REVIEW

Phase I and Phase II Enzyme Polymorphisms and Childhood Cancer

Ryan Swinney, Stephanie Hsu, Gail Tomlinson

ABSTRACT

Childhood cancers continue to be challenging clinical entities whose etiology, demographic characteristics, clinical progression, treatment efficacy, and outcomes remain incompletely understood. Research suggests that multiple environmental and genetic factors may play crucial roles in the pathophysiology of many of these malignancies.

Recent attention has been directed to the role of carcinogen metabolizing enzymes in the etiology and progression of cancer in both adults and children due to their multitude of polymorphic variants and their intimate interaction with environmental factors. In particular, xenobiotic metabolizing enzymes (XME), which are intimately involved in the activation and deactivation of many environmental carcinogens, have become an area of significant interest. Traditionally, these enzymes have been classified into either phase I or phase II enzymes depending on their substrates, activity, and occasionally based on their sequence in the metabolic pathways, and have been demonstrated to have numerous polymorphic variants. Phase I enzymes predominantly consist of cytochrome enzymes responsible for mixed function oxidase activity, whereas phase II enzymes are frequently conjugation reactions necessary for drug metabolism or the further metabolism of phase I enzyme products.

Current research has discovered numerous interactions between polymorphisms in these enzymes and changes in cancer susceptibility, treatment efficacy, and clinical outcomes in childhood cancer. Furthermore, studies of polymorphisms in these enzymes have demonstrated to have synergistic/antagonistic interactions with other XME polymorphisms and demonstrate variable influences on disease pathophysiology depending on the patient's ethnic background and environmental milieu. Continuing research on the role of polymorphisms in phase I and phase II enzymes will likely further elucidate the intimate role of these polymorphisms with environmental factors in the etiology of childhood cancer.

Key Words: xenobiotic metabolizing enzymes, childhood cancer, acute lymphoblastic leukemia, phase I enzymes, phase II enzymes

Childhood cancer continues to be the leading cause of disease-related death in children aged 0 to 14 years, with approximately 9,510 new cases and 1,585 deaths owing to malignancy expected in 2005.[1] Unlike adult cancers that are mostly carcinomas, childhood cancers derive from less differentiated tissue origins and are limited to a definitive range of ages at onset. Marked differences in cancer presentation, susceptibility, and prognosis have been noted in different ethnic groups and genders.[2] Even when children present with malignancies also seen in adults, their disease is often distinct in clinical presentation, treatment response, and long-term sequelae. In the case of acute lymphoblastic leukemia (ALL), children present with disease that is biologically distinct, responds extremely well to chemotherapy, and does not confer the same poor outcome as the adult form. Although treatment outcomes have improved for all childhood malignancies, childhood leukemia is noteworthy for its very high rate of disease-free survival, with approximately 80% of patients attaining prolonged disease-free remission.[3] These differences between adult and childhood cancer suggest that these malignancies represent very different origins, pathogenesis, and genetic etiologies.

Research has begun to elucidate the etiologic components in childhood cancer. The results suggest a multifactorial etiology composed of exogenous factors, including infection, environmental factors, and prenatal exposures.[2] However, pediatric cancers present a unique situation in which a limited amount of time has elapsed for carcinogenic factors to have caused sufficient genetic ''hits'' to accrue for cancer development, which contrasts with the etiologic model of most adult malignancies. Therefore, investigations have begun to look at the role of heritable

From the University of Texas Southwestern Medical Center, Department of Pediatrics (G.T.), University of Texas Southwestern Medical Center, Dallas, TX; Hamon Center for Therapeutic Oncology Research (S.H., G.T.).

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Address correspondence to: Dr. Gail Tomlinson, Department of Pediatrics, Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8593; tel: 214-648-4907; fax: 214-648-4940; e-mail: gail.tomlinson@utsouthwestern.edu.

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genetic mutations in the etiology of cancer coupled with developmental events and exposures. Mutations affecting the function of major cell-cycle regulatory genes, such as *TP53* and *RB1*, have shown functional roles in the development of sarcomas and retinoblastomas in children.[4–6] However, the majority of pediatric malignancies cannot be attributed to a single mutation in a major predisposition gene. The additive effect of low-penetrance genetic factors, including but not limited to polymorphisms in xenobiotic metabolizing enzymes (XMEs), may play a primary role in cancer development as well.[7–9]

XMEs consist of several classes of enzymes responsible for the metabolic activation and inactivation of carcinogenic substances, such as pesticides, petroleum products, and polycyclic aromatic hydrocarbons (PAHs), and account for the majority of interindividual variation in the ability to activate or deactivate certain carcinogenic agents. These enzymes have traditionally been categorized in phase I and phase II enzymes based on their reaction type, substrate, and sequence of reactivity. Phase I enzymes consist primarily of cytochrome P-450 (CYP450)-dependent mono-oxygenases that reside in the cytosol and are primarily responsible for increasing the hydrophilicity of lipophilic substrates through oxidative, reductive, or hydroxylation reactions. Many of the resultant products have carcinogenic properties. Phase II enzymes consist of cytosolic enzymes involved in conjugation reactions wherein hydrophilic moieties such as glutathione, nicotinamide adenine dinucleotide, and acetyl groups are attached to lipophilic substances and phase I enzyme products, thereby increasing their hydrophilicity and promoting subsequent excretion (reviewed in Sheweita[10]). Although both classes of enzymes exist in the majority of human tissues, their expression is notably increased in tissues involved in the active metabolism and excretion of carcinogenic materials, such as in the gastrointestinal tract, particularly the liver, and in the genitourinary system.

XMEs have become a popular area of investigation for cancer genetics research owing to their key role in carcinogen metabolism and their numerous functional polymorphisms. Investigators have documented several polymorphisms in the exons, introns, and promoter regions of XME-coding genes, which alter gene expression, protein translation, and enzymatic function (reviewed in Hirvonen[11]). Such polymorphisms can have significant impacts on cancer susceptibility based on the gene, the enzyme's substrate, the tissues within which the enzyme is predominantly expressed, and the extent of subsequent genotoxicity.[12,13] Recent studies have demonstrated a clear association between specific XME polymorphisms and the development of bladder, colon, lung, breast, and prostate cancers in adults.[14–17] Researchers have also demonstrated that such polymorphisms may affect important clinical aspects of cancer, including age at onset, disease progression, treatment efficacy, and patient outcomes.[18,19] Studies have also begun to explore the complex interactions of these polymorphisms with environmental exposures and lifestyle factors.[20] Furthermore, several articles have provided comprehensive reviews of these enzymes and their impact on adult cancers.[21] In contrast, little research has been conducted on the impact of XME polymorphisms on childhood cancer.

Preliminary studies have documented associations between certain phase I and II enzymes and childhood cancer, particularly in ALL.[7–9,22–26] However, the influence of these polymorphisms on the development of other common childhood cancers, such as neuroblastoma, lymphoma, osteosarcoma, and Wilms' tumor, remains largely unexplored, perhaps in part owing to a significantly lower number of cases relative to ALL. Polymorphisms in XME might also impact the clinical aspects of childhood cancer similar to those of adults, and initial studies have begun to support such conclusions.[26] Nonetheless, further research is needed to better clarify the full impact of XME polymorphisms on childhood cancer. Unlike with adult cancers, no comprehensive review of the impact of phase I and II enzyme polymorphisms on childhood cancer has been completed. Therefore, we constructed this review to integrate and summarize the current status of research on the impact of XME polymorphisms on cancer in children.

PHASE I ENZYMES

Phase I enzymes are functional drug-metabolizing agents and include a variety of mixed-function oxidases (mono-oxygenases). Phase I enzyme substrates are either activated to carcinogenic compounds or excreted efficiently owing to their increased hydrophilicity after phase I enzyme interaction. The most broadly studied phase I enzymes belong to the CYP450 family.

CYP450 ENYZMES

There are currently over 200 known, active CYP450 isozymes encoded for by approximately 60 genes. CYP450 enzymes are expressed mostly in hepatic cells, the primary site of detoxification, but are also found in other tissues, including lung, kidney, nasopharyngeal, and gastrointestinal tract tissue (reviewed in Sheweita[10] and Sheweita and Tilmisany[21]). The most common variants of CYP450 include CYP1A1, CYP2E1, CYP2D6, and CYP3A4 and CYP3A5. CYP450 enzymes are primarily associated with substrate oxidation, reduction, and/or hydroxylation to create more electrophilic derivatives and act as the terminal oxidase for the electron transport system of multifunction oxygenases, a necessary component for the biotransformation of many xenobiotic substances. CYP450 activates xenobiotics by oxygenation, thus increasing polarity and allowing easier excretion; however, polarization may also result in harmful metabolic activation of formerly inert carcinogens to more toxic or reactive substances. Specific population

variants in the CYP450 genes that may affect cancer susceptibility are discussed in detail below.

Although CYP450 enzymes are usually substrate nonspecific, enzymes may still exhibit substrate preference and isoenzyme specificity. Among the target substrates, PAHs are the most carefully studied. CYP450 enzymes activate PAHs into reactive intermediates via the reduction of the cyclic aromatic carbon ring. PAH activation produces ionized side-chain moieties that can randomly damage genes via the fusion of nucleotides into deoxyribonucleic acid (DNA) adducts, which can alter transcriptional activity and the eventual product protein's activity. Current research has presented contrasting results regarding the CYP450 enzyme that is most effective in causing DNA adduct formation resulting from PAH derivatives. Other CYP450 substrates include aromatic amines and amides and *N*-nitrosamines. Common *N*-nitrosamines include nitrites (cured meats and fish) and nonaromatic amines (drugs and medicines, agricultural chemicals, and food constituents and additives).

A variety of naturally occurring chemicals have been found to alter CYP450 activity. Chemical inducers of CYP450 stimulate CYP450 activity by enhancing transcription rates and are classified according to the affected isoenzyme type: 3-methylcholanthrene (3-MC) or phenobarbital (PB). 3-MCs target hepatic microsomal and nuclear CYP450 enzymes, whereas PBs target mitochondrial mixed-function oxidases. Hepatic nuclear CYP450s are also induced by microsomal CYP450 compounds, such as pregnenalone 16α-carbonitrile, PB, and β-naphthoflavone (β-NF). Interestingly, CYP450-inducing agents bind to aromatic hydrocarbon receptors rather than directly interact with the enzyme, creating a complex that is then transported to the nucleus, resulting in increased transcription and synthesis of CYP450 apoproteins, the precursors of the active CYP450 enzymes. Increased transcriptional activity and a high PB titer simultaneously trigger an increase in C^{14}-leucine, a chemical that down-regulates the degradation of the CYP450 proteins, thus prolonging the functional lifetime of the CYP450 enzyme. This positive-feedback loop further induces elevated CYP450 levels.

In contrast, chemical inhibitors of CYP450 convert the active enzyme to cytochrome P420, its inactive counterpart. Among CYP450 inhibitors are lipases and detergents, which alter the lipid environment or the protein structure of multifunction oxidases. Other inhibitors include chemical compounds such as carbon disulfide, heavy metals, and allyl-containing barbiturates. Researchers have demonstrated the ability of these inhibitors to destroy their target enzyme both in vivo and in vitro, thus preventing complete bioactivation of xenobiotics.

CYP1A1

Chemical oxidation via CYP1A1 enzymes usually produces metabolically active carcinogens, although CYP1A1 chemical induction varies throughout the body. CYP1A1 enzymes target large, planar molecules such as aromatic amines and aromatic amides, azo compounds, and mycotoxins, which are oxygenated to epoxides and other carcinogenic metabolites. These conformationally hindered products serve as poor substrates for phase II enzymes; thus, CYP1A1 enzymes are effective toxin inducers.[10] PAHs, β-NF, isosafrole, and tetrachlorodibenzo-p-dioxin (TCDD) induce CYP1A1 activity in kidneys, whereas polychlorinated biphenyl and TCDD are effective CYP1A1 inducers in murine lungs. CYP1A1 inhibitors vary in composition from flavonoids to sulfur dioxide in air pollutants.[27,28] Oxidation of aryl hydrocarbon hydroxylase (AHH), a CYP1A1-specific substrate located in human lung tissue, converts PAH benzo[*a*]pyrene 7,8-diol into its active carcinogenic form, a process that has been closely linked to an increased risk of lung cancer.[10] Interindividual variation of CYP1A1-mediated AHH activity has also been observed.[29]

There are currently two known, consistent polymorphisms on the *CYP1A1* gene.[30] The first occurs as a base substitution at base pair 6235 (T6235C) in the 3′ region of the gene. Detected by the *Msp*I restriction enzyme, this mutation is identified as the CYP1A1 *Msp1* variant allele or *Msp1* mutation or, more commonly, m1. The second polymorphism, A2455G, causes an amino acid change from Ile to Va, also identified as m2. The different CYP1A1 variant alleles are defined by the polymorphisms expressed. CYP1A1*2A is characterized by m1 only, whereas CYP1A1*2B contains both m1 and m2 variants. CYP1A1*2C is characterized by an m2 variant only. CYP1A1*4 carries a C4887A substitution, resulting in a Thr to Asp amino acid substitution at position 461. Additional information on these polymorphisms may be found on the Human CYP450 Allele Nomenclature Committee Web site (<http://www.cypalleles.ki.se>).

Increased transcription and messenger ribonucleic acid (mRNA) levels in ALL patients indicated an association between these coding disruptions and increased CYP1A1 inducibility, implicating increased risk of various cancers owing to higher concentrations of metabolized carcinogens.[29] In utero exposure to environmental PAHs, such as those found in cigarette smoke and cooked foods, has been linked to higher placental CYP1A1 activity and a modified risk of childhood ALL (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.1–3.1).[23] Similarly, Infante-Rivard and collegues observed that in utero and postnatal exposure to household pesticides, coupled with CYP1A1 mutations, significantly increased the risk of childhood ALL (varying OR [0.38–2.27] depending on the pesticide type and/or amount and in utero versus postnatal exposure).[25] Recent studies have reported a poor treatment outcome for childhood ALL patients expressing a CYP1A1 2A variant[26] and strong associations between CYP1A1 variant alleles and increased susceptibility to childhood ALL.[23]

Various correlations between allele genotype and cancer risk have been reported, although many remain

unconfirmed by subsequent studies, resulting in thethe impact of these variant alleles being poorly understood.[30–32] Other studies attempting to correlate enzyme activity to cancer susceptibility have suggested that the variant alleles influence enzyme inducibility rather than the *CYP1A1* gene; however, such claims also remain controversial, especially between different ethnic groups.[31,33–38]

CYP2E1

CYP2E1 enzymes oxidize compounds into more reactive states by creating free radicals on target substrates, which activates naturally occurring carcinogens, such as *N*-nitrosamines, into highly mutagenic agents through catalysis of bioactivating processes (ie, demethylation and denitrosation).[10,39] The reactive products can further increase cellular oxidative stress via other metabolic functions, including lipid peroxidation and carbon tetrachloride bioactivation, resulting in a marked increase in DNA adduct formation. Environmental and intracellular exposure to alcohol, ethanol, and tobacco smoke has also been observed to cause increased CYP2E1 activity. Higher CYP2E1 expression results in greater carcinogenic bioactivation, suggesting that individuals with this phenotype may incur an increased risk of CYP2E1 substrate–related cancers. Enzyme inhibition occurs with exposure to cis-1, 2-dichloroethylene, trans-1,2-dichloroethylene, and other chemical compounds (Table 1).[26]

Known variant CYP2E1 alleles include CYP2E1*2 (G227A exon 2 mutation), CYP2E1*3 (G1165A exon 7 mutation), and CYP2E1*5 (G1259C).[40] Clinical studies on pediatric cancers have correlated susceptibility to childhood ALL with CYP2E1 expression; however, reports from different institutions and patient cohorts have yielded conflicting results, causing difficulty in drawing consistent correlations between variant genotype and phenotypic effects. Among the reported trends, Canalle and colleagues observed no effect on childhood ALL susceptibility when CYP2E1*2 and CYP2E1*3 variants were considered independent ALL-inducing alleles (p = .485 and 0.795, respectively) but a significant increase in childhood ALL when coupled with CYP1A1, glutathione *S*-transferase M1 (GSTM1), and GSTP1 variant alleles (OR 10.3).[9] An independent study observed an increased risk of childhood ALL in patients expressing the CYP2E1*5 variant allele (OR 2.8) without a coupling effect from other phase I and/or phase II enzyme polymorphisms.[8] Because of current uncertainties regarding CYP2E1 polymorphism function and cancer susceptibility, Krajinovic and colleagues suggested future studies to clarify correlations between variant allele genotype and environmental carcinogen exposures and the resulting influence on cancer susceptibility.[8]

CYP2D6

CYP2D6 enzymes target pharmacologic drugs, such as debrisoquine and sparteine, and medications and inactivate them through total ring hydroxylation or oxidation.[10] Ethanol has been proven to increase CYP2D6 expression in murine brain cells,[41] whereas inhibition studies have identified amiodarone, quinide, and various herbal remedies as effective CYP2D6 suppressors.[42–46] CYP2D6 enzyme metabolizes 20% of current prescription drugs.[47] As such, it is the most extensively studied CYP450 subfamily, with over 40 identified mutations, each capable of distinct modulation of enzyme activity (<http://www.cypalleles.ki.se>). Carriers of CYP2D6 variants are categorized based on their effectiveness as drug metabolizers. Poor metabolizers are characterized by inactivated drug metabolism resulting from homozygous null alleles. Variants CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*6 are among the most common inactivating alleles, accounting for the genotype of 95% of European polymorphisms.[48–50] Intermediate metabolizers can be either carriers of one inactive allele or homozygous for an allele with reduced metabolic activity.[51,52] Among the decreased efficiency alleles are CYP2D6*10 (a Pro to Ser substitution) and CYP2D6*17, characterized by a possibility of three different amino acid substitutions (<http://www.cypalleles.ki.se>). Multiple copies of functional CYP2D6 genes, either interspersed or tandem, result in characteristically fast metabolism of target drugs. Recently, two additional alleles have been identified as ultrarapid metabolizers. CYP2D6*41 (C1584G) and CYP2D6*35 (G31A and C1584G) coupled with a CYP2D6*2 mutation result in extremely fast drug metabolism without duplicate genes.[53]

CYP2D6 variants have been extensively studied in adult cancers owing to the enzyme's demonstrated role in targeting chemotherapeutic agents such as tamoxifen into their effective forms. Associations with breast and lung cancer and various liver diseases have been investigated, although no correlation between genotype and cancer survival has been determined.[54,55] Despite being the most studied CYP450 subfamily, limited research has been conducted regarding CYP2D6 variant genotypes and pediatric cancers. One study attempting to correlate various XME variant alleles and susceptibility to childhood ALL did not find significant phenotypic effects for either CYPD6 allele studied (CYP2D6*3, CYP2D6*4) even when considered in multivariate analysis.[23]

Of particular interest regarding this CYP450 subfamily is the variance of allele expression between ethnic groups. For example, Ethiopians and Caucasians exhibit a high incidence of gene duplication, whereas Asians, Africans, and African Americans are more commonly categorized as intermediate metabolizers.[56–60] These ethnically specific allele distributions underscore the importance of considering the influence of environment and ethnic background on genetic cancer susceptibility.

As seen with CYP2E1, geographic differences resulting in different environmental carcinogen exposures may be an explanation for discrepancies in research data owing to novel gene-environment interactions. For example,

TABLE 1 Chemical Compounds Altering Phase I and II Enzyme Activity

Phase I/II Enzyme	*Substrates*	*Inducers*	*Inhibitors*	*Reference*
CYP450	Polycyclic aromatic hydrocarbons (PAHs), aromatic amines (dyes, textile pigments, paint, plastic, drugs, pesticides, rubber/tire/cable antioxidants), aromatic amides, *N*-nitrosamines (nitrites, nonaromatic amines) Pesticides: aldrin, atrazine, carbaryl, chlorfenvinphos, chlorpyrifos, lindane, parathion	3-Methylcholanthrene (3-MC) or phenobarbital (PB) chemicals, microsomal CYP450 compounds (pregnenalone, 16α-carbonitrile, PB, β-naphthoflavone (β-NF), aroclor	Lipases, detergents, chemical compounds: carbon disulfide, heavy metals, arllyl-containing barbituates, 2-allyl-2-isopropyl acetamide	10, 11, 25–27
CYP1A1	Aryl hydrocarbon hydroxylase, PAHs, halogenated polycyclics, aromatic amines and amides, azo compounds, mycotoxins, polychlorinated biphenyl	PAH, β-NF, 2AAF, tetrachlorodibenzo-p-dioxin, isosafrole	Flavonoids (3-hydroxy-, 5-hydroxy-, 7-hydroxy-, 3,7-dihydroxy flavones), sulfur dioxide	10, 11, 27, 28
CYP2E1	*N*-Nitrosamine, benzene, ethanol, halogenated solvents, xenobiotics within organic solvents, pesticides, plastic derivatives	Environmental alcohol, ethanol, exposure to tobacco smoke	Cis-1, 2-dichloroethylene, trans-1,2-dichloroethylene, protein kinase A	8, 9, 27, 40
CYP2D6	Pharmacologic drugs (debrisoquine, sparteine), medications (neuroleptics, antidepressants, β-blockers, antiarrhythmics, opioids)	Ethanol	Aminodarone, quinide, compounds in herbal remedies (substances in St. John's wort)	10, 11 46, 57
CYP3A4/5	Aflatoxin B1, osteosarcoma-treating compounds (ifosfamide, vinblastine, etoposide, doxorubicin), steroids (testosterone, cortisol), chemotherapeutic agents (epipodophyllotoxins), teniposide, cyclophosphamide, vindesine	Rifampicin, anticonvulsants, glucocorticoids (ie, dexamethasone), clotrimazole, phenytoin, phenylbutazone, omeprazole	Azole antifungal agents, macrolide antibiotics, liver disease, aging, erythromycin, triamterene	42, 56, 63–65
GST	Glutathione, electrophilic substrates, PAH epoxide metabolites, cyclophosphamide, nitroglycerin, L-phenylalanine mustard, chlorambucil, cyclophosphamide	Endogenous disulfides, phenolic antioxidants, flavonoids, intermediates in drug/compound metabolism, sulforaphane, relofen, indole-3-carbinol, D-limonen, PB, 3-MC, azo dyes, thiocarbamates, isothiocyanates, cinnamates	Acriflavine, allyl disulfide, trivalent antimony in erythrocytes	27
GSTM1/ GSTM3	CYP1A1-activated compounds, compounds in tobacco smoke (benzo[*a*]pyrene diol epoxide, 4-methylnitrosamin-1-3-pyridyl-1-butanone), acetaminophen, carbon tetrachloride, aflatoxin B1, epoxides		Glutathione analogues; GSTA shows some effect	10, 11, 23, 27, 74
GSTP1	Benzo[*a*]pyrene epoxide, acrolein, inhaled carcinogens, acetaminophen, carbon tetrachloride, aflatoxin B1	Diterpenses (cafestol, kahweol), α-angelica lactone, coumarin		7, 11, 26, 29
GSTT1	Monohalomethanes, epoxide metabolites of butadiene, 1,2:3,4-diepoxybutane, trihalomethanes			26, 84
NAT	Xenobiotics with primary aromatic amine or hydrazine structures, arylamines (4-aminophenyl, 2-naphthylamine), heterocyclic amines, arylhydrazines, arylhydroxylamines, dinaline, benzidine, 2-aminoflurene	*p*-Aminosalicyclic acid (ASA), sulfamethazine (SMZ)	Paclitaxel	10, 11, 22, 85
NAT1	Aromatic and heterocyclic amine carcinogens	ASA, SMZ	Hydroxylamine/nitroso intermediates of NAT1 substrates	85, 86
NAT2	Arylamine drugs (ASA, procainamide, SMZ, benzidine, isoniazid, hydralazine	ASA, SMZ		10, 11, 85, 86
NQO1	Benzene metabolites (quinones), nitroaromatic compounds (heterocyclic amines found in tobacco smoke)	Antioxidants, heavy metals, ionizing radiation, xenobiotics, ultraviolet radiation	Coumarins (dicoumarol), flavones, curcumin	94, 95, 98, 100

children in rural areas may receive high dosages of pesticides, whereas children in industrial cities may have greater exposure to air pollutants and other industrial carcinogens. A study from India exhibited unusually low allele frequencies of CYP2D6*4 in childhood ALL patients (0% homozygous variant; heterozygous: 9.1% cases versus 17.1% controls), which thereby suggests that the absence of CYP2D6 expression in these ALL patients presents a possible protective effect because of reduced ability to metabolize chemotherapeutic drugs; however, the size of the patient population allowed only for borderline significant results (OR 0.49, 95% CI 0.19–1.22, $p = .12$).[61] In a Portuguese patient population, however, Lemos and colleagues observed a high frequency of CYP2D6 variant in their studied leukemia cohort, which conflicted with previously reported leukemia genotype studies (50% vs 35.9%; $p = .06$).[24] As such, further research controlling for ethnicity and geographic and associated environmental factors would be necessary to determine more accurate, population-specific assessments of CYP2D6 variant functionality.

CYP3A4 and CYP3A5

CYP3A4 and CYP3A5 enzymes, located on chromosome 7q21.3-q22.1, account for 30 to 60% of total liver cytochromes and are involved in the oxidation of ifosfamide, vinblastine, etoposide, doxorubicin, and other chemotherapeutic agents, in addition to naturally expressed steroids (testosterone and cortisol) and aflatoxin B1.[62–64] In addition to liver expression, CYP3A4 and CYP3A5 enzymes have been located in small intestine surface epithelium, gallbladder, proximal renal tubular epithelium, and lutein cells in ovaries.[64] Increased CYP3A4 and CYP3A5 expression is linked to cellular exposure to rifampin and anticonvulsants, whereas azole antifungal agents, macrolid antibiotics, liver disease, and aging are attributed to CYP3A4 and CYP3A5 inhibition.[56]

The CYP3A4 enzyme is involved in the metabolism of epipodophyllotoxin, a chemotherapeutic drug whose inhibition influences the development of secondary myelogenous leukemias in association with gene translocations at chromosome band 11q23, the *MLL* (myeloid/lymphoid or mixed-lineage leukemia) gene.[65] Consequently, increased CYP3A4 expression has been speculated to increase the risk of leukemia. In a study on pediatric secondary cancer patients, the CYP3A4 variant allele was found in approximately 50% of blacks and 9% of white children diagnosed with secondary cancer, suggesting a potentially significant increase in susceptibility for secondary leukemias in blacks when compared with de novo cases. Surprisingly, when compared with the aggregate and control frequencies (15% and 19%, respectively), the CYP3A4 variant allele seemed to indicate decreased enzyme activity and thus reduced the risk of ALL as a secondary cancer in *MLL* translocation–positive patients, whereas CYP3A4 wild-type expression indicated an increased risk of treatment-related leukemia, although the ORs were incalculable owing to a lack of *MLL* (+), CYP3A4-V, secondary leukemia patients.[65] Recently, however, Collado and colleagues reported no association between the CYP3A4 variant allele and childhood ALL predisposition,[66] although the conflicting results could be explained by both studies involving small cohorts.

The pertinent polymorphism in CYP3A5 (A22893G), which is responsible for decreased metabolic activity of the enzyme, was studied by Blanco and colleagues and was not found to be associated with treatment-related malignancy in children from different ethnic backgrounds (black, white, Hispanic).[67] Similar studies in other childhood malignancies regarding CYP3A4 and CYP3A5 have not been performed at this time.

In addition to being CYP3A4 and CYP3A5 target substrates, ifosfamide, vinblastine, etoposide, and doxorubicin are effective chemotherapeutic treatments for osteosarcoma, suggesting a possible role of CYP3A4 and CYP3A5 as prognostic factors in chemotherapy resistance owing to their potential to inactivate these drugs. Dhaini and colleagues developed a quantitative fluorescence-based immunohistochemistry technique to measure CYP3A4 and CYP3A5 expression levels in osteosarcomas.[63] This technique uses molecular targets for CYP3A4- and CYP3A5-activated chemotherapeutic agents to stimulate CYP3A4 and CYP3A5 expression, thus demonstrating CYP3A4 and CYP3A5 efficacy as a biomarker of osteosarcoma treatment prognosis.[63] High CYP3A4 and CYP3A5 expression in tumors that metastasize suggests that CYP3A4 and CYP3A5 provide a protective mechanism for tumor cells and thus a poor prognosis for osteosarcoma patients.

PHASE II ENZYMES

Phase II enzymes are primarily linked to drug detoxification; the classic view of phase II enzymes is that of metabolizers of phase I enzyme products. However, phase II enzymes are not solely dependent on phase I enzyme activity because phase II enzymes are responsible for other metabolic activities as well. Common phase II enzymes include the glutathione *S*-transferase (GST) classes, *N*-acetyltransferases (NATs), and reduced nicotinamide adenine dinucleotide phosphate (NAD[P]H):quinine oxidoreductase 1 (NQO1).

GLUTATHIONE *S*-TRANSFERASE

GST enzymes are found in almost all mammalian hepatocytes and are responsible for catalyzing the conjugation of reduced glutathione (GSH) to electrophilic substrates.[21] In addition, GSTs catalyze glucuronidation, sulfonation, acetylation, and methylation using GSH and/or amino acids.[10] GSTs stimulate drug detoxification via a reduction in

free radicals and/or DNA sequestering by direct binding. Based on their primary structure, substrate specificity, and immunoreactivity, GST enzymes are categorized into four main families: GST α, π, μ, and θ (GSTA, GSTP, GSTM, and GSTT, respectively).[68] Despite these distinctions, GST enzymes also demonstrate overlapping substrate affinities and chemical induction or inhibition characteristics. GST enzymes target electrophilic substrates such as PAH epoxide metabolites, nitroglycerin, and chlorambucil for subsequent reduction. GST chemical inducers include endogenous disulfides, phenolic antioxidants, flavonoids, and intermediates in drug or compound metabolism. Likewise, GST activity is effectively inhibited by acriflavine, allyl disulfide, and glutathione analogues.[21] GST overexpression in tumors is characteristic of GST activation under oxidative stress.

GSTM1 and GSTM3

GSTM gene subfamilies are encoded by chromosome 1p13.3 and arranged in a 5′-GSTM4-GSTM2-GSTM1-GSTM5-GSTM3-3′ gene cluster.[69] GSTM1 exhibits a deletion mutation, resulting in a null phenotype and absence of GSTM1 protein production. Numerous studies have demonstrated an association of the null genotype or phenotype with increased cancer risk.[70–72] In addition, increased sister-chromatid exchange and chromosome aberrations have also been observed in conjunction with GSTM1 null genotype individuals,[73] behavior that may be associated with cancer risk predictivity.[72] Several GSTM1-specific substrates have been identified, including CYP1A1-activated compounds, benzo[*a*]pyrene diol epoxide, and other epoxides.[10,23,74]

The GSTM1 null genotype has been widely studied as an indicator of increased childhood ALL susceptibility. A study in French Canadian children demonstrated an association between GSTM1 null genotype with ALL (OR 1.8, 95% CI 1.2–2.6).[23] Furthermore, when GSTM1 null genotype is coupled with GSTP1*B, another risk-elevating variant allele, the risk of childhood ALL increased (OR 2.1, 95% CI 1.3–3.4), suggesting a strong gene-gene interaction.[7] Hall and colleagues reported GSTM expression in bone marrow indicative of a significantly increased risk of relapse in childhood ALL patients (OR 2.95, 95% CI 1.25–7.26, white blood cell count and age at diagnosis adjusted).[75]

Barnette and colleagues reported GSTM1 heterozygotes at an increased risk of childhood ALL compared with the null genotype (OR 5.661, 95% CI 2.581–12.415 or OR 4.3, 95% CI 1.8–10.2 depending on the variant; Table 2) in addition to glial brain tumors and osteosarcoma (OR; see Table 2).[76] Acknowledging others' conflicting reports regarding GSTM1 expression and pediatric cancer susceptibility, these investigators attributed their significant results to the patient population used, which exhibited the founder effect. Consequently, the investigators suggested that determination of genotype-based cancer susceptibility be assessed by geographic regions with uniform ethnic and genetic backgrounds. Also of interest is that Davies and colleagues demonstrated no association of the GSTM1 genotype on either susceptibility or outcome in ALL.[77]

Highly expressed in lung tissue, GSTM3 has also demonstrated a close association with lung cancer susceptibility in adults in addition to childhood ALL.[78] Better survival rates for childhood ALL were observed in children expressing the GSTM3 variant, although caution is advised in using their data because of a small GSTM3 cohort.[79]

GSTP1

GSTP1, located at chromosome 11q13, is highly expressed in lung tissue but is also expressed at lower levels throughout all body tissues, including bone marrow and blood.[7,10,70] Independent studies have reported conflicting associations between GSTP class enzymes and neuroblastoma.[80,81] Bourhis and colleagues described no significant correlation between GSTP expression and neuroblastoma but a significant tumor response when coupled with expression of P-glycoprotein, another drug-resistance mechanism against anticancer agents. Alone, P-glycoprotein expression exhibited multidrug resistance but also similarly exhibited no significant effect on neuroblastoma susceptibility.[82] Other studies either reported no significant impact on prognosis by GSTP1 genotype[80] or a minimally suggestive association between GSTP1 expression and neuroblastoma prognosis.[82] Thus, the true correlation between GSTP expression and neuroblastoma development and prognosis remains to be determined.

GSTP1 variants exhibit characteristics unique from all other GST enzymes, with each variant allele capable of producing a characteristic effect on childhood ALL susceptibility.[7] Whereas most GST variant genotypes correlate with increased susceptibility for childhood ALL, the GSTP1 variant genotypes, including GSTP1*B (I105V) and GSTP1*C (A114V), demonstrate a protective role against childhood ALL susceptibility. Increased ALL susceptibility was observed in individuals expressing the GSTP1*B mutant allele, whereas the GSTP1*C variant exhibited low expression in cases versus controls, suggesting an unexplored protective phenotype (OR 1.5, 95% CI 1.1–2.0; OR 0.7, 95% CI 0.4–1.1, respectively). Furthermore, varying susceptibilities to childhood ALL for each allele were correlated to altered enzymatic activity following exposure to different substrates. With an enumerate assortment of possible external and genetic factors influencing each unique GSTP1 variant genotype, it has proven difficult to isolate consistent trends across patient and ethnic groups. Such findings suggest further research on gene-gene and gene-environment interactions before concrete associations can be established.

TABLE 2 Reported Odds Ratios and 95% Confidence Intervals for Cited Studies

Variant	*Cancer Association*	N	*OR*	*95% CI*	p *Value*	*Reference*
CYP1A1*2A	Childhood ALL	170	3.9–5.55	1.1–3.1	—	23
CYP1A1+ ≥ 2 pesticides						
In utero	Childhood ALL	491	1.96	1.33–2.88	—	25
Postnatal	Childhood ALL	491	1.65	1.23–2.23	—	25
CYP1A1+ ≥ 2 insecticides, rodenticides						
In utero	Childhood ALL	491	2.18	1.5–3.17	—	25
Postnatal	Childhood ALL	491	1.8	1.32–2.44	—	25
CYP1A1*2 + CYP1A1*3 + GSTP1 non-null + GSTM1 null	Childhood ALL	113	10.3	1–111.8	.05	9
CYP2D6 functional allele genotype	Childhood ALL	118	0.49	0.19–1.22	—	61
GSTM1 null	Childhood ALL relapse (adjusted for WBC and age at diagnosis)	71	2.95	1.25–7.26	—	75
GSTM1 null + GSTT1 null	Childhood ALL	174	1.8	1.2–2.6	—	23
GSTP1*B + GSTM1 null	Childhood ALL	269	2.1	1.3–3.4	—	7
GSTM1*A heterozygous	Childhood ALL	326	5.661	2.581–12.415	< .001	76
GSTM1*B heterozygous	Childhood ALL	326	4.278	1.795–10.195	.001	76
GSTM1 heterozygous	Glial brain tumor	326	4.865	1.487–15.921	—	76
GSTM1*A heterozygous	Osteosarcoma	326	6.900	1.116–42.653	.038	76
GSTM1*B heterozygous	Osteosarcoma	326	16.000	2.775–92.244	.002	76
GSTP1*B	Childhood ALL	278	1.5	1.1–2	—	7
GSTP1*C	Childhood ALL	278	0.7	0.4–1.1	—	7
GSTT1 heterozygous	Childhood ALL	300	2.592	1.068–6.289	—	76
GSTT1 null	Childhood ALL relapse after prednisone treatment	420	0.25	NA	.07	83
GSTT1 null + trihalomethanes	Childhood ALL	491	9.1	1.4–57.8	—	85
NAT2 rapid acetylator	Childhood ALL	176	0.7	0.4–1.0	.03	89
NAT2 rapid acetylator + NAT1*4 homozygous	Childhood ALL	176	0.3	0.1–1.0	.03	89
NAT1*4 + NAT2 slow acetylator	Childhood ALL	176	1.9	1.1–3.4	.03	89
NAT2 slow acetylator	Childhood ALL	176	1.5	1.0–2.2	.03	89
NAT2 slow acetylator + GSTM1 null or + CYP1A1*2A (all homozygous)	Childhood ALL	176	2.7	1.4–4.9	.002	89
NAT2 slow acetylator + GSTM1 null + CYP1A1*2A (all homozygous)	Childhood ALL	176	3.1	1.1–8.4	.03	89
NQO1*2 heterozygous	Burkitt's lymphoma	71	1.81	1.04–3.15	.036	106
NQO1*2 heterozygous	Burkitt's lymphoma ≤ 9 yr at diagnosis	38	3.02	1.47–6.18	.003	106
NQO1 null/low expression + *MLL* gene rearrangements	Childhood ALL	36	2.54	1.08–5.96	.015	112
NQO1 C609T null/low expression + *MLL* gene rearrangements	De novo, B-lineage childhood ALL	39	2.47	1.08–5.68	.033	103
NQO1 C609T null/low expression + *MLL* gene rearrangements	De novo, B-lineage childhood ALL (ethnically matched population)	39	2.5	NA	.02	103

ALL = acute lymphoblastic leukemia; CI = confidence interval; NA = not available; OR = odds ratio; WBC = white blood cell count.

GSTT1

Located on chromosome 22, the GSTT1 variant exhibits a gene deletion with varying implications in ALL susceptibility.[68] GSTT1 enzymes are highly expressed in erythrocytes and target substrates such as monohalomethanes, epoxide metabolites of butadiene, and 1,2:3,4-diepoxybutane.[10] GSTT1 enzymes have also demonstrated excellent binding characteristics to steroid hormones such as glucocortocoids, which are often used in cancer treatment, and deletion is associated with the initial treatment response.[83]

The GSTT1 null genotype has demonstrated a strong association with childhood ALL susceptibility worldwide. In a comparative study between black and white children, Chen and colleagues reported significantly higher GSTT1 and GSTM1 ''double null'' genotypes in black children with ALL but relatively average expression in white children with ALL relative to their control groups (black expression 23.5% vs 3.9%; white expression 6.1% vs 8.0%).[84] Although this may initially suggest that the GSTT1 null genotype predisposes black children to ALL, the investigators propose further research into external environmental exposures as relevant factors in genotype expression and ALL susceptibility in black children.[84] Interestingly, in correlation with the previous study by Barnette and colleagues, an increased risk of ALL was observed in patients with at least one functional GSTT1 allele (non-null genotype); however, these results were similarly questioned because of previously mentioned population factors (e.g., founder effect) (OR 2.6, 95% CI 1.1–6.3).[76] Independent and geographically varied studies have presented supportive evidence regarding the GSTT1 null genotype and increased risk of childhood ALL.

Using GSTT1 enzymes' binding affinity for steroid hormones, Meissner and colleagues explored the effectiveness of GSTT1 as an indicator of chemotherapy treatment prognosis; patients with the GSTT1 null genotype exhibited a better response to prednisone treatment than patients expressing at least one functional GSTT1 allele (OR 0.25).[83] Other studies have focused on the influential role of environmental effects on cancer susceptibility. Trihalomethanes (THMs), such as chloroform, are chlorination by-products found in drinking water. Prenatal and/or postnatal exposure to THM in GSTT1 null genotype individuals was reported to induce a significantly increased risk of childhood ALL (OR 9.1, 95% CI 1.4–57.8).[85] This demonstrates the trend toward more comprehensive analysis of XME polymorphisms to include environmental effects and treatment prognosis and their implications when considered with various XME expressions.

N-ACETYLTRANSFERASES

NAT enzymes are involved in bioactivation (*O*-acetylation) and deactivation (*N*-acetylation) of chemical compounds. NAT enzymes catalyze the addition of an acetyl group from acetyl coenzyme A to the terminal nitrogen on target substrates, resulting in either increased toxicity or detoxification.[86] There are two distinct isoforms of NATs: NAT1 and NAT2. Despite their separate classes, NAT1 and NAT2 share a similar degree of substrate affinity, and both are located at chromosome 8p21.3-23.1. To date, 26 NAT1 polymorphisms and 29 NAT2 polymorphisms have been identified, and researchers are currently working to interpret the resulting phenotypic characteristics of this amalgam of polymorphisms.[86]

NAT1 and NAT2 are distinguished in part by their substrate-specific behavior (see Table 1). Among common NAT substrates are arylamines and heterocyclic amines, arylhydrazines, and arylhydroxyl amines.[21,86] *p*-Aminosalicyclic acid (ASA) and sulfamethazine (SMZ) have successfully induced both NAT1 and NAT2 activity in liver and bladder cells, although certain environmental conditions must also be met for functional induction (see below).[86]

NAT1

NAT1, or monomorphic NAT, is a ubiquitously expressed protein whose expression has been studied in liver and gut tissue, as well as in leukocytes and erythrocytes.[86] NAT1 enzymes target aromatic and heterocyclic amine carcinogens for oxidative reactions via *N*-acetylation and *O*-acetylation of aryl and heterocyclic amines.[87] Enzyme activation involves external chemical controls, including a variety of oxidative molecules. Both ASA (a NAT1 substrate) and SMZ (a NAT2 substrate) are effective NAT1 inducers, whereas hydroxylamine and nitroso intermediates of NAT1 substrates effectively inhibit NAT1 activity.[86,87] Although the NAT1 variant form has been largely established as an inducer of higher NAT1 enzyme activity in bladder and colon tissue,[10] a poor understanding of the functionality of the NAT1 genotype currently exists. NAT1 contains a C560A mutation that triggers the lowest erythrocyte NAT1 activity, whereas the homozygous mutant NAT1 C559T genotype demonstrates complete absence of erythrocyte NAT1 activity.[88] Such genotype variation and enzyme expression have implications for leukemia susceptibility owing to altered drug-metabolizing activity in the blood, although no present literature reflects such studies. Studies have failed to demonstrate an increased risk of childhood ALL with respect to NAT1 expression; however, Krajinovic and colleagues reported a significant combined effect in homozygous wild-type NAT1 (NAT1*4) individuals who also expressed NAT2 slow-acetylator genotypes (OR 1.9, 95% CI 1.1–3.4), whereas the NAT1*10 allele did not incur significant effects on ALL susceptibility.[89] The functional significance of NAT1 alleles is still uncertain, although NAT1 has been shown to control most prenatal *N*-acetylation activity.[89–91]

NAT2

NAT2, or polymorphic NAT, enzyme expression is highly tissue specific, with known expression in locations correlated with XME expression, such as liver and intestinal epithelium.[86] Among its target substrates are arylamine drugs (procainamide, SMZ), benzidine, isoniazid, and hydralazine.[10] ASA and SMZ are also effective NAT2 inducers.[85] NAT2 variants exhibit altered acetylation polymorphisms, yielding rapid, intermediate, and slow acetylators.[92]

Although suggestive research has surfaced, strong associations between NAT2 and cancer susceptibility remain elusive.[93] The NAT2 variant form has demonstrated an increased binding affinity to quinoline,[21] thus reducing quinoline's carcinogenic effects on target tissues; however, significantly altered susceptibility to a specific tissue cancer has been neither determined nor observed. Recently, Krajinovic and colleagues reported an increased risk of childhood ALL susceptibility in children with the NAT2 slow-acetylator phenotype (OR 1.5, 95% CI, 1.0–2.2) caused by variant allele genotypes, including NAT2*5A (T341C, C481T), NAT2*5B (T341C, C481T, A803G), NAT2*5C (T341C), NAT2*6A (C282T, G590A), and NAT2*7B (C282T, G857A).[89] The NAT2 slow-acetylator phenotype indicates the presence of two slow-acetylator alleles, whether homozygous or compound heterozygous. In addition to varied gene-gene interaction genotypes, a significant decrease in the number of NAT2 rapid-acetylator genotypes in the cases group was observed, indicating a possible protective role (OR 0.7, 95% CI 0.4–1.0). Individuals with NAT2 rapid-acetylator phenotypes who are homozygous for NAT1*4 exhibited an increased susceptibility to ALL (OR 1.9, 95% CI 1.1–3.4). Multivariant analysis with risk-elevating alleles also resulted in a significant increase in ALL risk. When considered individually, the presence of GSTM1 null and at least one CYP1A1*2A variant allele genotypes did not affect a significant increase in ALL susceptibility; however, when each was coupled with the NAT2 slow-acetylator phenotype, an overall two-genotype risk increase was observed (OR 2.7, 95% CI 1.4–4.9). Furthermore, when all three risk-elevating genotypes were simultaneously considered, the OR increased to 3.1 (95% CI 1.1–8.4).[88] This coupled-gene response is similar to that seen with the CYP2E1*2 and *3 genotypes described earlier.[9] These results provide a promising basis for future exploration of NAT2 variant influence on ALL susceptibility.

NQO1 ENZYME

NQO1 enzymes are highly inducible two-reductase enzymes that protect cells from benzene metabolites such as quinines via reduction reaction.[94,95] Oxidation via NQO1 produces relatively stable hydroquinone products, which are more easily removed by conjugation with GSH and GSH derivatives.[96] Effective NQO1 induction is achieved using antioxidants, heavy metals, and ionizing radiation, whereas coumarins, flavones, and curcumin are proven inhibitors.[97]

NQO1 is located on chromosome 16q22, with the most studied polymorphism occurring at base pair 609 (C609T), resulting in a Pro to Ser substitution at position 187, which has been shown to inhibit NQO1's ability to stabilize TP53, thus reducing the apoptotic response pathway.[98,99] Decreased NQO1 activity signals a C609T polymorphism such that homozygous variant individuals lack NQO1 expression completely, thus increasing susceptibility to the effects of carcinogens and subsequent greater accumulation of resulting genetic mutations in cells. Recent studies have demonstrated an association between NQO1 mutation and the risk of lung cancer in adults.[100] Similar studies of children with ALL have had mixed outcomes, with some studies supporting NQO1 activity to be associated with *MLL* translocation–negative ALL, whereas a different study by Sirma and colleagues did not find any association with leukemia.[101–103]

Mutant allele frequencies in ethnic cohorts yielded similar black and white distributions but higher frequencies in Hispanic and Asian populations. Independent studies by Nebert and colleagues and Kelsey and colleagues found similar increased genotype frequencies in Asian and Hispanic populations, with an average occurrence twice that of other surveyed ethnic populations.[104,105] In a recent report, Kracht and colleagues highlighted this ethnic skew, as did previous studies that have demonstrated a higher occurrence of childhood leukemias and lymphomas in Asian children living in the United Kingdom, especially those presenting with lymphomas between birth and 4 years of age.[106–108] Furthermore, a higher incidence of ALL and lymphomas has been noted in Hispanic children in the United States.[109]

Based on previous work by Rosvold and colleagues,[110] Krajinovic and colleagues also suspected that the decreased enzyme activity resulting from NQO1 allele mutations leaves individuals at greater risk of developing ALL.[8] In their recent study, Krajinovic and colleagues demonstrated that children with at least one NQO1 mutant allele were more susceptible to developing ALL (OR 1.7, 95% CI 1.2–2.4), whereas homozygous wild-type genotypes exhibited a seemingly protective effect.[8]

A similar hypothesis has been discussed, citing altered NQO1 levels among other XME mutations as risk factors for Hodgkin's disease survivors to develop acute leukemia, solid tumors, and non-Hodgkin's lymphomas as second cancers. Other factors considered included the age at which the treatment was initiated and the type of treatment used (thus, the effects of varying chemotherapy agents and interactions with altered XME levels).[111]

Owing to the NQO1 enzymes' additional roles in interaction and stabilization of the tumor suppressor protein p53,[101] low and null NQO1 genotypes have been studied for increased susceptibility to a variety of cancers, specifically childhood leukemias. *MLL* gene rearrangements in

chromosome band 11q23 have been studied in close association with NQO1 expression in infant acute lymphoblastic leukemia (iALL). According to Lanciotti and colleagues, *MLL* rearrangement does not affect iALL prognosis or other disease factors but instead acts solely to characterize two subsets of iALL, those positive or negative for *MLL* gene translocations.[101] Although high heterozygous/null NQO1 genotype frequency in ALL patients without MLL rearrangment was observed in Italian patients (72 vs 38%; $p = .006$),[101] Smith and colleagues and Wiemels and colleagues independently demonstrated a high NQO1 low/null genotype frequency association with *MLL* rearrangement in British and white American patient cohorts, respectively (OR 2.54, 95% CI 1.08–5.96; OR 2.47, 95% CI 1.08–5.68 for de novo, B-lineage ALL, respectively).[103,112] Varying gene-environment interactions resulting from different prenatal and postnatal carcinogen exposures may influence the conflicting observations on NQO1's influence on susceptibility to childhood ALL or acute myelogenous leukemia (AML). Lanciotti and colleagues also suggested Wiemels and colleagues' broader definition of infant age (≤ 18 months versus the standard ≤ 12 months), sample size, and parental carcinogen exposure as other possible factors influencing their seemingly conflicting NQO1 activity.[101] Future research controlling for such factors could provide further clarification as to the role of NQO1 genotype in iALL or AML susceptibility.

Similarly, in Kracht and colleagues' study on NQO1, genotype frequencies were considered among different patient cohorts, including de novo ALL, Burkitt's lymphoma, and non-Hodgkin's lymphoma cases.[106] A significantly greater incidence of NQO1*2 mutant allele was observed in Burkitt's lymphoma patients when compared with matched controls (OR 1.81, 95% CI 1.04–3.15), with increased significance in patients ≤ 9 years at diagnosis (OR 3.02, 95% CI 1.47–6.18). Kracht and colleagues were also unable to confirm the results of Smith and colleagues and Wiemels and colleagues and cited differences in environmental exposures as well as other gene-gene and/or gene-environment interactions as probable causes for their contrasting results.[103,106,112] Further studies correlating specific gene-environment interactions are suggested by all authors and would clarify the current contrasting results regarding the phenotypic implications of altered NQO1 genotypes.

FUTURE IMPLICATIONS

Genetic studies in cancer continue to be highly rewarding, and future studies should provide critical insight into the pathophysiology and treatment of several types of childhood cancer. With the advent of new and improved technologies, including allele discrimination genotyping, whole-genome sequencing, and microarray technology, researchers are able to perform studies more quickly, more cheaply, and with more precision than previously possible.

In the realm of pediatric malignancies, several studies are beginning to unravel potential causes of childhood cancer and describe some of the unique susceptibility and prognostic factors associated with them. In polymorphism analysis, areas with the greatest potential for discovery include studies of gene-environment interactions, synergistic or antagonistic relationships between polymorphisms, and the impact of ethnicity on the phenotypic expression of polymorphisms.

Gene-Environment Interactions

When investigating environmental factors associated with the development of childhood leukemia, several factors, including electromagnetic radiation, prenatal x-ray exposure, ionizing radiation, and viral infection, were identified. However, the most common exposures, electromagnetic radiation and viral infection, were shown to have only a mild impact on childhood susceptibility to leukemia, and children are rarely exposed to sufficient levels of prenatal x-rays or ionizing radiation to experience any leukemogenic effects. Furthermore, even when children are exposed to such factors, substantial interindividual differences in subsequent carcinogenic impact exist. For example, although many Japanese children were exposed to high levels of ionizing radiation after the bombing of Hiroshima, there was a varied increase in malignancy for all children exposed, and overall cancer rates increased only a minimal amount over the subsequent 5 years.[113] Similar examples exist in adult cancer studies; cigarette smoke is the most common environmental carcinogen, yet not all smokers develop pulmonary cancer. Investigators speculate that many of these environmental factors may require a coexisting polymorphism in an XME, DNA repair enzyme, or cell-cycle regulatory gene to sufficiently potentiate the tumorigenic effect of these environmental factors.

Preliminary studies have discovered that certain interactions between genetic polymorphisms and environmental factors can affect susceptibility to childhood cancer. In studies by Infante-Rivard and colleagues, a significant increase in leukemia incidence was seen in children with certain CYP1A1 variants whose mothers were exposed to pesticides while pregnant.[25] Subsequent studies also suggested a similar relationship between this polymorphism and prenatal exposure to cigarette smoke byproducts.[114] Other studies suggested that a rich array of gene-environment interactions exist and may account for a substantial portion of interindividual differences in cancer risk; however, owing to the relatively low number of annual cases of pediatric cancer and difficulties with parental recall bias, many studies will require long-term subject accrual and strict controls on data collection and analysis to yield conclusive results.

Gene-Gene Interactions

Unlike cancer-predisposing conditions such as Li-Fraumeni syndrome and hereditary retinoblastoma, most childhood cancers cannot be attributed to a single genetic factor but are instead likely due to multiple low-penetrance genetic factors. Subsequently, studies of individual polymorphisms and their impact on cancer risk have difficulty owing to the low impact on the cancer risk of single-gene polymorphisms and the low incidence of childhood cancer. In response, many investigators have begun to investigate collections of polymorphisms in the belief that low-penetrance, cancer-predisposing polymorphisms may have synergistic influences on cancer susceptibility when evaluated in aggregate. One area of particular interest has been the study of collections of polymorphisms in genes involved in the same biochemical process (ie, *MDM2*, *TP53*, and *CCND1* in cell-cycle regulation) or sequential reactions (ie, enzymes involved in genotoxin metabolism and enzymes involved in DNA repair).

The majority of pediatric cancer research investigating gene-gene interactions has focused on ALL owing to the relatively large number of cases when compared with other childhood cancers. Research by Krajinovic and colleagues demonstrated substantial increases in leukemia susceptibility when simultaneously evaluating multiple polymorphisms in XME enzymes such as CYP2E1 in isolation (OR 1.5) versus in combination with the variant NQO1 genotype (OR 1.9), among other variant allele couplings.[8,23] Related studies in other pediatric cancers are still in their initial stages, but preliminary data suggest that analogous effects may exist.[115] However, similar to studies on gene-environment interactions, these investigations must overcome problems with low numbers of pediatric cancer cases and the subsequent limitations in statistical power. Furthermore, owing to the high number of potential gene-gene interactions, studies must also control for the exponential increase in potential alpha errors with each additional gene polymorphism being studied.

Ethnicity Effects

ALL continues to be the leading cause of childhood cancer, accounting for between 25 and 30% of all cancer diagnoses. Current data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) report indicate that white children and Hispanic children have a uniquely elevated risk of developing ALL compared with black children (28 and 43 per million vs 15 per million, respectively).[116] Similar findings in other pediatric malignancies, such as Ewing's sarcoma, which has a strong ethnicity-specific predilection, suggest that certain undefined factors associated with ethnic status have a significant impact on susceptibility to malignancy.

Preliminary research investigating the interactions between polymorphisms in CYP450 enzymes and ethnic status have demonstrated novel interactions for several cancers in adults, including breast, prostate, lung, and colon.[117,118] Few studies of such interactions and their impact on susceptibility to childhood cancer have been performed at this time, with the only available studies being focused on ALL. Preliminary research on polymorphisms' status impact on ALL susceptibility focused on homogeneous populations of French Canadian and Turkish children[7,8,119] or heterogeneous populations of Brazilian children.[9] Although such studies provide insight about ethnicity and polymorphism interactions, they do not effectively reflect the unique ethnic milieu in the populations of the United States and many European nations, nor do they account for the elevated ALL susceptibility among Hispanics, a group that carries the highest risk of ALL. Preliminary research by our group suggests that ethnic status may affect the impact of different polymorphisms on cancer susceptibility. Specifically, we have found that Hispanic children with polymorphisms in CYP1A1 may be associated with an elevated risk of developing ALL when compared with their black and white counterparts (Swinney and colleagues, 2006, unpublished data). Nonetheless, further studies on the unique interactions between ethnic status and polymorphism status need to be pursued.

BROADER IMPLICATIONS

Although studies on the role of XMEs and pediatric cancer susceptibility have demonstrated promising results, a larger focus has been placed on correlations between altered XME activity and susceptibility to a variety of adult cancers and diseases.[120] Studies have also reported both beneficial and harmful modulation of enzyme activity by dietary compounds.

The adult cancers studied in relation to XME polymorphisms include cancers of the lung, bladder, and kidney. Because of their affinity for aromatic amines, a variety of phase I and II enzymes have been linked to altered risk of smoking-related cancers, namely, lung cancer. GST class enzymes and CYP1A1 variant genotypes have been associated with an increased risk of smoking-related cancers.[121] Interestingly, CYP1A1 protein expression has been found only in the lung tissue of smoking patients,[122] suggesting a possible harmful induction process owing to tobacco smoke inhalation. Although CYP1B1 protein expression in lungs remains controversial, mRNA expression has been found to be inducible in human lungs and alveolar macrophages.[122] The CYP2E1 PstI variant has been correlated with a significant increase in lung cancer risk, which is further amplified in conjunction with heavy smoking habits. Resulting disease consequences of the variant include earlier age at onset and lower exposure necessary for increased cancer susceptibility.[121] Other XME variants linked to lung cancer susceptibility include NAT1 slow acetylators, NQO1 wild type (in non-Hispanic and black Americans), and GSTM1 null genotype. The risk of urinary

bladder cancer, also a smoking-related cancer, has been shown to increase by a factor of 2.7 in heavy smokers exhibiting low-NAT2 slow acetylators. Interestingly, NAT2 slow acetylators and CYP1A2 variant carriers were found to be at increased risk of bladder cancer, but only smokers. Other factors affecting bladder cancer risk include occupational exposure to benzene, a phase I enzyme substrate.[10] Combined NAT and von Hippel-Lindau disease (VHL) studies have reported different genotype frequencies between NAT1 and NAT2 in clear cell renal cell carcinoma (CCRCC, a common VHL carcinoma).[123,124] Decreased enzyme activity in VHL transversion-carrying CCRCC patients has been observed as common NAT1 heterozygous phenotype behavior; however, studies attempting to correlate NAT2 expression with increased CCRCC risk in VHL transversion carriers ultimately failed to demonstrate significant values.[124]

Other studies have been conducted on the promising role of XME polymorphisms in a variety of common cancers. Sulfotransferase (SULT), a phase II enzyme, has exhibited varying effects on cancer susceptibility in different ethnic cohorts. The SULT*His^{213} variant allele seems to increase the risk of esophageal cancer in Taiwanese individuals and lung cancer in the US population but exhibited a protective effect against urothelial cancer in a Japanese cohort.[125–127] The SULT1A1*Arg^{213} variant causes an increased risk of earlier onset of breast cancer and greater susceptibility for secondary tumors.[128] Furthermore, homozygous SULT*His^{213} individuals are at an 80% higher risk of breast cancer, an odds risk that increases when coupled with alcohol consumption, high body mass, and other risk-elevating health factors. Further novel investigations on newly discovered XME polymorphisms continue to provide beneficial information on XME metabolism and cancer susceptibility.

Owing to the ubiquity of XME expression, studies regarding the effects of XME polymorphisms are applicable to medical studies extending beyond adult and pediatric cancers. CYP enzymes play an important role in the biosynthesis of skin endobiotics.[129] As a result, inhibition of CYP enzymes has exhibited harmful effects on the susceptibility for and degree of dermatologic diseases. Psoriasis affects approximately 2.1% of Americans and can develop at any age.[130] Therapeutic interventions include ultraviolet radiation exposure; however, interindividual variation in treatment response has been noted. A high frequency of CYP2S1 and CYP2E1 variant genotypes has been observed in patients with psoriatic plaques; treatment with ultraviolet exposure further induced enzyme activity, suggesting a genotype effect on psoriatic treatment response.[131,132] Other studies have found no association between the NAT2*4 rapid-acetylator allele genotype and psoriasis, but when investigators considered NAT2 rapid-acetylator phenotypes instead, an increased risk of earlier age at onset was observed (OR 2.7, 95% CI 1.3–5.5).[133]

An interesting and relatively new area of study is the effect of XME activity on treatment response. Because of steroidogenic characteristics of the skin, CYP1A1 activity has been suggested as a modulator of therapeutic efficacy of steroid treatments for androgenic skin disorders such as acne, hirsutism, and androgenetic alopecia, although no present studies have examined these possible disease associations. Retinoic acid is an important agent in epithelial tissue development whose metabolic pathway is highly CYP1A dependent; thus, inhibition of CYP-mediated retinoic acid metabolism allows for increased skin and plasma retinoid concentrations, suggesting a possible psoriasis prevention treatment method.[131]

Hypertension studies have also begun to examine the role of XME on susceptibility and treatment efficacy.[134] Hypertension is most common in the elderly who are at greater risk of adverse drug reactions owing to increased medication consumption. The antihistamines terfenadine and astemizole serve as substrates for CYP3A enzymes; however, inhibition of CYP3A by macrolide antibiotics caused cardiotoxicity, leading to their withdrawal from the market.[134] In addition to drug interactions, certain XME polymorphisms have been shown to influence blood pressure response to diuretics, β-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and clonidine (ADD1, GNB3, NOS3, ACE, among others), although the observed single- and multiple-gene effects were relatively small.[135] SULT1A1 activity modulation during hypertension and hypotension, along with the enzyme affinity for hormones, suggests future research into possible therapeutic methods via SULT1A1 induction and/or inhibition. Current research methods and tools are capable of examining gene-environment interactions, associations between haplotypes and multilocus genotypes, and larger sample sizes, although no such research has yet been attempted.

Although often overlooked, dietary habits are also an important factor in treatment efficacy, especially in young women. Drug administration complications are common among smokers, dieters, pregnant and/or lactating women, extensive alcohol consumers, and individuals taking oral contraceptives. Mixed-function oxidase drug-metabolizing rates in dieting individuals, usually younger females, are easily modulated through reduced substrate availability owing to competition from tissue needs.[136] With increased media attention on dieting methods, drug administration efficiency is becoming of greater interest and concern among physicians. Studies have been conducted regarding disease prevention and risk reduction through dietary monitoring. Dietary catechol-containing phytochemicals, such as polyphenols found in teas, inhibit the *O*-methylation of catechols via inhibition of catechol-*O*-methyltransferase (COMT), a phase I enzyme. COMT serves as a neurotransmitter or neurohormone whose presence increases the bioavailability of certain central nervous system chemicals essential to the prevention of

neurodegenerative activity seen in Parkinson's disease patients. COMT inhibition decreases dopamine metabolism, causing greater oxidative damage in the exposed neurons, characteristic of neurodegenerative changes linked to Parkinson's disease.[137] Patients undergoing hypertension treatment are advised not to drink grapefruit juice because of its ability to down-regulate CYP3A4 in small intestine walls, thus reducing first-pass metabolism and drug efficacy.[134] In contrast, antioxidants, such as those found in garlic and tomatoes, alter the activity of xenobiotic metabolizing agents by decreasing the phase I enzyme activity associated with carcinogen activation and increasing phase II enzyme activity, a process for toxin excretion.[138]

Owing to their ubiquitous expression, XME interactions have been subject to studies in association with a variety of adult and pediatric cancers, diseases, and treatment outcomes. Although many remain skeptical as to the functional significance in variant screening for cancer susceptibility,[139] current research efforts are focusing on developing methods that may allow for future genotyping to assess disease risk. Screening for potentially harmful diseases immediately after birth could enable physicians to calculate and predict disease risks and prognosis, thus alerting patients and their families, who may be at increased risk. Some investigators have even begun to study the effects of SULT variants on mortality rates, perhaps in the hope of eventually being able to predict individual life spans. Current studies on single-gene polymorphisms and limited studies on gene-gene interactions provide a strong basis for future studies correlating the effects of broader gene-gene and gene-environment interactions.

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