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Pharmacogenomics insights into precision pediatric oncology

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Purpose of review
Pharmacogenomic insights provide an opportunity to optimize medication dosing regimens and patient outcomes. However, the potential for interindividual genomic variability to guide medication dosing and toxicity monitoring is not yet standard of care. In this review, we present advances for the thiopurines, anthracyclines and vincristine and provide perspectives on the actionability of pharmacogenomic guidance in the future.

Recent findings
The current guideline on thiopurines recommends that those with normal predicted thiopurine methyltransferase and NUDT15 expression receive standard-of-care dosing, while ‘poor metabolizer’ haplotypes receive a decreased 6-mercaptopurine starting dose to avoid bone marrow toxicity. Emerging evidence established significant polygenic contributions that predispose to anthracycline-induced cardiotoxicity and suggest this knowledge be used to identify those at higher risk of complications. In the case of vincristine, children who express CYP3A5 have a significantly reduced risk of peripheral neuropathy compared with those expressing an inactive form or the CYP3A4 isoform.

Summary
The need for adequately powered pediatric clinical trials, coupled with the study of epigenetic mechanisms and their influence on phenotypic variation and the integration of precision survivorship into precision approaches are featured as important areas for focused investments in the future.

Keywords
chemotherapeutic agents, pharmacogenomics, precision oncology

INTRODUCTION
Pharmacogenomics is one of the earliest and most impactful applications of genetic insight into the practice of medicine; with its roots tracing back to the recognition that genetic variation in the enzymes involved in drug metabolism can significantly impact drug response and toxicity [1]. With the advent of advanced high throughput molecular technologies, the field of pharmacogenomics continued to evolve from the study of single genes and enzymes to genome-wide assessments of individualized drug responses. Pharmacogenomic insights pose an opportunity for providers to optimize medication dosing regimens based on the genetic characteristics of individual patients, and while it has yet to become standard of care, preemptive pharmacogenomics testing has gained considerable popularity among patients and their families, as well as medical providers. The application of pharmacogenomics-based interventions in pediatric oncology is of particular interest, however significant challenges to widespread implementation remain.

To help facilitate the use of genotype results in medical decision-making, the Clinical Pharmacogenetics Implementation Consortium (CPIC) was established to provide freely accessible peer-reviewed, and evidence based guidelines for gene-drug pairs that can be utilized to incorporate pharmacogenomic results into patient care. To date, CPIC has created and curated information on
THIOPURINES

The thiopurines, including azathioprine, 6-mercaptopurine (6-MP), and thioguanine, were among the first identified drugs to be impacted by clinically significant genetic variations in drug-metabolizing enzymes. Both thioguanine and 6-MP are utilized in the treatment of pediatric acute lymphoblastic leukemia (ALL) and acute myeloid leukemia, and depending on the protocol, can be required for up to 2 years as an integral part of maintenance chemotherapy for pediatric ALL treatment [3]. Thiopurine toxicity is widespread and includes pancreatitis, hepatitis, sinusoidal obstruction syndrome, and myelosuppression [4*], with variations in key enzymes (namely, TMPT and NUDT15) resulting in heightened risk for life-threatening bone marrow toxicity [5].

The first thiopurine pharmacogene to be implicated in the development of thiopurine toxicity was TMPT, which catalyzes the methylation of 6-MP and its downstream metabolites to facilitate their removal and preclude any further bio-activation [6]. There is inherited variation in thiopurine methyltransferase (TPMT), with certain allelic variants resulting in increased production of thioguanine nucleotide metabolites and ultimately resulting in further nucleic acid damage and cell death [4*]. To date, CPIC provides information on more than 40 star allele haplotypes for the TMPT enzyme [2], with different star alleles and suballeles having variable metabolic activity and predisposition to thioguanine toxicity [7]. This information has been used to assign pediatric patients with a metabolic phenotype (based on the predicted functional activity of their haplotype), and is subsequently used to designate normal, intermediate, or poor metabolizers of thiopurines.

To date, the 1 allele correlates with normal thiopurine metabolic function, TMPT2, 3A, 3B, 3C, 4, 11, 14, 15, 23, 30, and 41 are termed as ‘no function’ alleles, and all other star alleles are denoted to be of uncertain function. The most common TPMT phenotype is TPMT1/1, which accounts for 90% of enzyme variants across most bio-geographical and ancestral groups [8] and represents individuals that are ‘normal metabolizers’ of thiopurines. Alternatively, those that carry a star allele of uncertain function have been designated as ‘possible intermediate metabolizers’, those that carry a normal function allele in combination with a no function allele (e.g., TMPT1/2 and TMPT1/3A) have been deemed as ‘intermediate metabolizers’, and those with two ‘no function’ alleles are considered to be ‘poor metabolizers’ of thiopurines. These metabolic phenotypes were among the first to be

specific pharmacogenes with the goal of defining genetic variants associated with star allele haplotypes, assessing functional status for each haplotype, and assigning a predicted phenotype for each possible diplotype [2]. Although CPIC has certainly facilitated the translation of pharmacogenomic knowledge from the bench to the bedside, the potential for interindividual genomic variability to guide medication dosing and toxicity monitoring on a widespread level has yet to be fully actualized.

In this review, we focus on pharmacogenomic advances for three frequently used drug classes in pediatric oncology, with thiopurines being the only one for which specific CPIC recommendations are available in the United States, and with anthracyclines and vincristine chosen as representative classes of chemotherapeutic agents for which emerging pharmacogenomic evidence will likely inform clinical management in the future. Lastly, we provide perspectives on future advances in the field of precision pediatric oncology and the actionability of pharmacogenomic guidance in the future.
The anthracyclines, including doxorubicin and daunorubicin, are another important class of chemotherapeutic agents utilized in the treatment of a variety of pediatric leukemias, lymphomas, and sarcomas [11,12]. Anthracyclines function as antineoplastic drugs by intercalating into DNA, disrupting topoisomerase IIα-mediated DNA repair, and ultimately causing DNA damage and cellular apoptosis [13]. Despite the indication for anthracyclines in numerous chemotherapy protocols, their use is often complicated and subsequently limited by anthracycline-induced cardiotoxicity (ACT). ACT is widespread and ranges from asymptomatic systolic dysfunction to overt congestive heart failure [14,15], and while the exact mechanism of ACT continues to be debated, free-radical-mediated oxidative damage and mitochondrial dysfunction are believed to play a significant role in the development of cardiotoxicity [11,15].

ACT is dose-dependent and cumulative, with those receiving higher doses and chest irradiation at greater risk. Cardiotoxicity can be observed in patients treated with lower doses of doxorubicin or daunorubicin, indicating that variations in individual susceptibility can play a significant role in risk stratification [14,16,17]. Given that conventional biomarkers often remain within normal limits until myocardial damage has ensued, the detection of early and asymptomatic cardiotoxicity remains a critical challenge. With nearly 60% of all pediatric cancer survivors having a history of prior anthracycline and/or chest radiation exposure [18,19], there is an ongoing need to identify genetic risks, discover predictive biomarkers, and implement standardized screening protocols to mitigate life-threatening ACT.

To date, numerous single-nucleotide polymorphisms (SNPs) and genes have been associated with the development of ACT. In a genome-wide model that utilized International HapMap cell lines, 137 SNPs spanning 30 genes were found to be significantly associated with daunorubicin cardiotoxicity, with many of these genes involving the phosphatidylinositol signaling system, glycosylphosphatidylinositol-anchored proteins, and axonal pathways [20]. Subsequent studies found that polymorphisms in genes encoding for ATP-binding cassette (ABC) transporters were associated with ACT [21–24], with ABCB1 rs2235047 and ABCC1 rs4148808 variants associated with a higher risk of ACT across various pediatric malignancies [23,24], and the ABCC5 rs7627754 TT genotype [21] as well as ABCC1 gene variants (rs3743527 and rs246221) associated with ACT in pediatric ALL [22]. On a larger scale, the rs6759892 variant in UGT1A6 (which encodes for a glucuronosyltransferase) has been found to be significantly associated with the development of ACT [23–25], and more recently, a genome-wide association study implicated the missense variant rs2229774 in Retinoic acid receptor gamma (RARG) (which encodes for a retinoic acid receptor) with heightened susceptibility to ACT [26]. Conversely, variants in genes encoding for solute carriers (which play an important role in the absorption and excretion of drugs) appear to confer a lesser risk of ACT, with the genetic variants rs7853758 in SLC28A3, rs9614091 in SLC10A2, and rs4877847 in SLC28A3 found to be protective against the development of ACT for more than 5 years after completion of...
Several allelic variants of CYP3A5 have been identified, with the CYP3A5 *1/*1 expressers on one end of the genotypic spectrum and CYP3A5 nonexpressers (*3/*3 genotype) on the other, with those expressing at least one copy of the CYP3A5 *1 allele found to exhibit greater expression than those who are homozygous for other variants. CYP3A5 *3 creates a premature codon that alters mRNA splicing and results in a truncated protein [38], and as a result, CYP3A5 *3 homozygous individuals produce attenuated levels of functional CYP3A5 protein. Given that vincristine is preferentially metabolized by CYP3A5 [39,40], its clearance is dependent on the presence of a functional CYP3A enzyme. As a whole, Asians and African Americans have a higher prevalence of nonfunctional CYP3A5 alleles (CYP3A5 *6, *7), while a relatively large percentage of Caucasians express a CYP3A4 *22 isoform with intermediate metabolic capacity [41*], suggestive of an increased risk for VIPN in these subpopulations and the potential use of CYP3A4 and CYP3A5 activity as future biomarkers for vincristine efficacy and toxicity.

**CONCLUSION**

Pharmacogenes can account for major interindividual differences in drug bioavailability, efficacy, clearance, and toxicity. Genetic polymorphisms are able to partially explain this variability, and when the data are available, allow stratification of individual patients into metabolic phenotype...
categories, although these genotype–phenotype relationships are infrequently considered due to a general lack of evidence-based recommendations to guide clinical decision making. In this mini-review, we highlighted three drug classes with noteworthy pharmacogenomic insights, with thiopurines as the only class to have widely recognized and accepted pharmacogenomics-based dosing guidelines. Although perhaps the most significant limitation to the implementation of pharmacogenomics is the lack of adequately powered pediatric clinical trials during development and postmarketing surveillance of drugs, other barriers include the storage of genomic data in the electronic health record and the lack of coverage of genotype testing by many insurance plans.

Additional research is needed to evaluate phenotypic variations in pharmacogenes that are mediated via epigenetic mechanisms. This is an important dimension of genomics research that remains largely underdeveloped in the field of pharmacogenomics, a somewhat surprising finding when considering the potential impacts of xenobiotics, diet, and lifestyle choices (such as smoking and alcohol use) on drug metabolizing enzymes. It is likely that complex gene-environment–lifestyle interactions account for many of the observed inconsistencies described in the medical literature, and clinicians must be cognizant that pharmacogenes, like other genes, are subject to complex interactions that define specific response profiles. As such, development of precision-based approaches to account for such interactions is necessary to personalize drug treatments in ways that optimize clinical efficacy and minimize toxicity.

Finally, it is important to integrate the principles of precision survivorship into the practice of precision medicine. Clearly, the chemotherapeutic and cell-based therapies being utilized in pediatric populations have long-lasting consequences on the health and wellbeing of these patients. In an era where childhood cancer survivors are living years beyond their diagnosis, efforts should be made to capitalize on the advances in genomic medicine to mitigate the side effects of chemotherapy and preserve the quality-of-life of patient survivors for the years to come.

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Saiz-Rodriguez M, Almenara S, Navares-Gomez M, et al. Effect of the most relevant CYP3A4 and CYP3A5 polymorphisms on the pharmacokinetic parameters of 10 CYP3A substrates. Biomedicines 2020; 8:94. The article examined the influence of CYP3A4 and CYP3A5 polymorphisms on pharmacokinetics and enzymatic function in healthy volunteers receiving single doses of several drug substrates. Evidence was presented that in CYP3A4 mutant allele carriers, substrates exclusively metabolized by CYP3A4 showed a higher normalized area under the curve and a tendency toward reduced normalized clearance.


