerable to Japanese patients (49). This observation has led to the Japanese practice, in many instances, of replicating, in Japan, clinical trials examining new chemotherapeutic-based regimens that have already been done in the United States or Europe—simply to determine optimal and tolerable dosing. For example, Ogawara and colleagues studied in 2002 the combination of carboplatin and paclitaxel, a long-standing doublet used in the treatment of lung cancer in the United States, to determine whether the combination was tolerable in Japanese individuals (50). Although the authors concluded that the regimen was practicable, a surprisingly large number of patients (70%) had grade 4 neutropenia. Similarly, Takei and colleagues did a feasibility study using standard Western doses of carboplatin and paclitaxel in Japanese ovarian cancer patients, but they found that the incidence of grade 4 neutropenia (80% in the Japanese cohort) was dose-limiting (51). Watanabe and colleagues in 2003 (49) studied 34 Japanese patients to determine the tolerable doses of an induction chemotherapy regimen that included cisplatin, docetaxel, and 5-FU following a U.S.-based trial for head and neck cancer patients. The study found that although the overall doses of cisplatin, docetaxel, and 5-FU were 80% of the total doses given in the

United States, Japanese patients still had a 19% incidence of grade 3 or 4 neutropenia, compared with only 8% in the U.S. population. Despite the decreased overall doses, and possibly in concordance with the increased rate of toxicity, the overall rate of disease response in the Japanese patients was not significantly different from the response rate in the U.S. patients (approximately 88%).

Another study addressing this idea was published by Millward and colleagues who treated 68 lung cancer patients with carboplatin and paclitaxel (52). Because the trial was conducted in both Australia and Singapore, the patient population in this study included both Caucasians and Asians. Surprisingly, the disease response rate for Asians was 65%, compared with only 31% for Caucasians ($P = 0.01$). Moreover, although the end-of-study incidence of febrile neutropenia for the overall study population was 26%, an initially treated cohort of Asians showed a 50% incidence of febrile neutropenia, leading the study investigators to institute a mandatory dose-reduction for carboplatin in all subsequently enrolled Asian individuals. Despite this dose-reduction, the incidence of febrile neutropenia in the subsequently enrolled Asians was still 40% (6 out of 15). It is important to note that although a majority of Asians in this study received lower doses of carboplatin than their Caucasian counterparts, their tumor response rate was more than twice as high, suggesting that the same germline genetic determinants and environmental factors may be contributing to greater toxicity and greater response. This hypothesis, and the elucidation of such determinants in Asian individuals, deserves ongoing investigation.

## Challenges of Studying the Genetic Aspects of Pharmacoethnicity of Cancer Therapies

Discovery of pharmacoethnic differences in the clinical arena often requires the systematic analysis of large, diverse populations to dissect the genetic or biological mechanism of these differences. In many cases, this type of clinical endeavor—which in certain instances may require international collaboration—is simply not feasible. In particular, demonstration of genetic polymorphisms governing a chemotherapy-related outcome is often difficult because of five common limitations: (1) clinical trials of pharmacoethnicity require diverse populations; (2) chemotherapeutic induced outcomes are likely multigenic requiring large sample sizes; (3) chemotherapy cannot be administered to healthy volunteers; (4) potentially important polymorphisms may be generally uncommon; and (5) multiple-SNP testing, with its resultant generation of false-positives, must be appropriately considered (see "Challenges and Limitations to Studying Pharmacogenomic Pharmacoethnicity in Cancer Therapies," summarized with potential solutions, in Table 2).

To consider these limitations in detail, much of the difficulty stems from the fact that chemotherapeutic susceptibility is likely a complex genetic trait (53), meaning that the combined effects of many different genes and polymorphisms interact to determine the ultimate phenotype. To detect the individual genetic loci governing the phenotype, the study of a large number of individuals is needed because each locus either has a very modest individual effect, or is relatively infrequently found (54). Thus, in some cases, reportedly "negative" pharmacogenomic trials may simply be underpowered to detect the actual modest-effect or rare loci. Moreover, acknowledging that more than one gene is likely involved also requires that the investigators pre-identify a number of candidate genes (55) to be studied (each with a rationale for their likely importance; this is commonly done by involving the known mechanistic or metabolic pathways for a given drug). Alternatively, one can employ an upfront unbiased approach in which all loci are considered without preference, as in genome-wide association studies (GWAS) (ref. 56). The latter are beset with their own important considerations, not the least of which are the need for involvement of experienced statistical geneticists, accounting for multiple-SNP testing (57), and dealing with the usually large number of generated false positives through use of separate "discovery" and "replication" sets or via another means of validation.

If one further uses pharmacoethnicity to posit that ethnic-specific determinants exist, individuals from each ethnic group need to be well represented in the cohort of study to allow comparison of interethnic effects. This usually requires multicenter (and perhaps international) collaboration. An alternative approach focuses on phenotypically enriched populations (such as East Asians, as reviewed above). This approach could allow detection of ethnic-specific polymorphisms in the enriched population, which would then have to be subsequently interrogated for their presence, and role, in other populations.

It should be noted that almost all of the clinical evidence and studies of cancer pharmacoethnicity thus far have been limited to three broad ethnic groups: Asians, Caucasians, and African Americans. Considering the United States alone, this body of data ignores the many other ethnic groups (such as U.S. Hispanics and American Indians, as two examples) for which there are almost no information on cancer pharmacoethnicity. Finding ways to incorporate these groups into cancer chemotherapy trials of large enough size to draw meaningful, ethnic-specific pharmacogenomic conclusions remains an unmet need (58).

Finally, even when some of the above hurdles in the arena of pharmacogenomic research can be overcome, and despite the great promise of the field, significant time will be needed before most pharmacogenomic discoveries reach fruition in clinical practice.

## Preclinical Models: Using the International HapMap Project as a Discovery Tool

To overcome the difficulty of embarking on large pharmacogenetic trials to study interethnic variation in drug sensitivity, we and others have developed a preclinical, unbiased genome-wide approach to identify ethnic-specific SNPs and genes responsible for susceptibility to a given chemotherapy (59). This experimental model employs well-genotyped Epstein-Barr virus-transformed B-lymphoblastoid cell lines (LCLs) established from healthy individuals in the International HapMap Project.[3] Because the HapMap project includes cell lines derived from individuals representing 11 distinct ethnic groups (including approximately 100 individuals per ethnic group), it can be used as a tool toward discovery of the genetic contribution to pharmacoethnic differences in chemotherapeutic susceptibility. The ethnic groups of the HapMap currently include the Yoruba in Ibadan, Nigeria; Japanese in Tokyo, Japan; Han Chinese in Beijing, China; Caucasians in the United States (Utah residents with ancestry from northern

[3] The International HapMap Project (2008), http://www.hapmap.org/abouthapmap.html.

and western Europe); Maasai in Kinyawa, Kenya; Luhya in Webuye, Kenya; Chinese in the metropolitan Denver, CO area; Gujarati Indians in Houston, TX; Tuscans in Italy; individuals of African ancestry in the Southwest United States; and individuals of Mexican ancestry in Los Angeles, CA.

Upon treatment of HapMap cell lines with a chemotherapeutic agent, each cell line's unique sensitivity to drug-induced cell growth inhibition can be measured as a phenotype (59). These phenotypes can then be subjected to GWAS using the publicly available HapMap genotypes to identify potential SNPs contributing to cytotoxicity. As a means to prioritize SNPs that act through their effect on gene expression, we have also made publicly available the exon-level gene expression for many HapMap cell lines.[4] Therefore, SNPs, host genes, and target genes can be identified for follow-up studies. This cell-based method is unique in that it represents an unbiased, comprehensive (genome-wide) approach that takes into consideration the multigenic nature of cellular susceptibility to a drug. Most importantly, the model avoids giving chemotherapy to unaffected family members for genetic studies.

One could postulate that finding observed *in vitro* interethnic differences in cellular susceptibility using LCLs might serve as a viable preclinical model for the genetic analysis of clinical differences in toxicity. With the advantages of being a renewable, richly genotyped model system that does not have the clinical and environmental confounders of studies in human subjects, LCL studies permit a relative emphasis on (and thereby potential identification of) the effects of genetics on the phenotype (60). If population differences in sensitivity to a given drug are indeed observed (and especially if the differences mimic those observed for the drug in clinical settings), then that scenario provides an excellent opportunity for attempting to evaluate the potential genetic causes of the ethnic difference without the upfront need for genotyping large numbers of human subjects of adequate ethnic diversity. Indeed, using HapMap LCLs, Huang and colleagues recently reported on the genetic determinants associated with sensitivity to carboplatin, cisplatin, daunorubicin, and etoposide (59, 61–63). In two of those examples, LCLs derived from Caucasians were more susceptible to cytotoxicity induced by carboplatin and daunorubicin than LCLs from Africans, and these observed interethnic differences led to the determination of unique, "ethnic-specific" genetic variants (SNPs and their related genes) associated with chemotherapeutic cytotoxicity susceptibility in each population (61, 63). In contrast, LCLs derived from Africans were more susceptible to the growth-inhibitory effects of cytarabine than Caucasians (64). A unique pharmacogenetic signature of SNPs explained much of the variation for each population. Ethnic-specific signatures were secondary to either polymorphic SNPs in one population, which were monomorphic in the other, or due to significant associations of SNPs with cytotoxicity or gene expression in one population but not the other.

Conversely, one could similarly hypothesize that, in contrast to ethnic-specific genetic signatures, using LCLs from different HapMap populations might permit identification of SNPs governing susceptibility "across-populations" if the same SNPs (or those in linkage disequilibrium) can be shown to be important in several populations. "Cross-population" SNPs could still explain interethnic differences in susceptibility if the risk allele frequencies of the SNPs varied greatly between populations. In other words, if Asians, for example, are particularly susceptible to the effects of platinum agents, then we would expect that cross-population platinum susceptibility SNPs would have very high risk allele frequencies in Asians, but lower frequencies in other populations. Moreover, cross-population SNPs would be especially useful in a clinical setting since they could potentially be tested in any individual entering a clinical trial, regardless of the individual's ethnicity.

The real power of the HapMap LCLs lay in the fact that large numbers of ethnically identified individuals are readily available for study, and approximately 6,000,000 SNPs in these individuals' genotypes are already known. Still, as we have alluded to, SNPs discovered using LCL studies should be regarded as preclinical candidates that then need to be validated in a clinical setting. Testable SNPs could either be ethnic-specific or cross-population, depending on the intended ethnic diversity of the clinical trial. Not only should the number of testable candidate SNPs be exponentially increased by the use of the HapMap resource (especially through unbiased approaches as outlined above), but the efficiency of subsequent clinical trials should be improved. Clinical testing of several novel LCL-derived candidate SNPs is already underway in our research group. Simultaneously, we continue to explore the preclinical discovery of new germline genetic signatures underlying chemotherapeutic pharmacoethnic differences.

## Implications of Cancer Pharmacoethnicity Research

As we stated at the outset, identifying genetic variants contributing to interethnic variation in sensitivity to chemotherapeutic drugs has the potential to favorably impact cancer care and the greater mission of personalized medicine in several ways. For one, recognition of pharmacoethnicity invites one to capitalize on the principle that a population enriched for a given phenotype is ideal for the study of genetic variants responsible for that phenotype. In this way, pharmacoethnic differences may identify especially informative populations in which to begin a search for genetic susceptibility determinants. Secondly, improved awareness of pharmacoethnic factors has the chance to inform clinicians—and patients—about ways to explain and potentially reduce existing health disparities in cancer care, a subject that is receiving increasing attention and that cannot be understated (13, 65). Third, identification of ethnic differences in chemotherapeutic susceptibility is a step toward the more exact goal of identifying interindividual differences, even within ethnic groups. The latter, ideally, will consider each individual's genetic make-up in the context of environmental and regional differences that may also be important within a population. As genetic admixture among ethnic groups continues to increase, consideration of the individual and his or her genetic ancestry will only become more important (66). Lastly, and perhaps extending to areas outside of clinical medicine, pharmacoethnicity research and the awareness it brings to acceptance of the similarities and differences between individuals (especially awareness of the nuances of ethnic admixture) carries the hope of improving the ethics of how ethnicity is considered in clinical studies and in our world.

[4] Gene Expression Omnibus, GSE7761, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health (2007), http://www.ncbi.nlm.nih.gov/geo/.

## Conclusions

Recognition of the widespread presence of pharmacoethnicity in cancer therapeutics is important in worldwide drug discovery and development. Appreciation of ethnic-specific differences is often hindered by the need for large, diverse populations in clinical trials. Alternatives include the study of unique, ethnic populations enriched for a specific clinical phenotype, along with complementary preclinical methods such as those utilizing the International HapMap cell lines for pharmacogenomic discovery. Realization of the importance of pharmacoethnicity could improve chemotherapeutic tolerability and effectiveness for all, and will further our progress toward the ultimate goal: individualization of chemotherapy based on a person's unique genetic make-up, not on one's ethnicity.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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