The effect of organic solvents and CYP1A1, CYP2E1, GSTM1 polymorphisms on the development of acute lymphoblastic leukemia in Quebec children

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I. Abstract

Background: Childhood acute lymphoblastic leukemia (ALL) is a complex disease whose etiology remains largely unknown. Both genetic and environmental factors are believed to be involved in leukemogenesis. Several epidemiological studies have looked at the role of various parental environmental determinants such as smoking, alcohol, radiation, pollution and other chemical exposures, often finding null or contradictory results. The role of many potential environmental determinants remains unclear. It has long been suspected that organic solvents are carcinogens. They are common in the workplace and are potentially important sources of exposure in mothers during various time periods: preconception, pregnancy and postnatal. These time windows are vital for the developing fetus and exposures to carcinogens through the placenta or breast milk could lead to DNA damage. In addition, variants in xenobiotic metabolizing genes that biotransform various chemicals entering the body, in particular CYP (cytochrome P450) and GST (glutathione S-transferase) genes, have equally been linked to the development of ALL. As such, it is quite possible that variants in CYP and GST genes affect the biotransformation of chemicals such as organic solvents in the fetus or infant, leading to increased DNA damage and potentially cancer. Studying this interaction may be important in understanding the disease's etiology.

Methods: I analyzed the effects of maternal occupational exposure to organic solvents during pregnancy and breastfeeding on the risk of developing ALL in the offspring. The effects of organic solvents from household activities were also investigated in breastfeeding mothers during the postnatal period. In addition, I analyzed the joint effects of case genetic variants in certain likely functional xenobiotic metabolizing genes (CYP1A1, CYP2E1 and GSTM1) with organic solvent exposures. The data was taken from a large population based case-control with 790 cases and 790 controls recruited from Quebec, Canada. The data included state-of-art determination of occupational exposures, household exposures to various environmental exposures, and genotyped DNA samples from the study participants and their parents. Chemists and industrial hygienists ascertained occupational exposures in mothers using the so-called expert

method. Maternal household exposures to organic solvents used in various activities such as furniture stripping, painting or electronic repair were assessed through interviews with parents. The data was analyzed using logistic regression and Poisson log-linear models based on case-control, case-only and case parent-trio designs.

Results: Associations were found between occupational organic solvents during pregnancy and ALL (notably with alkanes, mononuclear aromatic hydrocarbons and mineral spirits) though these have previously been published. No associations were found between household exposures to organic solvents and ALL during the breastfeeding period. Significant main effects were found between case GSTM1 null and CYP1A1 *4 variants and ALL. Additionally, individuals with one copy of the CYP1A1 *2A variant and GSTM1 null had a significant odds ratio of developing ALL at 1.68 (95% CI: 1.03-2.75) as compared to an individual with neither. Offspring with the GSTM1 null variant whose mothers were occupationally exposed to aliphatic alcohols and aliphatic ketones, specific chemical families of organic solvents, during pregnancy had a lower risk of developing ALL than carriers of the wild type carriers. The case-parent trio analysis did detect a harmful interaction effect between offspring with the CYP1A1 *2B variant and maternal occupational exposure to any type of organic solvent during pregnancy. Similarly, the case-only analysis found important harmful interaction effects between the CYP1A1 *2A and *4 variants and maternal occupational exposures to any type of organic solvent during pregnancy and protective interaction effects between GSTM1 null variants and this same exposure. Among mothers who breastfed, exposure to organic solvents from household activities from one year before pregnancy to date of diagnosis did not generally increase the risk of ALL; however, there was evidence to suggest that the GSTM1 null and CYP1A1 *2A variants modified the effect of solvent exposure from furniture stripping, and likewise for the CYP2E1 *5 variant with certain activities involving exposure to electronics. The CYP1A1 *2A variant also appeared to significantly modify the effect of latex and/or acrylic paint exposures in a breastfeeding mother on the risk of ALL.

Discussion: Although the study had limited power to uncover statistically significant

interactions, the results suggest a role on the incidence of childhood ALL for gene variants involved in the metabolism of carcinogens in the presence of environmental prenatal or breastfeeding exposure to organic solvents.

Résumé

Introduction: La leucémie lymphoblastique aiguë (LLA) chez l'enfant est une maladie complexe dont l'étiologie reste peu connue. On pense que des facteurs génétiques et environnementaux sont impliqués dans la carcinogenèse. Plusieurs études ont analysé le rôle de certains déterminants environnementaux chez les parents, tels que le tabac, l'alcool, la radiation, la pollution et une variété de produits chimiques, donnant des résultats souvent nuls ou contradictoires. Le rôle des déterminants environnementaux n'est clairement défini dans la littérature. Des chercheurs soupçonnent que les solvants organiques sont des carcinogènes importants et qu'ils sont présents dans plusieurs lieux de travail. Ces produits chimiques ont le potentiel d'être une source d'exposition chez la mère durant les différentes périodes du développement du foetus, notamment, la préconception, la gestation et le postnatal. Ces périodes sont essentielles pour le fœtus et les solvants organiques ont le potentiel de traverser le placenta ou le lait maternel et léser l'ADN du fœtus ou le nouveau-né. De plus, quelques variantes dans les gènes xénobiotiques du métabolisme, qui transforment une multitude de produits chimiques absorbés par le corps, en particularité des variantes dans les gènes CYP (cytochrome P450) et GST (glutathion S-transférase), ont été associées avec la LLA chez l'enfant. Tout cela suggère que les variantes dans les gènes CYP et GST perturbent la biotransformation des produits chimiques, tel que les solvants organiques, chez le fœtus et le nouveau-né, menant à des dommages à l'ADN et possiblement des néoplasies. L'interaction entre les variantes et les solvants organiques peut être cruciale pour comprendre l'étiologie de ce cancer chez l'enfant.

Méthodes : J'ai analysé les effets de l'exposition maternelle aux solvants organiques pendant la gestation et l'allaitement sur le risque de développer la LLA. Les effets de l'exposition aux solvants organiques retrouvés au domicile ont aussi été investigués chez

les mères qui allaitent. De plus, j'ai analysé l'effet cumulatif des variantes dans les gènes CYP et GST (notamment CYP1A1 *2A, *2B, *4, CYP2E1 *5 et GSTM1 nulle) et les expositions aux solvants organiques au travail de la mère pendant la gestion et celles au domicile pour les mères qui ont allaité. Les données ont été sélectionnées d'une étude cas-témoin réalisée au Québec, recrutant 790 cas et 790 témoins. Cette étude a accumulé de l'information extensive sur les professions et les expositions chimiques au travail ainsi que les expositions à domicile. Des échantillons d'ADN ont été recueillis des participants et de leurs parents. Les expositions d'occupation ont été déterminées par des chimistes et hygiénistes industriels utilisant une méthode experte alors que les expositions au domicile ont été évaluées par des entrevues avec les parents. Les données ont été analysées avec des modèles de régression logistiques et log-linéaire (régression de Poisson) basés sur les concepts de cas-témoins, cas-seul et trio cas-parent.

Résultats : Des associations ont été découvertes entre les expositions professionnelles chez la mère pendant la gestation et LAL (notamment des alcanes, des hydrocarbures monocycliques et des essences minérales post 1970), par contre ces résultats ont déjà été publiés. Aucune association n'a été trouvée entre les expositions de solvants organiques au domicile chez la mère et LAL pendant l'allaitement. Des effets statistiquement significatifs ont été trouvés entre les variantes GSTM1 nulle et CYP1A1 *4 chez l'enfant et la LLA. Les individus qui possèdent une copie de la variante CYP1A1 *2A et de la variante GSTM1 nulle, ont un risque significatif de développer la LLA comparé à des individus avec aucune de ces variantes. Les progénitures d'une mère qui a été exposée à des solvants organiques au travail, particulièrement les alcools aliphatiques et les cétones aliphatiques, qui possèdent la variante GSTM1 nulle, ont un risque inférieur de développer la LLA comparées aux progénitures qui ont le gène de type sauvage. Le trio cas-parent suggérait un effet d'interaction nocive entre la variante CYP1A1 *2B chez la progéniture et l'exposition de la mère à n'importe quel solvant organique au travail pendant la gestation. De même, le type d'étude cas-seul a détecté un effet d'interaction nocive entre les variantes CYP1A1 *2A et *4 et l'exposition de la mère à n'importe quel solvant organique au travail et une interaction protectrice entre la variante GSMT1 nulle et cette même exposition. Le risque de développer la LLA pour les progénitures des

mères qui ont allaité et qui ont été exposées à des solvants organiques lors d'activités au domicile pendant une année avant la gestation jusqu'à la date de diagnostic n'était pas élevé. Par contre, les variantes GSMT1 nulle et CYP1A1 *2A ont modifié l'effet des solvants relié à des activités de décapage de meubles et d'électroniques. La variante CYP1A1 *2A a aussi modifié l'effet de l'exposition chez la mère qui allaite aux peintures de type latex et/ou acrylique sur le risque de développer la LLA chez l'enfant.

Discussion: Ces résultats suggèrent la possibilité que les variantes des gènes étudiés interagissent avec l'exposition aux solvants organiques pendant la gestation ou l'allaitement chez la mère, pour influencer le risque de développer la LLA chez l'enfant, malgré le pouvoir limité de l'étude pour détecter des interactions statistiquement significatives.

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II. List of acronyms

AH	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
bHLH	basic-helix-loop-helix
CI	confidence interval
COR	case odds ratio
CSE	chronic-solvent induced encephalopathy
CYP	cytochrome P-450
FAB	French-American-British
FAF	family allowance files
G x E	gene x environment
GST	glutathione S-transferase
GWAS	genome wide association studies
HSC	hematopoietic stem cells
IARC	International Agency for Research on Cancer
LRT	likelihood ratio test
MAH	mononuclear aromatic hydrocarbons
MLL	mixed-lineage leukemia
MTHFR	methylenetetrahydrofolate reductase
NHL	non-Hodgkin lymphoma
OR	odds ratio
PAH	polycyclic aromatic hydrocarbons
PCR	polymerase chain reaction
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
RT PCR	reverse transcriptase-polymerase chain reaction
RR	relative risk
SNP	single nucleotide polymorphism
XM	xenobiotic metabolizing

III. Introduction

Childhood acute lymphoblastic leukemia (ALL) is a complex disease representing approximately 25% of all pediatric tumors [1]. The genetic abnormalities of childhood leukemias have been well defined, more so than many other cancers [2], however the etiology underlying its development remains unknown [3,4]. It is now widely believed that both environmental and genetic factors play a role in the etiology of complex diseases [3,5].

There have been several epidemiological studies looking at parental environmental exposures such as smoking, alcohol consumption, pollution, pesticides, occupational chemicals including organic solvents, household chemicals including paints and thinners, radiation and proximity to power lines and the development of childhood ALL. The results in the literature are contradictory or null for the majority of these factors [6-8]. For childhood cancers, investigators have often explored parental exposures, in particular in the work environment, that occur during preconception, pregnancy and postnatal periods due to their relevance for offspring development. Numerous studies have found associations between occupational or household exposures to organic solvents and ALL and also between certain occupations involving exposures to organic solvents and ALL in both mothers [9-16] and fathers [9,12,13,15-34]. The evidence however, is not consistent. Organic solvents are ubiquitous. They are widely used in industrial and household settings. They are found in chemicals such as paints, glues, dry-cleaning fluids, gasoline, degreasers, varnishes and thinners [35]. Investigations have largely focused on paternal occupational exposures rather than maternal exposures, although some evidence suggests the effects of these exposures on ALL, may be stronger in mothers during preconception and pregnancy [9,11,15].

It is believed that initiation of carcinogenesis in diseases such as ALL may occur in utero [36], therefore studying maternal exposures can provide important insights into the disease's etiology. A fetus will be exposed to low weight non ionic chemicals which include several organic solvents and various drugs (naproxen, diazepam etc) from placental

transfer [37-39]. The fetus and the placental organ have the capacity to biotransform chemicals into ultimate carcinogens, which could cause DNA damage such as adduct formation [37,38,40,41]. Infants are generally not exposed to chemicals such as organic solvents directly, but can be exposed when the chemicals are transferred from the mother along with the breast milk. Organic solvents can enter the breast through passive transfer from plasma and accumulate and/or be biotransformed in adipose tissue [42-44]. Organic solvents, including countless other chemicals, have often been detected in breast milk [35,44-47]. The pregnancy and breastfeeding periods are therefore important time windows to study the etiologic mechanism underlying the development of childhood ALL.

A carcinogen will become active after it is biotransformed by phase I and phase II enzymes. Phase I enzymes interact with the compounds and are responsible for hydrolysis, reduction and oxidative reactions. Phase II enzymes detoxify the metabolites arising from phase 1 through reactions that transform the active metabolites into non-reactive and water-soluble compounds [3]. Several investigators have studied fetal and infant metabolism of carcinogens and have detected the presence of fetal xenobiotic metabolizing enzymes, such as CYP and GST isoforms, phase I and phase II enzymes respectively. These enzymes have also been found active in several tissues of the fetus starting at gestational ages of approximately 12 weeks [37,41,48]. It is therefore quite possible that child variants in the CYP1A1, CYP2E1 and GSTM1 genes affect the way carcinogens, such as organic solvents, are biotransformed. These variants could potentially increase or decrease the level of harmful metabolites in fetal and/or infant cells, which could lead to DNA damage and important somatic mutations.

Maternal exposures to organic solvents during pregnancy or breastfeeding coupled with genetic susceptibility of the fetus/infant with respect to relevant genes may have a combined action leading to an increased risk of developing ALL. The role of these environmental and genetic susceptibility factors in the etiology of the disease is an important aspect to explore especially because parental exposures to such chemicals in the workplace at crucial reproductive periods are common. If such exposures are found to be harmful, exposures could be avoided to reduce risks of cancer in their offspring.

IV. Literature Review

Section 1: Acute Lymphoblastic Leukemia (ALL)

Childhood ALL is a complex disease representing approximately 25% of all pediatric tumors [1] and although the treatment cure rate is high (with between 76 to 86% of children surviving and disease-free at 5 years), the etiology behind its development remains unknown [2,3,49,50].

Carcinogenesis

Carcinogenesis is a complex process by which cell growth, differentiation and normal processes are disrupted and deregulated. Genetic and epigenetic changes are both important components of this process and are caused by chromosomal or point mutations, which are either spontaneous or induced by environmental carcinogens. The mechanism behind the development of tumors or cancer is traditionally categorized into three steps: initiation, promotion and progression. Initiation usually occurs when somatic or germ cells are exposed to a carcinogen that mutates the DNA. This step is irreversible [41].

Subtypes of Acute Lymphoblastic Leukemia (ALL)

There are four main types of childhood leukemias: acute lymphoblastic, acute myeloid also known as acute nonlymphocytic, chronic lymphocytic and chronic myeloid (the latter being very rare in children at 4% of leukemic cases in the United States). Before molecular techniques were available, childhood leukemias were evaluated with morphological evaluations using the French-American-British (FAB) classification. Using this system, 80% of childhood leukemias were classified as ALL and 20% were acute myeloid leukemias (AML). More recent immunotyping studies have shown that of the ALL cancers, 75% are classified as B lineage and 15% are T lineage. Both B and T

lineage leukemias display improper rearrangement of immunoglobulin receptor genes, resulting in abnormal lymphocyte phenotypes [49].

Molecular analyses and recurrent genetic aberrations of ALL

Molecular analyses have shown many recurrent genetic abnormalities in childhood leukemia cells [50]. These have been well defined, more so than many other cancers [2]. They include translocations where a proto-oncogene forms a fusion gene with another gene, more commonly the immunoglobulin gene enhancer on chromosome 14 or other important transcription factors. This deregulates the transcription of the genes, resulting in chimeric fusion genes or protein products with altered activities. Some translocations involve antigen receptor genes, but most are fusions of fragmented gene pieces. The product of these fused genes play important roles in the development of cancer through the deregulation of important cell pathways involved in cell differentiation and proliferation [49,51]. Translocations have been used as important prognostic tools whereby some patients with certain translocations are given different therapies [50,52]. Although translocations are common in childhood ALL, many cases also display cells with high hyperdiploidy (over 50 chromosomes) or hypodiploidy (too few chromosomes) [49]. Different chromosome numbers have been shown to lead to different prognoses (high hyperdiploidy between 51 and 65 chromosomes and near haploidy between 23 and 29 chromosomes, independently predict prognoses) [52]. Furthermore, many genetic abnormalities remain largely uncharacterized [49].

The various subtypes of childhood leukemias have varying incidences in different age groups. These subgroups also have their own common genetic aberrations; therefore there are many potential advantages in studying etiologic risk factors in homogeneous disease subgroups.

Infant acute lymphoblastic leukemia peaks at the age of 6 months with several affected individuals carrying translocations on the 11q23 chromosome; such genetic aberrations are associated with a poorer prognosis. These translocations are given the name mixed-

lineage leukemia (*MLL*) because unlike most translocations, they are not associated with a particular hematoepoietic lineage. In infant ALL cases, the common *MLL* gene translocation is t(4;11). MLL has been shown to regulate HOX gene expression, known to be important in leukemogenesis [36,50]. Infant leukemia represents approximately 5% of childhood leukemia cases [51].

The more common form of childhood ALL, *B cell precursor ALL*, peaks between the ages of 2-5 in developed countries. Developing countries do not have as clearly a defined peak in incidence [36,51]. The most common translocation for this subtype is the t(12;21)(p13;q22). Approximately 25% of childhood ALL cases have this TEL-AML1 gene translocation[50]. Aside from the common translocations, chromosomal hyperdiploidies with more than 46 chromosomes are also present and identify another B-precursor ALL subtype [36,50]. Additionally, there exists other molecular subtypes for B-cell childhood leukemia such as TCF3-PBX1, BCR-ABL1 and t(1;19)(q23;p13) [50,53].

T-precursor ALL has been associated with many genetic aberrations (such as translocations) found in various transcription factors [50]. Unlike infant and B-cell ALL, T-cell ALL has no prominent age peak [54]. The most common genes affected are basic-helix-loop-helix (bHLH) genes, MYC, TAL1(SCL) and LYL1. The two latter genes are important in the development of erythroid cells usually not expressed in T-lympoid cells, whereas MYC is normally expressed in T-lympoid cells. The translocations in these genes sometimes occur near certain enhancers (such as the TCRβ-chain locus on chromosome 7, band q34 or α/δ-chain locus on chromosome 14, band q11) thereby activating these genes and downstream target genes as well. When the genetic rearrangements seen in T-cell ALL cases are found near enhancers, they often involve many regulatory genes encoding LMO1 and LMO2, HOX11, HOX11L2, other major HOX genes, TLX1, TLX3 and MLL. Fusion genes in some aforementioned proto-oncogenes are also common in T-ALL. Additional investigations have uncovered several NOTCH1 mutations in all T-cell subtypes [50,53].

Molecular studies conducted on leukemic cells have found recurring genetic aberrations in certain key cellular pathways in lymphoid development, cell cycle regulation, tumor suppression, apoptosis and drug responsiveness. Additionally, there have been several association studies linking these genetic aberrations with the development of leukemias. Certain genetic aberrations lead to poorer prognosis and are used for treatment options. In addition, investigators have determined that these genetic abnormalities change and evolve throughout disease progression [53].

Section 2: Acute Lymphoblastic Leukemia (ALL): Risk Factors

Sex, age and genetic syndromes

As previously described, childhood leukemia affects different age groups, with a peak at 2-5 years; therefore age is an important predictor of ALL development. It is also well known that the disease affects more males than females [2]. In addition to age and sex, certain inherited genetic syndromes such as Down Syndrome, Bloom's syndrome, ataxia-telangiectasia and Nijmegen breakage syndrome (accounting for less than 5% of ALL cases) have long been recognized as important known risk factors for leukemia [7,55].

Socioeconomic status and industrialization

The highest incidence of ALL is found in white populations of North America, Western Europe and Oceania as well as in the Chinese of Hong Kong and Singapore (approximately 40 per million for these high risk populations). Lower rates are seen in former socialist countries of Eastern Europe, in Japan and much of Latin America, with parts in the Middle East reporting approximately 20-30 cases per million. Some studies have shown a correlation between incidence and socioeconomic status, except among Hispanic populations in California, Florida and Costa Rica. Ecological studies provide evidence that increased socioeconomic status is associated with an increase in the incidence of ALL (where peaks of social development coincide with peaks in ALL) [56]. Aside from ethnic and socioeconomic differences, another possible hypothesis for ALL

incidence variations could be tied to increased industrialization, leading to increased environmental exposures to chemical contaminants [51].

Inherited genetic variants: genome wide association studies

Genetic variations are believed to have important roles in the incidence of ALL. Several candidate gene studies have associated ALL with a few DNA variants in carcinogen-metabolizing genes. Some genome wide association studies (GWAS) have also provided important insight into the role of some inherited variants on the development of ALL [2]. Two variants in the ARID5B gene (a transcription factor gene necessary for embryonic development, cell type-specific gene expression and cell growth regulation) in a 2009 European study were found to be significant predictors of childhood ALL; in addition, this variant was a strong predictor of the B-hyperdiploid ALL subtype [57]. Another European GWAS also found a single nucleotide polymorphism (SNP) in the ARID5B gene to be a significant predictor of ALL and the B-hyperdiploid ALL subtype. The study reported SNPs in the IKZF1 and CEBPE genes to be associated with childhood ALL (these genes are believed to be involved in transcriptional regulation and differentiation of B-cell progenitors)[58]. A case-control study done in 2010 found that the association between ARID5B polymorphisms and B-hyperdiploid ALL existed in black patients as well. Finally, a GWAS recently published in 2010 found 6 SNPs to be strongly associated with pediatric ALL in the following 4 genes: HAO1, EPB4IL2, C2orf3 and MAN2A1[59]. Although genes have an important role, the current research paradigm proposes that determinants other than genetic are involved in the development of cancer and that many such environmental and genetic determinants are likely implicated. Studying these determinants one at a time is unlikely to provide a clear picture of the strength or the role of each [3].

Parental Genetics

The affected individual's genetic susceptibility is an important factor in the development of ALL, but maternal susceptibilities may be important as well [3,60]. It has been suggested

that the mother's genotype could impact the child's health during pregnancy. The mother's genotype could affect how the carcinogens in the womb are being biotransformed, in turn affecting the degree and capacity to which the fetus is exposed to carcinogens [3]. Studying parental mediated effects is important for the study of childhood cancers.

Section 3: Transplacental Carcinogenesis

Steps of Carcinogen Metabolism

Carcinogens are usually not reactive and cannot cause damage until after they are metabolized into their active counterparts. There are two general steps required for the biotransformation of these compounds:

1) Phase I enzymes interact with the compounds and are responsible for hydrolysis, reduction and oxidative reactions.

2) Phase II enzymes detoxify the metabolites arising from phase 1 enzymes through the following reactions: glucuronidation, sulfonation, acetylation, methylation and conjugation with glutathione or amino acids. These reactions transform the active metabolites into non-reactive and water soluble compounds [3].

Enzymes in the Cytochrome P-450 family are known to partake during phase 1 whereas enzymes from the glutathione S-Transferase (GST) enzymes are involved in phase II [3,61,62]. The carcinogens are predominately broken down in the liver, creating active metabolites, which can mutate DNA by creating for example DNA-adducts. If these adducts are not repaired by DNA repair mechanisms, a genetic variation can arise when DNA polymerase falsely replicates this damaged site [41]. The main cellular pathways usually affected in cancer are: cell cycle genes, tumor suppressor genes (p53, K-ras), DNA replication and repair genes and xenobiotic metabolizing genes [3].

Transplacental Carcinogenesis

Xenobiotic metabolism in a pregnant woman can occur in the liver and in the placenta [40]. Fetuses and pregnant mothers are especially susceptible to carcinogens. Nonionic organic compounds, which have a low molecular weight and are lipid-soluble, can easily cross through the placenta [37,38]. The majority of studies exploring placental transfer of molecules have focused on drugs because their exposures are easier to monitor. Drugs such as diazepam have been found in fetal tissues, increasing in concentration as gestation progresses [40]. The same increases in fetal concentrations were seen for the drugs naproxen and rosiglitazone [40]. If the chemicals that cross the placental barrier have carcinogenic properties, the permanent effects of the exposure may only be seen some time after birth [38]. As described above, a carcinogen is usually metabolized by enzymes and transformed into ultimate carcinogens (by-products of the chemical), but metabolism can also be enzyme independent. If the latter is true, the effect on the developing fetus can be significant if the chemical can cross the placenta. If the carcinogen is enzyme dependent, there are two ways exposure to the fetus could occur: either the mother's tissues or the placenta biotransform the chemical which then enters the fetus or the fetus metabolizes the chemical in its own tissues [38]. Some animal experiments have shown that when the fetus is developing, it is sensitive to DNA adduct formation from carcinogens due to a lack of DNA repair enzymes, physiologic immaturity and a high rate of replication and fast development [3,41].

Presently, there is enough evidence demonstrating the carcinogenic potential of over 50 compounds, mixtures of compounds or chemical processes. Many of these compounds have been experimented on and have shown teratogenic or transplacental carcinogenic activities in animals. The induction of tumors has been seen in experiments where direct-acting chemicals are introduced during the late fetal stages [41]. Rat and mouse fetuses have been shown to be much more susceptible to such chemicals than adults, starting at 11 days gestation, when organogenesis begins, and especially a few days before birth when the organ system is quite vulnerable. In some monkey species, this vulnerability occurs during the equivalent of a human's first trimester. For enzyme-dependent carcinogens, a mouse or rat fetus at a later stage is said to be more vulnerable because fetal enzymes

may begin to metabolize carcinogens only at this late stage. The associations between fetal exposures to carcinogens and the onset of disease have not as clearly been seen in human epidemiological studies, though a important example was seen with exposures to diethylstilberstrol during the first trimester and it's future induction of vaginal adenocarcinoma in the female offspring [38,63].

Section 4: Environmental Exposures to Organic Solvents

Environmental contaminants and ALL

Although environmental determinants are believed to play a role in the etiology of childhood ALL, epidemiological evidence does not yet convincingly prove their role in the disease's etiology. Only ionizing radiation and exposure to certain chemotherapy drugs in offspring have been shown to cause ALL. The literature shows inconclusive or null results for the involvement of parental occupations, maternal reproductive history, parental smoking, parental alcohol consumption, maternal diet, use of prenatal vitamins, organic solvent exposure (household and occupational in parents), drinking water contaminants, outdoor pollution, pesticide exposure and residential power-frequency magnetic fields in both parents and offspring, and immunological factors particularly infections in offspring [6-8].

Transplacental exposures

Different chemicals will be transferred from the maternal bloodstream via the placenta to the fetus at different rates or quantities. The most common transfer mechanism is passive diffusion although it is known to also occur through active transport, facilitated diffusion, phagocytosis and pinocytosis [40]. The transfer depends on several factors such as the molecular size of the compound and it's lipophilicity, the degree of ionization, the degree of binding to blood components and placental tissue. The placenta has a lower ability for protecting against the entry of lipophilic compounds, as opposed to lipophobic compounds (bound by proteins in order to travel in the bloodstream) into the fetus. The

level of active carcinogen in the fetus will also depend on how fast the toxins are metabolized into active constituents [39-41].

Many studies have determined the presence of several cigarette-smoke components, such as cadmium, in both cord blood and placental tissue, indicating that certain smoke toxins have the potential to cross from mother to fetus. Exogenous exposures entering the fetus have been suggested to create DNA damage in the placenta and/or fetus. Correlations exist between the amount of adducts found in maternal and fetal tissues with exposures to known cigarette toxins [41,64-67]. Certain studies and a recent meta-analysis have even linked bulky DNA adduct formation in the placenta and/or the fetus to other environmental toxin exposures, such air pollution (fossil fuel, outdoor pollution (such as Ozone), industry pollution etc) [67-69].

Organic Solvents

Solvents are defined as substances, which dissolve or suspend compounds to form a solution. Organic solvents are a subset of these substances and are defined by the presence of carbon in their chemical makeup. Aliphatic solvents contain carbon atoms positioned in chains whereas aromatic solvents contain carbon atoms in ring formation [70]. Organic solvents are present and widely used in industrial and household settings. They are found in chemicals such as paints, glues, dry-cleaning fluids, gasoline, degreasers, varnishes and thinners [35]. As such, studying exposures to such chemicals in the context of occupations is plausible due to their presence in various industrial settings. The majority of epidemiological studies in the literature assessing the risk of chemical exposures such as organic solvents use occupational data.

In a 1991 publication, Siemiatycki provided a high-quality classification of organic solvents observed in most occupations. In addition, he described their uses, definitions and characteristics while also summarizing associations between their occupational uses and cancers [71]. An update and adaptation of this work for a paper based on the ALL study

was produced; it includes a list of frequently encountered occupational organic solvents regrouped in chemical families[10]. It is shown below in table 1.

Table 1: Chemical families and names of commonly used occupational organic solvents. Codes are as in[71] &[10]

Chemical Family	Chemical Name	Chemical code
Aliphatic alcohols	Methanol	232
	Ethanol	233
	Isopropanol	234
	Ethylene glycol	235
Chlorinated alkanes	Carbon tetrachloride	237
	Chloroform	238
	Methylene chloride	239
	1,1,1-Trichloroethane	240
Chlorinated alkenes	Trichloroethylene	242
	Perchlorothylene	243
	Ethylene dichloride	300
Aliphatic ketones	Acetone	248
	Methyl ethyl ketone	304
Mononuclear aromatic hydrocarbons (MAH)	Benzene	252
	Toluene	253
	Xylene	254
Aliphatic esters	Ethyl acetate	302
	Diethyl ether	250
	Turpentine	280
	Carbon disulfide	266
	Butyl cellosolve	306
Chemical families of mixture components	**Mixture Name**	**Chemical code**
Alkanes and MAHs	Mineral spirits post-1970	202
	Mineral spirits pre-1970	203
	Leaded gasoline	191
	Unleaded gasoline	299
	Aviation gasoline	190
	Kerosene	195

Information in table obtained from Infante-Rivard 2005[10].

Various animal studies show that some single agent organic solvents cause the development of certain cancers; few animal studies have looked at organic solvent mixtures. The solvents among some aliphatic chlorinated compounds that have been

demonstrated to cause cancer in animals include trichloroethylene, tetrachloroethylene, carbon tetrachloride, methylene chloride and chloroform [70].

In humans, investigators believe organic solvents also have carcinogenic potential. These exposures could create DNA damage (such as DNA-adducts) and lead to important mutations involved in the pathogenesis of cancers. Many organic solvents also lead to organ toxicity, therefore both organ toxicity and genetic damage appear to be possible consequences of organic solvent exposure [70].

Several organic solvents have been linked to many cancers in epidemiological studies [70,71] and as a result have been evaluated by the International Agency for Research on Cancer (IARC). Table A1 in appendix A provides a summary of several commonly used organic solvents in the workplace along with their use and IARC evaluation. There also exists a range of mixtures of organic solvents, also commonly used in the workplace [75]. Table A2 (also in appendix A) summarizes some important mixtures of organic solvents with IARC evaluations and their uses.

Organic Solvent Affinity

Labreche and Goldberg (1997) [43], describe the affinity of some widely used organic solvents for tissues and body fluids. The affinity is quantified by a partition coefficient, which is the ratio of the concentrations of the molecules in the two tissues being compared. Alcohols and ketones are hydrophilic and will be highly concentrated in the blood, but will have lower concentrations in fat tissue. The aromatic solvents such as benzene, styrene, toluene and xylene and the halogenated solvents such as methylene chloride, trichloroethylene and tetrachloroethylene are hydrophobic and will be highly concentrated in the fat as opposed to the blood (the air/blood ratio is smaller than the fat/blood ratio). These characteristics are important because they determine where in the body, the solvents will be at their greatest concentration. The alcohols and ketones will be highly concentrated in the blood, the liver and kidneys (blood rich organs) whereas the aromatic solvents and halogenated solvents will be concentrated in adipose (fatty) tissues.

The breasts have a large number of lipid cells, but also have an abundant blood supply and are therefore a good target for many organic solvents and their metabolites [43].

Organic Solvents and Breast Milk

The widespread use of organic solvents in the workplace makes it an important group of chemicals to study in the breast milk of lactating workers. Organic solvents are highly volatile and have often contaminated water sources [35,42]. They can easily enter a human body through ingestion, inhalation or skin absorption [43] and their concentration will be proportional to the solubility and lipophilicity of the chemical. Breast milk has a high fat content, therefore, lipophilic chemicals are more likely to accumulate in it (higher milk-to maternal plasma ratio leads to higher concentrations) [72]. The lifespan of organic solvents in the human body is quite short, therefore for accurate measurement of the solvents they must be measured shortly after exposure [73]. Despite their less persistent nature, there have been several studies showing the presence of organic solvents such as tetrachloroethene, benzene, chloroform, methylene and xylene in breast milk [35,45,47,74,75].

Fluids secreted by the apocrine glands are found in the lactating and non-lactating tissues of the breast. These fluids are recycled through an absorption mechanism whereby the fluids enter the lymphatic vessels from the ductal cells and finally enter the capillary blood vessels. The recycling of fluids may lead to an accumulation of endogenous and exogenous molecules in the breast fluid. Contaminants, such as organic solvents have been found in higher concentrations in breast tissue as compared to neighboring adipose tissues. The breast tissue may not metabolize or clear the chemicals as quickly as other tissues. Metabolic oxidative and reductive by-products may remain within the breast tissues a sufficient length of time to initiate carcinogenesis through DNA damage or alteration and damage from radial reactions [43]. Some studies have shown that breast milk can be positive on the Ames test (which shows mutagenic capacity in sample bacteria) and this was more common in pregnant farm workers, exposed to several chemicals, as compared to urban habitants [43,76].

Organic Solvent and Breast Milk Hypothesis

It has been hypothesized that exposure to organic solvents may be an important risk factor for breast cancer. The contact of these chemicals with important breast tissues may eventually lead to the initiation or promotion of carcinogenesis in the breast [43]. This hypothesis may also be relevant to the transfer of organic solvents into breast milk and future carcinogenesis in the feeding infant. Infants may be vulnerable to chemical contaminants from breast milk due rapid growth and development of their tissues, although there is not yet enough evidence to fully support this hypothesis [44,45].

Issues with current breast milk and chemical contamination studies

Many studies have analyzed chemical contamination of breast milk in lactating women and have problems such as small sample sizes and important biases (selection or information biases such as misclassification of exposure) [35]. The sampling and analyses of breast milk remain inconsistent across available studies. Some studies have selective sampling or do not describe their study methods making comparisons of different breast milk samples quite difficult [44,45]. In addition, available studies analyzing the exposure levels of chemical contaminants in breast milk have pooled data from several populations. Although pooling has many practical advantages it does have limitations such as ignoring the heterogeneity found in these different populations and providing a single average estimate, which may not be very indicative or useful. Women with different demographic characteristics such as age, parity and duration of breastfeeding, which affect exposure levels, are also grouped together. Investigators also use different techniques to measure exposure levels [35]. Due to the small number of epidemiological and toxicological studies looking into chemical contamination of breast milk and the transfer to offspring, there are no established clinical criteria for normal and abnormal breast milk concentrations. Guidelines need to be established in further studies [44].

Section 5: Organic solvents and cancer association studies

Organic Solvents and ALL

For several decades, investigators have been finding associations between occupational exposures in parents and risk of cancers in their offspring. The first study to find associations between occupations where parents were exposed to organic solvents and childhood leukemia was conducted by Fabia and Thuy and published in 1974. Most studies on childhood ALL look at the following time windows due to their etiologic importance: prenatal, gestation and postnatal. Organic solvents will affect the offspring differently during these time windows, through consequences such as organ, cell or DNA damage. The fetus or infant will be more or less vulnerable to certain chemicals during different time periods.

Many studies have shown evidence of positive associations between occupational exposures involving certain organic solvents in fathers (such as to paints, thinners, lacquers, glues, pigments, motor vehicle solvents, solvents in general, hydrocarbons, chlorinated solvents, mononuclear aromatic hydrocarbons or specific solvents such as 1,1,1 trichloroethane, carbon tetrachloride, benzene, xylene and toluene) and ALL in different time windows, either preconception, pregnancy or postnatal [9,10,13,16-21,77-79]. Some studies have examined associations between certain occupations in fathers, (such as painters, machinist, smiths or motor vehicle repairmen/drivers, rubber manufacturers, building finishers and related trades workers, wood treaters, machine repairmen) and childhood ALL or leukemia in general, where a broad range of chemicals, including organic solvents and hydrocarbons, could be responsible for the observed effects [12,23-25,27,80]. Others have found no significant associations with paternal exposure to organic solvents and /or occupations where solvents are regularly used and ALL [15,24,27-34]. Residing in proximity to petrol stations and repair garages during the postnatal period, where organic solvents are regularly used, has also been positively associated with the development of childhood ALL [81].

More studies have examined paternal occupational exposures, especially occupational hydrocarbons, than maternal occupational exposures. This is primarily due to the fact that many women used to work in the home, making the number of exposed women too low for analyses [13,28]. This is less true today and several studies have now been examining maternal chemical exposures, finding positive associations for mothers who have exposures to solvents such as benzene, toluene, gasoline, mononuclear aromatic hydrocarbons, alkanes, all hydrocarbons and solvents, paints and lacquers during pregnancy, preconception and postnatal time windows [9-15]. Certain studies, which analyzed similar occupational exposures to organic solvents in both parents, found more significant and stronger associations in mothers as compared to fathers, usually in the pregnancy and preconception time periods [9,11,15]. Contrarily, other investigations have yielded no associations between maternal exposure to solvents or maternal occupations with probable exposures to solvents and ALL [33,81,82]. An earlier study in 1987, found positive associations between childhood leukemias and maternal exposures to paints, lacquers and petroleum products during pregnancy and nursing [16]. The investigators considered breastfeeding a possible source of exposure for offspring. Very few published papers have contemplated breastfeeding as a mechanism of transfer, especially for organic solvents and its effects on ALL.

A 1998 review reports the findings of studies that have focused on parental occupational exposures and the risk of childhood cancers. There was strong evidence in support of exposure to organic solvents in paternal occupations causing childhood leukemias and lymphomas in all 5 published studies (with some odds ratios (OR) over 3.0 for exposures such as solvents in general, chlorinated solvents, benzene, carbon tetrachloride and trichloroethylene). In addition, several studies had analyzed risks of ALL associated with paternal exposures to paint and pigments (where there is a strong possibility of organic solvent exposure) and most of them found positive associations with ORs at 1.5 or greater. The same results have been shown for paternal exposures to motor related solvents and/or exhaust fumes [83]. A more recent review (2006), evaluated all the available evidence for occupational exposure to solvents and hydrocarbons in both mothers and fathers in different time windows. Results suggested that parental exposures to organic

solvents in the workplace likely have harmful effects on the development of childhood ALL but the evidence remains inconclusive [51].

Fewer studies have contemplated the effects of household exposures to organic solvents in parents and children on the effect of childhood ALL. Organic solvent contamination of household drinking water during pregnancy was associated, though not quite statistically significant, with the development of childhood ALL [14]. Freedman et al (2001)[84] found significant associations with a high level of artwork activity and medium level of electronic repair in children during their childhood (leading to exposure to solvents) and the development of ALL. Household painting of over 4 rooms during preconception was also associated with childhood ALL. There were a few borderline significant associations with other activities by case children during childhood involving organic solvents such as furniture stripping and auto/truck maintenance. Infante-Rivard (2005)[10] found no significant associations between household exposures to organic solvents and childhood ALL during pregnancy. Lowengart et al (1987)[16] published a paper whereby they show evidence suggesting maternal household exposures involving organic solvents had an effect on childhood ALL.

Appendix E summarizes the results of several studies that have looked for associations between occupational or household exposures to organic solvents to mothers or fathers during preconception, pregnancy or postnatal time periods.

Organic solvents and adult leukemia

In addition to increased incidence of cancer arising in offspring due to organic solvent exposure, many studies have found increased risks of adult leukemias as well. In humans, benzene is toxic to bone marrow and has been shown to cause chromosomal abnormalities. Exposure to benzene is now generally accepted as a risk factor for acute leukemia [70]. The latest assessment of benzene in 1987 by the IARC found benzene to be a sufficient cause for the development of leukemia by acting through the liver, mammary and bone marrow[85] (see Table A1 of Appendix A). Many studies have found significant

associations between certain workers, their exposure to benzene and acute leukemias mostly myeloid or monocytic cell types [70,86-90]. Occupational exposures have also been seen to cause several hematological disorders, such as aplastic anemia and myelodysplastic syndrome [89,91,92]. A problem with many of the earlier studies done on benzene is that they did not take into account other organic solvents that workers also handled. There is confounding and it is difficult to separate out the effects [86].

Several occupational groups such as painters, professional drivers (exposed to gasoline, diesel and their exhaust fumes) and rubber manufacturers have been associated with adult onset leukemia [77,79,93].

Section 6: Limitations with current case-control studies looking at organic solvents and childhood ALL

There are some problems with the methods used to measure occupational solvents in adults across various studies. It is difficult to measure how much an individual is exposed to, especially if the exposure level is low. Investigators have therefore relied on occupational groups heavily exposed to organic solvents [70]. These studies have shown inconsistent results probably due to exposure levels that are unmeasured or poorly measured [28,94]. The exposures encountered in the workplace are often self-reported. This method could lead to inaccurate or even biased information and therefore misclassification of exposures. These same problems are seen in studies of childhood ALL looking at parental occupational exposures. After originally publishing results from self-reported occupational exposures in parents of children with ALL and control parents, McKinney et al recently refined their chemical exposure measurements to minimize misclassification and validated their new exposure classification with an expert hygienist. They found that their original responses contained many misclassified exposures, resulting in different conclusions once reanalyzed. It is therefore recognized that experts and systematic coding with a validated classification system may improve information bias, which alters the measures of effect [21]. The validity and reliability of the

questionnaire or interview should also be assessed in order to correct for bias, though most studies have not verified these sources of error. There is also significant variation between studies because job titles are coded differently or different job exposure matrices are used [21].

There are also a wide variety of cytogenetic abnormalities in ALL, affecting certain age groups differently and when all ALL cases are grouped together, you are potentially missing some associations between certain chemical or physical determinants with one particular karyotype or abnormality [22]. Due to this problem, some investigators are using cytogenetic classifications as their outcomes, such as was done by Scleo et al (2009)[22] looking at the effect of paints and solvents on the different cytogenetic subtypes.

Another common problem is that it is very difficult to separate the effects of one organic solvent from the next due to the phenomenon that most people exposed to one chemical family are highly likely to be exposed to several others. Such high collinearity makes separating the effects quite difficult [28].

Finally, it is also a common occurrence for studies to test for multiple exposures and find both positive and negative associations for many solvents and mixtures [9,10,20] raising the potential for false positives and false negatives when there is multiple testing.

Sections 7: Candidate Xenobiotic Metabolizing Genes

Cytochrome P-450 genes

Active metabolites are in part moderated by the cytochrome P-450 (CYP) mixed-function oxidase complex. CYP enzymes are monooxygenases containing a heme group and operate as the terminal oxidase in the electron transport chain and use NADPH-P450 as a cofactor. There are 14 CYP families described in humans with families 1 to 3 known to metabolize xenobiotics, while the remainder metabolize endogenous substrates [37]. As with other genes involved in the metabolism of carcinogens, these can carry multiple

polymorphisms, which in turn, for some of these variants, can be translated into different amino acids and proteins. These polymorphisms can therefore lead to differences in enzyme activity and metabolism [3].

CYP1A1

Of the CYP genes, CYP1A1 (of the CYP1 family) is very important for the activation of aromatic amines, polycyclic aromatic hydrocarbons and constituents in cigarette smoke [3,37,95]. CYP1A1 enzymes are mainly active in extrahepatic tissues such as the lung, whereas CYP1A2 is concentrated in the liver [96]. Cytochrome P-450 genes are transcriptionally activated by polycyclic aromatic hydrocarbons (PAH), polyhalogenated dioxins and furans. Animal experiments have shown that these metabolites bind to the aryl hydrocarbon (AH) receptor on the cell surface and form a receptor ligand complex, which then migrates to the nucleus where it binds to xenobiotic DNA elements. A higher affinity to the receptor, will theoretically lead to more cytochrome P-450 activation, which mediates the response against the toxins. The level of cytochrome P-450 enzymes in the bloodstream, along with other detoxifying enzymes and endogenous nonprotein thiols will determine the amount of active carcinogens found in the fetus [41].

There are three identified polymorphisms of CYP1A1, thought to account for the variability seen in humans of the enzyme aryl-hydrocarbon hydroxylase (AHH) [62]. Using restriction enzyme *Msp*I, a polymorphism caused by a point mutation was found in the 3' flanking region (T → C transition), downstream from the polyadenylation site, of CYP1A1 and was termed *m1* (T6235C) [97].

A genetic variant was later found in the coding region of exon 7 and causes an amino acid substitution whereby Valine replaces Isoleucine at codon 462, which is near a heme-binding region. This polymorphism is termed *m2* (A4889C)[97]. Another variant was found in exon 7 of CYP1A1 and is a C to A substitution at position 4887 (resulting in a asparagine amino acid rather than a threonin at codon 461). This third polymorphism, named *m4* is located right next to *m2*, in the 3' region [98].

Both the *m1* and the *m2* polymorphisms have been associated with differing levels of AHH activity, resulting in different rates of carcinogen metabolism [99]. The Val (*m2*) variant has a two-fold higher catalytic enzyme activity than the wild type Ile polymorphism [62]. These differing activity levels have been associated with increased risk of lung cancer, particularly squamous cell carcinoma [62,97,100], while other studies have shown no change in enzyme activity or kinetics with the *m2* and *m1* polymorphisms compared to the wild type [99]. There are several other polymorphisms of CYP1A1 studied in association with diseases such as ALL [101], though they are not described here.

CYP2E1

The CYP2E1 gene metabolizes low molecular weight organic compounds such as benzene, ethanol, aromatic and halogenated solvents, alkanes, alkenes, *N*-nitrosamine and other organic solvents [102-105]. This gene is also known to reduce dioxygen to free radical species, which participate in lipid peroxidation and oxidative stress [103]. In human adults, CYP2E1 enzymes are also abundant in the liver, but evidence suggests they are also expressed in extrahepatic tissues such as in the lung, leukocytes, umbilical vein endothelial cells and the brain [37,106]. When CYP2E1 metabolizes carcinogens such as benzene, the metabolites accumulate in the bone marrow. The metabolism of benzene has been extensively studied and begins with oxidation by CYP2E1 to benzene oxide [91]. Some of the by-products such as reactive quinones, created through oxidation of these metabolites are believed to create DNA damage [107].

There exists a large inter-individual variation in the level of CYP2E1 enzyme with several known polymorphisms resulting in variable toxicity of its substrates [105,108]. There are also inter-ethnic differences in the frequencies of CYP2E1 polymorphisms, for example, 5% of European are heterozygous for the CYP2E1 *5A polymorphisms whereas 37% of Asians are heterozygous [105].

There are various genetic variants (nucleotide substitutions) in the CYP2E1 gene, 5 of which are at positions -1019 (*Rsa*I polymorphism, base pair G is converted to C), -1165, -1259 (*Pst*I polymorphism), -991 and finally -771 [109]. One of the main polymorphisms of interest (CYP2E1 *5B), associated with increased transcription, occurs at position -1019, in the 5' flanking region of the CYP2E1 gene and is believed to be located within the binding site for the transcription factor HNF-1 [103,109]. HNF-1 is an important regulator of liver specific expression and is thought to be active in both the enhancer and promoter regions of the human albumin gene [109]. When this polymorphism was amplified and fused to a reporter gene, it yielded 10 times more activity that the wild type genotype, but there were no quantitative differences in the amount of formed DNA-protein complexes [109]. The PstI polymorphism is thought to also impact transcription levels and has been associated with increased risk of developing lung cancer in smokers [62].

Glutathione S-Transferase (GST) Genes

The GSTM1 gene is part of a multifunctional family of genes, which produce multiple soluble enzymes, called the glutathione S-Transferase (GST). There are four main identified protein families: Alpha, Mu, Pi and Theta (or GSTA1, GSTM1, GSTP1, GSTT1 respectively). These enzymes protect tissues from oxidative stress and electrophiles by conjugating hydrophobic and electrophilic compounds with a reduced gluthathione resulting in the detoxification of active metabolites. As with the other xenobiotic metabolizing genes discussed, there exists many polymorphisms within the GST genes which increase or decrease enzyme activity [110]. The GST enzymes are an important for detoxification and reduce the adverse effects of reactive metabolites, therefore the level of their expression could be a pivotal component of an individuals susceptibility to carcinogenesis [111]. GST genes have been found to be involved in the metabolism of various exogenous carcinogens.

GSTM1

GSTM1 is highly expressed in the liver and detoxifies arene oxides, including the carcinogenic metabolite BP-diol epoxide [62,112]. The Mu genes were found to exist in a cluster about 20 kilobase pairs apart and were mapped to chromosome 1p13.3. One of the GSTM1 variants arises from a homozygous deletion (null allele) caused by homologous recombination, rendering the enzyme inactive. Using the map, investigators were able to localize the right and left junction regions (identical 4.2 kilobase regions that flank the GSTM1 gene). Recombination of these repeat regions cause the deletion and in most cases, produces a 7.4 kilobase *Hind*III fragment, whereby 10.3 and 11.4 kilobase *Hind*III fragments were deleted [113]. Its prevalence varies across different populations and is present in approximately 50% of Caucasians [3,68].

CYP1A1, CYP2E1 and GSTM1 genes in the fetus

The placenta is quite capable of metabolizing and detoxifying several exogenous chemicals and is an important component of fetal protection [37-39,41,114-118]. Various phase I and phase II enzymes, especially CYP enzymes, present in the placenta are detected at various levels throughout gestation; they partly determine the amount of xenobiotic molecules entering the fetus [40,117]. In addition, the cytochrome P-450 enzymes have been seen active in human fetal livers and extrahypatic tissues starting at a gestational age of 12 weeks, although in smaller quantities (20 to 70%) than in the adult. The rate of xenobiotic metabolism is also lower than in adults. Many chemical substrates such as caffeine, benzo(a)pyrene and polycyclic aromatic hydrocarbons have been found to be actively metabolized in the human fetus [37,41,48]. Studying fetal metabolism is difficult due to some of the experimental restrictions. As a result, many studies are done in animal models, particularly mouse, rat and sheep models, but comparisons are problematic due to physiologic differences between animal models and humans [111,119,120]. However, human fetal livers resemble sheep models, making them widely used in experiments assessing fetal protein activity. Fetal xenobiotic metabolism is complex and depends on the anatomy and biochemistry of the developing liver, but the liver is active and does metabolize chemicals at an early gestational age [120].

CYP1A1 has commonly been found in fetal cells and is believed to play an active role in xenobiotic metabolism. CYP1A1 has been found primarily in hepatocytes [37,95,120,121]. The evidence of the presence of CYP2E1 enzymes in the fetus is more contradictory. Several studies using techniques such as the reverse transcriptase-polymerase chain reaction (RT-PCR) method, have found no evidence of CYP2E1 in fetal cells ranging from less than 11 weeks to 30 weeks in gestational ages [37,121-123]. Contrarily, a study by Carpenter et al provided evidence that CYP2E1 was present in fetal cells through reverse transcriptase reaction with RNA, beginning at a gestational age of 16 to 24 weeks. The authors also confirmed that embryonic CYP2E1 expression could be further induced by the presence of xenobiotics such as ethanol or clofibrate [124]. A more recent study in 2003 confirmed the previous results, detecting the presence of CYP2E1 expression in 18 out of 49 fetal samples in the second trimester and 12 out of 15 fetal samples in the third trimester. It appears as though the quantity of CYP2E1 enzyme is very variable across subjects and generally increases up until the age of 90 days where it becomes somewhat constant [125].

Much less is known about fetal expression levels of GST enzymes as compared to adult levels. A study done in 1990, found evidence of GSTM1 and GSTP1 in lung and kidney tissues of fetal samples [126]. Recently, two major isoenzymes from the family GSTA have been isolated in hepatic and extrahepatic tissues of human fetal samples from the second trimester; however the fetus has reduced capacities to metabolize peroxidative substrates as compared to adults [111]. Similar results were found in a 2001 study, where investigators sought information on GST expression levels at gestational ages of 8 and 13 weeks. Substantial GST enzyme activity was found in the samples (except for GSTT1, which was not present in embryonic or fetal tissues). GSTP1 was the main GST family present in both samples (at 8 and 13 weeks) in all tissues except for the kidney where GSTA1 was the main protein family expressed. GSTA1 and GSTM1 were generally more moderately expressed across all tissues analyzed [116].

Investigators recently detected mRNA expression of six cytochrome P450 and 11 glutathione S-transferase isoforms, including CYP1A1, CY2E1 and GSTM1, in

hematopoietic stem cells (HSC) in the fetal liver (second trimester). The CYP isoforms had lower detectable levels than in the total liver cell population, unlikely to lead to DNA damage, though the GST isoforms in the HSC were found to have substantial metabolic activity towards the substrate 1-chloro-2,4-dinitrobenzene [127].

Section 8: Candidate Xenobiotic Metabolizing Gene Association Studies

Several genetic variants have been studied in association with ALL in children. Of these, a few have been commonly linked to ALL: CYP1A1*2A, CYP2E1*5, CYP2E1*5B, CYP2D6 *3, NAT1 *4, NAT2 slow acetylator, GSTM1 null, and GSTP1B[101]. The candidate genes of interest in this project (CYP1A1 (*2A, *2B and *4), GSTM1 (null) and CYP2E1 *5) have been studied in the context of acute lymphoblastic leukemias.

Two reviews in 2005 and 2006 found evidence to support the claim that some variants in the GSTM1, CYP1A1 and CYP2E1 genes are risk factor for acute leukemia [3,128]. A 2010 meta-analysis and systematic review researched all the candidate genes studied in ALL case-control studies. Combining the odds ratios, they found 7 variants out of 25 studied in the literatures to be significant risk factors and 1 variant to be a protective factor. Of the genes studied here, GSTM1 null (OR=1.16; 95% confidence interval (CI): 1.04-1.30), CYP1A1 *2A (OR=1.36; 95% CI: 1.11-1.66) and CYP2E1 *5B (OR=1.99, 95% CI: 1.32-3.00) were found be have significant pooled odds ratios across the various studies [129].

Comparing across various studies can be quite difficult, especially when different populations and sample sizes are used, however some general trends are discernable. The variants studied in this project have several contradicting results in the literature, described below.

CYP1A1 and childhood ALL association

The CYP1A1 polymorphisms are widely studied in etiologic research of childhood ALL. Many studies have found no or borderline significant associations [18,101,102,130-134]. For the *2A polymorphism, Kraijnovic et al (1999)[135], using a Quebec population, found a positive association with childhood ALL.

CYP2E1 and childhood ALL association

Fewer studies have looked at the effect of CYP2E1 on the risks of developing childhood ALL. A few recent studies have shown no significant associations between ALL and CYP2E1 variant [102,107,131], though one study found a positive and significant association between heterozygotes of the *5 variant and childhood ALL (OR 3.4 (1.3-9.1) [136]. This gene is particularly interesting with regards to organic solvent metabolism.

GSTM1 and childhood ALL association

The GSTM1 null variant and its association with childhood ALL has also been studied by several investigators. The results have been inconsistent, with several papers finding null or non significant associations between the null variant and ALL [102,130,131,134,137-139] and several finding significantly harmful associations [132,135,136,140-143]. These aforementioned studies have various sample sizes in different populations, making comparisons difficult. If various populations have a different prevalence of the GSTM1variant and possibly different environmental exposure levels to chemical carcinogens, this may explain why various studies are finding different effects [143]

Combined effects between various polymorphisms of interest on childhood ALL

Many studies have looked at gene-gene combined effects between the three genes discussed above or in different combinations with other important genes in the xenobiotic and DNA repair pathways. Investigators have found strong associations when CYP1A1 was combined with other variant genotypes [3,102,133,144], for example, many studies have

found substantially increased risks to cancers such as ALL and gastric cancers, when individuals had a variant CYP1A1 genotype in conjunction with the null variant of GSTM1 as compared to an individual who had neither [135,144-146]. Darazy et al (2011) [145] found a 36.5- fold increased risk of gastric cancer when CYP1A1 *2A and GSTM1 null variants were combined.

There is also ample evidence of an association between GSTM1 genes and other GST genes such as GSTP1 and GSTT1 [132,138,140,141]. A positive association has equally been documented for combined CYP2E1 polymorphims or combinations between CYP2E1 polymorphisms and various other gene polymorphisms such as NQO1 *2 and MPO *2 [103,107]. Despite the evidence for a combined effect, there are several studies also finding no associations when gene variants are combined [137,139,143]. Chen et al (1997) [138] documented an association between GSTM1 and GSTT1 variants in a black population, but none in a white population.

The presence of several variants in the xenobiotic metabolizing pathways may decrease overall transformation efficiency and increase risk of ALL through increased quantities of ultimate carcinogens, whereas one variant may have less of a pronounced effect [102].

The results of the candidate xenobiotic metabolizing gene association studies described above are detailed in appendices C and D (descriptions of the studies, population, strengths, limitations and results are provided).

Section 9: Xenobiotic metabolizing genes and organic solvent gene-environment interactions

Gene-environment Interactions and childhood ALL

It is very possible that CYP and GST variants biotransform their chemical metabolites differently than wild type, leading to increased risks when parents or the child are

exposed to these xenobiotic chemicals during important developmental periods (preconception, pregnancy and infancy or postnatal). In particular, there have been no studies looking at how variants in xenobiotic metabolizing genes may modify the effect of parental exposures to organic solvents on childhood ALL; however there have been some studies, though very few, looking at the joint effect of xenobiotic metabolizing gene variants with other environmental exposures in ALL. Below are a few examples of studies finding joint effects between xenobiotic metabolizing gene variants and organic solvents on the risk of developing various diseases. Joint effects between various gene polymorphisms and environmental exposures previously studied in ALL are also described.

CYP2E1 and organic solvent gene-environment interaction

A recently published paper explored the role of CYP2E1 as a possible effect modifier of the association between solvent exposure and non-Hodgkin lymphoma (NHL) in a large sample of primarily Caucasian women. Other genes, such as GSTM1, known to function in the metabolism of several organic solvents such as benzene were included. The authors found significant interaction between the CYP2E1 rs2070673 polymorphism and occupational exposures to dichloromethane, carbon tetrachloride and methyl chloride. Homozygous variant individuals had increased risk as compared to wild type individuals. This variant therefore appeared to modify the effect of these organic solvents on the risk of NHL [147]. Effects have equally been seen between heterozygotes of the CYP2E1 *5B variant and chronic-solvent induced encephalopathy (CSE) (OR: 6.1 (1.9-20.0)), further linking the metabolism of solvents with the CYP2E1 gene [104].

Several other studies have looked at CYP2E1 as a potential effect modifier of benzene and poisoning. A case-control study done in China on 100 subjects, found that the CYP2E1 *Rsal/PstI* variant did not modify the effect between benzene exposure and benzene poisoning [148]. Another study done in 2004, confirmed these results [149]. Contrarily, results in Kim et al (2007)[91], showed that individuals with the *Rsa*I variant

produced fewer benzene metabolites than the wild type, suggesting that CYP2E1 variants may reduce the metabolism of benzene.

GSTM1 and organic solvent gene-environment interaction

The GSTM1 polymorphisms have been shown to modify the effect of occupational exposures to xenobiotics such as trichlororethylene (chlorinated solvent) on renal cell cancer in adult workers (OR of 2.7 for GSTM1+ with a 95% confidence interval of 1.18-6.33) [150]. There is also some evidence to suggest GSTM1 and GSTT1 may be involved in the metabolism of styrene, particularly inside lymphocytes. The null variants increase genotoxic effects in lymphocytic cells [151]. Soderkvist et al 1996 [152] looked at exposures to organic solvents in 60 patients as a possible risk factor for chronic toxic encephalopathy. In addition, they looked at GSTM1 null as an effect modifier and found that subjects exposed to high levels of organic solvents with the GSTM1 null had elevated relative risks of 7.9 (1.1-4.8), whereas individuals with wild type GSTM1 equally exposed to high levels of organic solvents also had an elevated relative risk at 4.5 (0.8-4.1) though the effect was not as high. Landtblom (2003) [153] found that GSTM1 null did not modify the risk of organic solvents on the risk of developing multiple sclerosis in 50 adult patients with multiple sclerosis. Small sample sizes in both studies made interaction effects difficult to assess.

Gene-environment interaction and childhood ALL

Sinnett et al (2006) [3] report the results of a study where variants of methylenetetrahydrofolate reductase (MTHFR) appeared to modify the effects of folate on childhood ALL. Additionally, Infante-Rivard has published several articles showing that CYP1A1, CYP2E1 or GSTM1 child variants modified the effects of several parental environmental exposures [154-157]. In one such study, CYP1A1 *4 increased risk of maternal smoking during particularly the second and last trimester and increased the risk of low-level paternal smoking during the postnatal period on childhood ALL. The CYP1A1 *2B variant however, decreased the risk of prenatal maternal low-level smoking exposures

and of paternal postnatal high levels smoking exposures[156]. Similarly, another study by Infante-Rivard showed that the GSTM1 null and CYP2E1 *5 variants modified the risks of prenatal maternal alcohol consumption on childhood ALL, whereas only the CYP2E1 *5 variant modified postnatal maternal alcohol exposure[155]. Further examples of effect modifications were seen between the CYP2E1 *5 and GSTT1 null variants and postnatal and prenatal exposures to drinking water contamination of trihalomethanes and between maternal exposures to indoor insecticides during pregnancy and CYP1A1 m1 and CYP1A1 m2 variants[154,157].

Clavel et al (2005) explored interaction effects between maternal smoking, coffee or alcohol consumption during pregnancy and functional polymorphisms in phase I and phase II xenobiotic metabolizing genes (CYP1A1, GSTM1, GSTT1, GSTP1, EPHX1 and NQO1). CYP1A1 *2A and GSTM1 null appeared to increase the risk of maternal smoking on the risk of childhood leukemia as compared to the wild type polymorphisms, whereas NQO1 *2 appeared to have reduced the risk of maternal exposure to coffee and childhood leukemia. The wild type GSTP1 genotype appeared to have increased the risk between alcohol drinking and childhood leukemia as compared to the variant genotype [134].

V. Objectives

1) To assess the main effects of exposures to occupational organic solvents in mothers during the pregnancy and postnatal (breastfeeding) time windows on the risk of the offspring developing ALL

2) To assess the main effects of household exposures to organic solvents and paints through various activities in breastfeeding mothers during the postnatal time period (from birth of proband to date of diagnosis) on the risk of the childhood ALL

3) To determine effects of the CYP1A1 -2A, 2B and 4, CYP2E1 5 and GSTM1 null xenobiotic metabolizing gene polymorphisms on the development of childhood ALL

along with their combined effects

4) To explore the joint effects of exposures to occupational organic solvents and the candidate genetic variants (mentioned above) in offspring during the pregnancy and postnatal periods on the risk of the offspring developing ALL

5) To explore joint effects of household activities involving organic solvent exposures and painting and the candidate genetic variants (mentioned above) in breastfeeding mothers during the postnatal period

VI. Methods

The data for this project was taken from a population based case-control study, which was conducted between 1980 and 2000. There was extensive demographic, environmental and genetic data obtained from the participants. Details about this case-control study are also described elsewhere [10,156-159].

Case ascertainment

Through 1980 to 1993, cases between the ages of 0 and 9 years old were recruited from tertiary care centers in the province of Quebec and between the years of 1994 to 2000, cases up to the age of 14 were included in the study. A case was established as having ALL if diagnosed by a hematologist or oncologist in a tertiary center. Almost all acute lymphoblastic leukemias cases are treated at tertiary care centers due to governmental policy in the province of Québec. With all tertiary centers in the province targeted in this study, the cases therefore arised from a defined study base (the population of Québec). It is assumed that only a small number of children, if any, were treated outside of the province due to universal healthcare.

Control ascertainment

Between the years of 1980 to 1993, one control patient per case was recruited from family allowance files (FAF) from the Régie des Rentes du Quebec. All families with children in Canada are awarded family allowances by the government, therefore all children were deemed included in this data source. Ten children were randomly selected from this list, based on the distribution of expected cases, matched on sex, age and region of residence at time of diagnosis. For the years 1994 to 2000, controls were selected from the provincial universal health insurance files obtained from the Régie de l'Assurance Maladie du Québec. This database captures all children registered to the universal health plan, therefore is a very complete population database. The latter replaced the family allowance files because residential addresses were available, whereas the FAF largely moved to bank address for direct deposit. The same procedure was used for the selection of controls for both databases.

Exclusion criteria / Participation rates

Children with the following characteristics were excluded from the study: adopted, living with foster families, families spoke neither French nor English, did not live in Canada or had no parent available for interview. After exclusion criteria were applied, 848 cases were eligible for the study. Of these patients, the parents of 790 cases agreed to participate in the study (participation rate 93.1%). For the controls, 960 met the inclusion/exclusion criteria and the parents of 790 of these patients were interviewed (participation rate of 86.2%). The primary reasons for nonparticipation were the following: confidential phone numbers, refusal to participate or the family could not be contacted.

Exposure coding and acquisition

Mailed questionnaires were sent to the parents of the participants to inform them of the purpose of the study. Afterwards, trained interviewers contacted the parents by telephone to schedule an appointment for the interview. The interview was conducted over the

telephone using detailed questionnaires. These questionnaires assessed information on general risk factors and confounders, but also included assessment of a complete job history of the mother and father starting from the age of 18 until the end of pregnancy. Specific information about the job included the job title, dates, type of industry and the industry's name and address. More specific questions about the jobs held 2 years before and up to the birth of the affected child were collected through a semistructured questionnaire and included questions on activities of the company, activities of the employees, room or building types where employees worked, machine maintenance procedures, materials used, goods produced, details about the work environment, use of protective work gear and the presence of chemicals, gases, dusts, fumes, biocides, oils, solvents and radiation sources. An open-ended question was used to probe women further on typical activities done in the workplace. Even more details concerning exposures, environment, tasks and time spent doing these tasks were requested from individuals whose job was in the most frequent list or whose job was at a high risk of the occupational exposures mentioned above. Additional information on the most frequent job titles, the number of working women and the average number of jobs held are described in Infante-Rivard et al[10].

A team of trained chemist and industrial hygienists who have extensive experience in assessing occupational exposures in population case-control studies coded the organic solvents and other chemical exposures. The complete list of exposures included over 300 known compounds and mixtures. Each occupation was assigned a standard Canadian industrial title (three digit level) and job title (at the seven digit level), derived from Statistics Canada [160,161]. The experts determined whether parents were exposed to specific solvents, or solvent mixtures and to which degree. They did so by integrating several sources of information: 1) the job history provided by the parents, 2) knowledge gained from coding thousands of jobs in the same geographical area and 3) personal knowledge of the industries. This *expert strategy* has been previously described in the literature [162,163]. All of the organic solvents described in the study (see Table 1) are often used as industrial solvents, however some of these chemicals are used as chemical reagents or

fuels (such as benzene or gasoline respectfully). Additional details about exposure ascertainment are described elsewhere [10].

Household activities and painting data acquisition

Exposures to organic solvents from various household activities including painting, in several members of the home including the affected child if above the age of five, were also acquired through the general questionnaire. The time window was from one year before pregnancy to date of diagnosis. These activities included: furniture stripping, model building, silkscreen printing, electronic or radio operator amateur, electronic equipment repair (television, radio, stereo and other), use of large electronic tool equipment (circular saw, table saw, band saw, other), use of a sewing machine, maintenance or repair of truck and/or car, electronic video games and painting. More detailed questions were ascertained for the use of electronic video games if the affected child was exposed and for the sewing machine activities if the mother was exposed. For painting, additional information was requested such as type of paint, application method and more specific information concerning the time periods when exposures occurred (1 year before pregnancy, pregnancy and from birth of child to date of diagnosis).

Genotyping

Genetic material was obtained from study participants and their parents through the collection of blood samples, buccal swabs and mainly saliva samples. The two latter types of samples were collected by mail. DNA was extracted from the cell pellet samples using QIAamp DNA blood kits (QIAGEN, Mississauga, Ontario, Canada), which were used in compliance with the manufacturer's instructions.

There were three polymorphisms in the CYP1A1 gene studied using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in cell pellets: m1 (T6235C in the 3´ flanking region), m2 (A4889G) and m4 (C4887A in exon 7) [98]. Three allele variants were defined based on the presence of these variants: *2A (only m1

inherited variant), *2B (both m1 and m2 inherited variants) and *4 (only m4 inherited variant). For the genotyping of CYP2E1, a polymerase chain reaction (PCR) allele-specific oligonucleotide hybridization assay was used to identify the polymorphism at position -1259 where there is a G to C base pair substitution (allele CYP2E1*5). More details on the PCR techniques are described elsewhere [103]. The genotyping of the null GSTM1 variant (deletion) was done through PCR-based assays with the use of internal controls. The methods are described in Infante-Rivard et al. (2006) [164].

The studied variants are not somatic mutations that arise from DNA damage, but are inherited variants, which are passed on at conception. These variants are transmitted over generations and likelihood that they arose during cancer development is extremely small [156].

Analyses

For the following analyses, the occupational organic solvents were classified based on their chemical families or mixture: alkanes (C5-C17), aliphatic alcohols, chlorinated alkanes, chlorinated alkenes, aliphatic ketones, mononuclear aromatic hydrocarbons, mineral spirits (post-1970), unleaded gasoline, leaded gasoline and solvents in an all inclusive category (described in Table 1 of the literature review). The exposure level of these chemicals is dichotomous: no exposure versus any exposure (the latter includes possible, probable and definite exposure).

Maternal exposures to organic solvents from household activities were also assessed as a dichotomous variable (list of the household activities is mentioned above). Breastfeeding mothers were exposed if they performed one of the household activities from one year before pregnancy to date of diagnosis. Exposures to organic solvents from painting was also assessed in breastfeeding mothers from the date of birth of the affected child to the diagnosis. A breastfeeding mother was considered exposed if she participated in any painting activities in the household. In addition, type of paint used (latex, oil, acrylic or other) was assessed along with how the paint was applied (jet, brush or roller).

The distribution of important demographic characteristics in the study group is described. These characteristics included age of child at diagnosis, sex of child, maternal education, maternal race, maternal age at diagnosis, family income at diagnosis, breastfeeding, breastfeeding duration, paternal and maternal family history of cancer and maternal history of smoking (before and during pregnancy).

Hardy-Weinberg equilibrium

For the CYP1A1 and CYP2E1 polymorphisms in the control patients, Hardy-Weinberg equilibrium was tested as a control measure using Stata11 (Stata Corporation, College Station, TX). This test reassures as to the appropriate selection of the control group, which should represent the underlying population study base. The GSMT1 deletion variant cannot be tested because the heterozygotes cannot be differentiated from the homozygotes [112].

Case-control analysis

The main effects of the candidate gene polymorphisms, along with their combined effects (where all genes are included in the model simultaneously) on the development of childhood ALL were assessed using the case-control design with a conditional logistic regression model (Stata11, (Stata Corporation, College Station, TX). Effects on ALL were also calculated for maternal exposures to occupational organic solvents during the pregnancy and breastfeeding period. In addition, effects of organic solvents from household activities were assessed in breastfeeding mothers during the postnatal period. Gene x environment (G x E) interaction effects between child's genes and maternal occupational or household exposures to organic solvents during pregnancy and breastfeeding were also assessed. In the analyses, the CYP genes (CYP1A1 *2A, *2B and *4 along with CYP2E1 *5) either followed an additive genetic model (where there is a r-fold increased risk for one variant allele and a 2r-fold increased risk when there are

two), or a dominant model (where having one or two variant alleles produces the same increased risk)[165]. The dominant model was used when the number of individuals with two variant copies was too small to assess risk. The GSTM1 gene followed a recessive model (where two copies of the variant allele are necessary for there to be an increased risk [165]) in all analyses, with either a null or present genotype.

Confounders were determined using a change-in-estimate method [166]. A list of a priori possible confounders found in the literature included maternal smoking, maternal education, family income, maternal history of cancer and maternal age. These confounders were important for both the pregnancy and breastfeeding periods [9-11,18,20-23]. Breastfeeding duration was included as a possible confounder during the breastfeeding period only. Maternal genotype was not adjusted for in the models because if it is used as covariate in a case-control analysis, the risks from the child's genotypes will be attenuated by the risks from the mother's genotypes [167]. For the joint effects between maternal exposures to occupational or household organic solvents and child gene variants along with the genotype main effects, models were adjusted for maternal race, as a measure against population stratification bias [168].

Likelihood ratio tests were used for model selection to assess the significance of the interactions, whereby models including interaction were compared to models excluding interaction (1 degree freedom under chi-square). Effects were reported as odds ratios with 95% confidence intervals. To assess effect modification effects, the adjusted odds ratios of the organic solvents were reported across genotype strata (in subjects with the wild type genotypes and in subjects with the variant genotypes).

Case parent trio analysis

Interaction effects were also explored with a case-parent trio and a case-only analysis. The case-parent trio also provided estimates for the gene effects on childhood ALL. The case-parent and case-only analyses have more power to detect interaction than the case-control [168-170] and can be valuable additions to the case-control analysis.

The case-parent trio design analyzes the transmission of alleles from the parents to the affected offspring [168]. The analysis was performed using a log-linear poisson regression model accounting for maternal mediated effects, which are the effects of the maternal genotypes on the risk of the offspring developing the disease. The maternal genotype can be an important aspect of the uterine environment [171] and can possibly account for varying levels of organic solvent metabolites in the womb. The same concept can be applied during breastfeeding, where the breast tissues may differentially metabolize organic solvents depending on the mother's genotype. This design therefore adjusted for maternally medicated effects and estimated the gene and G x E effects in the CYP loci using LEM software. The log-linear model was proposed by Weinberg et al [171] and further extended to include G x E interactions [172]. It conditions only on child's disease status to model the expected counts of all possible child and parental genotype categories. The design is robust to population stratification bias. Genotype relative risks are estimated and missing genotype data were imputed using the built-in expectation-maximization algorithm in LEM [173]. Departure from a multiplicative model was assessed in the case-parent trio. Likelihood ratio tests were performed comparing models, which included the interaction variable to those excluding the interaction variable (2 degrees of freedom under chi-square). The case-parent trio effects are reported as genotype relative risk estimates with 95% confidence intervals

For the GSTM1 null variant, it is not possible to use the conventional log-linear model for trios because there are only two categories in the genotype. Instead, a likelihood ratio test with 1 degree of freedom was used to estimate gene effects in the program R (the R script provided by Buyske et al (2006)[112]). It was not possible to estimate G x E effects with this variant using the case-parent trio analysis. The approach conditions on parental mating types and can also incorporate missing genotypes. The GSTM1 gene followed a recessive genetic model.

Case-only analysis

The case-only design was performed using unconditional logistic regression adjusting for the same confounders as the case-control analyses, also adding sex and age of child (due to unmatching), in the statistical program Stata 11. For this analysis, the outcome becomes the susceptibility variant among cases. If the variant had two alleles, the heterozygote variant and the homozygous variant were collapsed into one category (dominant model) [174] and the case carrying 1 or 2 alleles was considered a "case" while a case with no copy of the variant was considered a "control".

This study design provides a COR (case odds ratio) estimate between the genetic variant and the organic solvent exposure. The interpretation of the odds ratio in a case-only analysis is a function of the odds ratio of the marginal effects of the genotype and exposure along with their joint effect in a case-control study: $COR = OR_{ge} / (OR_e \times OR_g.) \times Z$ (where OR_{ge} is the joint G x E odds ratio, OR_e is the effect of the exposure and OR_g is the effect of the genotype). COR is the case-only odds ratio and Z is the odds ratio in controls subjects (calculated similarly to the case-only OR, though with controls). It is equivalent to the interaction estimate obtained from a case-control if the genotype and exposure independence assumption is held. The case-only design is more efficient than the case-control and the case-parent trio to detect interactions [154,175].

The statistical significance of the estimates in all study designs was assessed using the 95% confidence interval. For the case-parent and case-control designs, G x E interaction effects were considered significant if the likelihood ratio test (LRT) p-values were above 0.05.

VII. Results

Demographic characteristics

The demographic characteristics were evenly distributed between the cases and the controls (see Table 2), with the exception of the number of mothers who breastfed, as 54% of control mothers breastfed compared to 48% in the cases.

Case-Control Analysis

Xenobiotic-metabolizing (XM) genes

The CYP1A1 *2A, *2B, *4 and CYP2E1 *5 polymorphisms were all in Hardy-Weinberg equilibrium, with p-values for the Pearson chi-squared greater than 0.05.

The effects of the proband CYP1A1 *2A, *2B, *4, CYP2E1 *5 and GSTM1 null polymorphisms on the development of childhood ALL are summarized in Table 5a. Two genetic models were utilized to determine the odds ratios of the CYP1A1 *2A and CYP1A1 *2B variants: additive and dominant models (where +/- and -/- variants were combined into one category). The odds ratios of the CYP1A1 *2A polymorphism, adjusted for maternal race, were harmful though borderline significant. In the additive model, the heterozygotes had an adjusted odds ratio of 1.34 (95% CI: 0.96-1.88) and the homozygote variants had an adjusted odds ratio of 1.81 (0.92-3.54). Alternatively, under the dominant model, the odds ratio was 1.34 (0.93-1.92). As for the CYP1A1 *4 polymorphism, the heterozygotes had a significant protective odds ratio of 0.46 (0.24-0.87). For both the CYP1A1 *2B (under additive and dominant models) and CYP2E1 *5 variants, the effects were null. Lastly, the odds ratio for the GSTM1 null variant was borderline significant, though not a strong predictor, at 1.23 (0.92-1.64).

Two additional analyses were performed where the candidate polymorphisms were included in a conditional logistic regression model, adjusting for maternal race. The first excluded CYP1A1 2B due to its collinearity with CYP1A1 2A. Using linear

combinations of CYP1A1 2A (+/-;+/+) and GSTM1 null, individuals with both these variants had a 1.68 (1.03-2.75) higher odds ratio of developing childhood ALL as compared to individuals with neither. The second analysis, excluded CYP1A1 2A, due to its collinearity with CYP1A1 2B. A linear combination of CYP1A1 2B (+/-;+/+) and GSTM1 null contrasted with neither showed a borderline significant risk at 1.78 (0.94-3.36). No other combinations in both analyses of either two, three or four genotypes at risk showed any significant risk of developing childhood ALL.

Occupational exposures

Occupational prenatal exposures to organic solvents regrouped under families and mixtures had some important and significant effects (Table 3). Maternal education was included in the regression models as a confounder. Mononuclear aromatic hydrocarbons (MAH) had a harmful and significant adjusted odds ratio of 1.65 (95% CI: 1.04-2.62). For mineral spirits (post-1970), the effect was also harmful at 1.91 (95% CI: 1.00-3.66). Alkanes were borderline significant with an adjusted odds ratio of 1.68 (95% CI: 0.95-2.96). All the other chemical families, mixtures and solvents in general had either nonsignificant or null effects.

During the breastfeeding period, the number of women exposed to solvents was much lower than during pregnancy (13 case-mothers and 9 control-mothers versus 149 case-mothers and 148 controls-mothers respectively). Due to the small exposure numbers, measures of effect could not be estimated for several chemical families and mixtures. Most of the odds ratios during the breastfeeding period were nonsignificant or null with wide confidence intervals, except for the effect of aliphatic alcohol, which was quite high at an adjusted odds ratio of 3.57 (95% CI: 0.75-17.04). Exposure to the category defined as any organic solvent yielded a higher estimate than during pregnancy, though the results were not significant (2.00 (0.68-5.85)). Two confounders were utilized for the breastfeeding period analyses: maternal age and education. The multivariate results for the maternal exposures to occupational organic solvents during breastfeeding are equally described in Table 3.

Occupational organic solvents and G x E interactions during pregnancy

G x E interaction effects between maternal occupational organic solvent exposure during pregnancy and proband GSTM1 null, CYP1A1 *2A and CYP2E1 *5 variants were studied. Due to the small number of study participants with the CYP1A1 *2B and CYP1A1 *4 polymorphisms, these variants were not included in the G x E interaction models. The latter polymorphisms were, however, included in the G x E interaction models when the exposure was to any organic solvent (described in Table 8) using a dominant model. The effects for exposures to specific chemical families and mixtures across genotype strata are described in Table 6.

Comparing the effects of exposures to maternal occupational organic solvents across the GSTM1 genotype strata showed generally lower odds ratios in individuals with the variant, however no significant differences between the estimates (with LRT p-values over 0.05) were found, except for exposures to aliphatic ketones. For this chemical family, the odds ratio in wild type individuals was 7.81 (0.95-64.32) and 0.83 (0.26-2.69), in the variant carriers, resulting in an LRT p-value of 0.04. The odds ratios of the aliphatic alcohol exposures across the GSTM1 genotypes were almost significantly different, where the odds ratio for the exposure in wild type subjects was 1.40 (0.79-2.47) compared to in subjects with the null variant at 0.69 (0.40-1.22) with an LRT p-value of 0.08.

For the CYP1A1 *2A polymorphism, the multiplicative interaction effects between maternal occupational organic solvent exposures and this gene were all nonsignficant with LRT p-values all above 0.05.

Lastly, for the CYP2E1 *5 polymorphism, the odds ratio for maternal exposures to any organic solvent in the wild type subjects was 0.91 (0.63-1.31) and 3.01 (0.64-14.20) in the variant allele subjects, showing a borderline- significant interaction with an LRT p-value of 0.13. The same trend with the CYP2E1 *5 was seen with aliphatic alcohols,

where the odds ratio in wild type individuals was 0.87 (0.56-1.34) and in variant individuals was 4.24 (0.69-26.08), with an LRT p-value of 0.08.

Several of the interaction effects between GSTM1 null and the chemical families showed that exposure among carriers of the null variant resulted in a protective effect while exposure among wild type carriers increased risk. The interaction effects between the CYP variants showed the opposite trend, where exposures to chemical families of organic solvents among the carriers of variants resulted in harmful effects while exposures among wild type carriers were largely null. The difference between the interaction effects for the CYP variants and wild type carriers however, were not statistically significant.

Occupational organic solvents and G x E interactions during breastfeeding period

Due to the low number of breastfeeding women occupationally exposed to organic solvents, it was not possible to evaluate the role of G x E interactions.

Organic solvent exposure from household activities

The effects of exposure to organic solvents from household activities from one year before pregnancy to the date of diagnosis, and to painting from birth of child to date of diagnosis in breastfeeding mothers are shown in Table 4. Certain activities had some strong effects, such as exposure to organic solvents from use of large electronic tools (OR=1.94 (0.90-4.22)) and being an electronic or radio operator amateur (OR=4.97 (0.54-45.48)). The effects of painting were somewhat important (OR=1.44 (0.89-1.99) and applying the paint with a brush showed an increased risk (OR= 1.63 (0.91-2.91). Some paint types were also associated with an increased risk of ALL in the offspring, namely oil paint and other types that do not include oil, acrylic or latex (OR= 1.55 (0.64-3.78) and 4.62 (0.53-39.85) respectively). The confounding variables included in these household models are the same as with the occupational solvents during breastfeeding:

maternal age and education. The estimates were not confounded by duration of breastfeeding or smoking.

Organic solvent exposures from household activities and G x E interactions in breastfeeding mothers from one year before pregnancy to the date of diagnosis

The number of breastfeeding women exposed to organic solvents from the following activities: model building, silkscreen printing, electronic or radio operator amateur, TV/radio/stereo/or other electronic equipment repair and maintenance or repair of trucks was relatively low. Finding G x E interaction for these variables was not always possible for proband SNPs at the CYP1A1 and CYP2E1 loci. The same was generally true for the GSTM1 null variant though interaction effects in these activities, other than silkscreen printing, were uncovered using the case-only analyses.

Among breastfeeding mothers, a significant difference was observed between the risk for furniture stripping in offspring with the wild type CYP1A1 *2A polymorphism (OR= 0.85 (0.54-1.34) and that in offspring with the variant (+/-; +/+) (OR=2.35 (0.88-6.26; LRT p-value=0.05). No other significantly harmful interactions were seen between this variant and other organic solvent exposures from household activities in breastfeeding mothers. All other comparisons in the other polymorphisms had LRT p-values above 0.15.

Case-Only Analysis

Occupational organic solvents and G x E interactions during pregnancy

The adjusted G x E interaction effects are reported in Table 7, except when the exposure is to any solvent, which is described in Table 8. The interaction effects between the majority of maternal exposures to occupational organic solvents and the proband GSTM1 null variant were protective, consistent with several findings in the case-control. The interaction effect of aliphatic alcohols and GSTM1 null was significant (OR=0.54 (0.32-0.90). When the exposure is defined as any organic solvent, the interaction odds ratio was

very protective and significant at 0.65 (0.43-0.99). The case-control analysis detected an interaction effect between GSTM1 null and aliphatic ketones and this was also seen in the case-only at 0.73 (0.25-2.11), although the effect was not statistically significant.

The effect of the CYP1A1 *2A variant and maternal exposure to any occupational organic solvent during pregnancy was 1.59 (1.00-2.53). For the CYP1A1 *4 variant, the OR with exposure to any organic solvent was 2.13 (0.91-4.96). All other interaction effects involving the CYP1A1 *2A polymorphisms were non significant, with some odds ratios being protective in some chemicals and others increased. Finally, for the CYP2E1 *5 variant, the interaction odds ratios were generally harmful, except when the exposure was aliphatic alcohols where both the case-only and the case-control showed a protective but non-significant effect.

Organic solvent exposures from household activities and G x E interactions in breastfeeding mothers from one year before pregnancy to the date of diagnosis

Interaction effects for exposures to organic solvents from household activities including painting in breastfeeding mothers and proband genes found similar results to the case-control analyses (see Table 10). The interaction estimate between GSTM1 null and furniture stripping was significant (OR=1.84 (1.08-3.13). For the CYP1A1 *2A variant, the interaction effect with painting was close to significant at 1.37 (0.90-2.11) and significant with latex and/or acrylic paint at an odds ratio of 1.54 (1.00-2.38). All other results for the CYP1A1 *2A polymorphism were nonsignficant. There was a strong interaction found between the CYP2E1 *5 variant and exposures to electronic equipment repair at an odds ratio of 18.28 (1.48-226.12). A similarly important, though not significant, interaction was seen between CYP2E1 *5 and exposures to electronic or radio operator amateur activities (OR=4.16 (0.82-21.28).

Case-Parent Trio Analysis

Xenobiotic-metabolizing (XM) genes

The effects of proband CYP1A1 *2A, CYP1A1 *2B, CYP1A1 *4, CYP2E1 *5 and GSTM1 null polymorphisms on the development of childhood ALL using a case-parent trio design are summarized in Table 5b. A genotype relative risk model was used for all the CYP polymorphisms, whereas a recessive genetic model was used for the GSTM1 variant. The main effects of the CYP variants were nonsignificant. The only important difference between the case-control and case-parent results arose in the effect of the CYP1A1 *4 variant, whereby it was significantly protective in the case-control, but had a null effect in the case-parent trio (relative risk of 0.99 with a 95% CI of 0.55-1.77). For the GSTM1 variant, the relative risk using the case-parent trio design was found to be significant at 1.43 (1.20-1.70).

Occupational organic solvents and G x E interactions during pregnancy

For the case-parent trio analyses, interaction effects found between occupational exposures to organic solvents during pregnancy and proband CYP1A1 *2A variant are shown in Table 7, except for exposures to any solvents which are in Table 8). The G x E relative risks between alkanes and 1 copy of the CYP1A1 *2A variant was 1.81 (0.32-10.06) with an LRT p-value at 0.18. For chlorinated alkenes, the interaction effect for 1 copy was 1.62 (0.15-17.69) with an LRT p-value of 0.18. For MAH exposure during pregnancy, the interaction relative risk with 1 copy was 2.48 (0.65-9.46) and with 2 copies was 5.93 (0.27-131.17) giving an LRT p-value of 0.29. Finally, the G x E effect between 1 copy of the CYP1A1 2A variant and all solvents was 1.52 (0.79-2.95) and for 2 copies was 5.36 (0.76-37.41) with an LRT p-value at 0.19. The joint effect between 1 copy of the CYP1A1 2B variant and exposure to any solvent was significant at 1.57 (0.52-4.73) giving an LRT p-value of 0.03. All other exposures had null interaction effects with the CYP1A1 variants and large LRT p-values.

The G x E effects between the offspring CYP2E1 *5 variant and maternal occupational organic solvent exposures during pregnancy were nonsignificant with LRT p-values all above 0.85. There were no particular patterns, with most interaction effects bordering the null.

Organic solvent exposures from household activities and G x E interactions in breastfeeding mothers from one year before pregnancy to the date of diagnosis

The interaction effects between proband CYP1A1 and CYP2E1 *5 polymorphisms and exposures to household activities including painting in breastfeeding mothers on the development of childhood ALL were all nonsignficant with LRT p-values over 0.05 (details are described in Table 10). The interaction effect between CYP1A1 2A and organic solvents from large tools was relatively high at 2.17 (0.51-9.30) with an LRT p-value of 0.22. The interaction effect between CYP1A1 2A and oil painting was also high at 3.10 (0.31-31.05), though the 95% confidence interval was quite wide with a large LRT p-value at 0.56. Thus, there was no particularly strong pattern for any of the interaction effects across the household activities.

VIII. Discussion

Main effects of organic solvents

Maternal occupational exposures to alkanes, MAH and mineral spirits during pregnancy were shown to be risk factors for the development of ALL in their offspring (ORs of 1.68 (0.95-2.96), 1.65 (1.04-2.62) and 1.91 (1.00-3.66) respectively). These results were previously published by Infante-Rivard et al 2005 [10]. There are no other studies in the literature that have analyzed exposures to *specific* chemical families or mixtures of occupational organic solvents in mothers during pregnancy. In addition, our results found no effect of maternal occupational exposure to any solvent as a broad category during pregnancy and ALL, which corroborates previous null findings [15,82]. For instance, Schuz

et al similarly found a non-significant effect between maternal exposure to solvents during pregnancy and pediatric cancer cases at an odds ratio of 1.3 (0.8-1.9) [11].

However, our results are different from other studies, which have found significant harmful associations between maternal organic solvent exposures in the workplace and development of ALL [18,176]. McKinney et al found a strong effect of maternal exposures to organic solvents during pregnancy at 2.7 (1.6-4.6), using a job-exposure classification system and a large sample size of 1881 pediatric leukemia and lymphoma cases [21]. Shu et al found some significant effects of maternal exposures to organic solvents during pregnancy and ALL at an odds ratio 1.9 (1.0-3.6). Strong effects were also found for other maternal solvent exposures such as degreasers and cleaning agents, turpentine, paint removers and paints (any or spray) [9]. It is difficult to compare these results because of the different methods used to determine exposures. Moreover, the exposure environments were different. Nevertheless, the discrepant results probably suggest that much more work is needed, in particular with respect to exposure assessment, to understand the impact on ALL of prenatal exposures to organic solvents.

There were few breastfeeding mothers occupationally exposed to organic solvents during the early postnatal period. It was not possible to obtain estimates for several chemical families and mixtures of organic solvents. A pattern was discernable for aliphatic alcohols, where 10 cases were exposed as compared to 3 controls (adjusted OR 3.57 (0.75-17.04)). The effect of occupational exposures to any organic solvent in breastfeeding mothers was higher than during pregnancy at 2.00 (0.68-5.85), though the results were not statistically significant. There are few available studies in the literature, which analyzed exposures during the breastfeeding period and ALL. None have looked at specific maternal occupational exposures to organic solvents and ALL. Our results do suggest the possible transfer of organic solvents, in particular aliphatic alcohols from mother to child through the breast milk increasing the risk of ALL. Future studies would be needed to confirm these findings, however, due to small exposure numbers, finding a larger sample size in this population with enough exposed women would be quite difficult and unlikely to be fruitful. Interestingly, as reported by Shu et al (1999)[9], some

volatile organic solvents may have an important impact during pregnancy through placental transfer and not during the postnatal period from breastfeeding. Our results suggest similar findings due to varying effects of certain chemical families during pregnancy and breastfeeding, though a larger sample size for the breastfeeding period would be required to confirm this hypothesis.

The exposure to organic solvents from household activities in breastfeeding mothers from one year before pregnancy to date of diagnosis yielded no strong or significant results, other than organic solvent exposure from use of large electronic tools (OR: 1.94 (0.90-4.22)) and from being an electronic or radio operator amateur which had a strong harmful effect (OR=4.97 (0.54-45.48)). Exposure to painting, in breastfeeding women, during the postnatal period was not particularly strong (OR= 1.33 (0.89-1.99). Applying the paint with a brush however, had a stronger effect (OR=1.63 (0.91-2.91)) as well as using oil paint or a paint other than latex, acrylic and oil. Paint application could be an important component of exposure, either by increasing exposure time or changing the degree of direct contact with the paint compounds. There have been few studies looking at maternal exposures to such household chemicals during preconception, pregnancy and postnatal periods and ALL, though our results were consistent with previous findings. Lowengart et al (1987)[16], found an effect between paint and lacquer exposure in mothers during pregnancy and nursing at 1.8 (p-value 0.03). Freedman et al found a modest effect of painting in mothers during preconception (postnatal maternal exposures were not assessed)[84]. A more recent study shows the effects of household paints and solvent finding a significant effect for frequent users of paint (either mother, father or offspring) and ALL at 1.74 (1.25-2.43) during all time windows. However, maternal exposures to paints at any time period or specifically during pregnancy showed nonsignificant results. Frequent use (by either mother, father or offspring) of solvents in the household was associated with borderline significant result (OR=1.31 (0.95-1.81)). No other maternal exposures to organic solvents in this paper during any time period for the mother yielded significant results [22].

Using the change-in-estimate method, maternal age was an important confounder in the model for occupational organic solvents during breastfeeding, but was not an important confounder during pregnancy. Increased age in the mother could have physiological impacts on quality and quantity of breast milk thereby affecting the risk of ALL. Several studies have documented effects of increased maternal age on risk of ALL or other leukemias such as AML [177-179].

Main effects of xenobiotic metabolizing gene variants

Certain xenobiotic metabolizing gene polymorphisms of interest in the CYP and GST gene families were also studied. The genetic frequencies of CYP1A1 *2A, *2B and our GSTM1 null polymorphisms in this Quebec population were consistent with other studies looking at a Caucasian population (over 95% of the population in our study is Caucasian) [103,134,135,139,143,180]. Krajinovic et al (1999)[135] note that the CYP1A1 *4 genotype frequency in the their study of French Canadians was 5%, slightly more elevated than what other studies reported in Caucasian populations. In our results, 8% of controls were heterozygous for the CYP1A1 *4 variant, also more elevated than that found in previous studies and consistent with Krajinovic et al (1999)[135]. Pakakasama et al found that 0.5% of their Turkish control population had this variant allele, a much smaller proportion [132]. This allele frequency is therefore quite variable across populations.

The effect of the CYP1A1 *2A variant on the risk of developing ALL was harmful in the case-control and quite close to significant for heterozygotes, homozygotes and a combined category of the two (1.34 (0.96-1.88), 1.81 (0.92-3.54) and 1.34 (0.93-1.92), respectively). The effect of the CYP1A1 *2B variant (combination of m1 and m2), was similar to that of *2A, but not close to significant for heterozygotes, homozygotes or a combined category of the two (1.32 (0.80-2.19), 1.75 (0.64-4.81) and 1.43 (0.83-2.46) respectively). In the case-parent trio analysis generating genotype relative risks, the effects for both variants were null. Effects of this size, close to significant, for the 2A (or m1) variant have been seen in the literature for ALL [130,133,136], whereas others have been nonsignificant or null [132,134]. Three studies found significant effects between this variant

and ALL, one of which is a study done by Joseph et al in a Keralite population in Southern India, finding significant results for both the m1 and m2 mutations and another with similar results in a Brazil population [102,144]. A study performed in a French Canadian population by Krajinovic et al [135] found similar results to ours, but they were statistically significant for a combined heterozygotes and homozygotes category of the variant *2A (OR=1.8 (1.1-3.1)). For the combined 2B variant category, the odds ratio was not significant at 0.9 (0.4-1.8) and again quite similar to our results. Contrarily, one study found a protective effect of the 2A variant, though the estimate was not statistically significant [131]. Pakakasama et al found a protective though non-significant effect of the 2B variant at an odds ratio of 0.4 (0.1-1.4) [132]. Our results are therefore quite similar to the results found in the literature and do not point to a large role of the *2A or *2B genes in the development of ALL.

The effect of the CYP1A1 *4 polymorphism was statistically protective in our case-control design. Heterozygotes had a protective odds ratio against childhood ALL at 0.46 (0.24-0.87). However, in our case-parent trio, the effect became null. Few studies have looked at this polymorphism in ALL studies, though another study using a French Canadian population found a protective effect, though not significant at 0.6 (0.3-1.2). A study by Pakakasama et al (2005)[132], in a Thailand population, found no significant effects when they looked at this variant in combination with the variants 2A and 2B. Although our findings were very significant for the case-control, our case-parent results did not confirm these findings. Having the variant may confer an advantage against the development of ALL, though additional studies are required for this variant in the context of ALL. In addition, the concentration and activity of CYP1A1 *4 in the fetus and infant would have to be studied further to understand its role.

There is increasing evidence of the harmful effects of GSTM1 null in ALL. Many studies from various ethnic populations, such as India, Thailand, Iran, Portugal, China, Phillipines and Turkey have found significant, or very close to significant, harmful effects in children with this variant and development of ALL [132,136,140-144,181,182]. Protective effects of this variant was seen in only one study by Pigullo et al (2007)[137] done in Italy,

with an odds ratio of 0.71 (0.47-1.07). Other investigations have yielded null effects [102,130,134,139]. The results from our study are quite consistent with many that have found harmful effects. Although our case-control estimate of the null variant was borderline harmful at 1.23 (0.92-1.64), our case-parent trio results were quite significant at 1.43 (1.20-1.70). This is also consistent with another study done in a French Canadian population which found GSTM1 null to be a harmful and significant predictor of ALL at an odds ratio of 1.8 (1.2-2.6) [135]. The null variant may lead to a change in the biotransformation of chemical exposures entering the fetus or child causing an increased risk of ALL. GSTM1 enzymes have been shown to be present in the fetus [111,116,126].

Fewer studies have analyzed the effects of CYP2E1 variants on ALL. The effect of the *5B mutation on enzyme activity has not clearly been defined, though some in vitro studies have shown changes in expression [129]. At a biological level, it is uncertain how this variant could impact metabolism leading to potential DNA damage. Two studies found significant harmful effects of this variant on the risk of ALL. The first was carried out in a Turkish population and found an odds ratio of 3.4 (1.3-9.1) [136] and the second in a French Canadian population with an odds ratio of 2.8 (1.2-6.7) [103]. Unlike the latter studies, our results do not show any significant effects of this variant neither in the case-control or the case-parent trio. A few other studies done in a variety of ethnic backgrounds (in Brazil, Spain and Turkey) have also found either null or non-significant results with the CYP2E1 *5B variant and ALL [102,107,131].

The combined effects between GSTM1 null and CYP1A1 *2A were significant at an odds ratio of 1.68 (1.03-2.75) indicating that individuals with both these variants, as compared to an individual with neither had an increased risk of developing ALL. Such results have been previously described in the literature (including the French Canadian population study) [102,135,146] with one study finding a combined odds ratios as high as 9.68 (1.13-83.05) [144]. It appears that having two variants in xenobiotic metabolizing genes, particularly in phase I and phase II genes may lead to increased ALL risk. This may be due to important disruptions or changes in the biotransformation of various chemicals. No other combinations were statistically significant or particularly strong.

Overall G x E patterns and differences between the study designs

Interaction effects between the *GSTM1 null variant and occupational organic solvents* in the case-control and case-only study designs were generally protective. The case-only estimates were more precise than the ones from the case-control estimates, though the patterns were very similar in both study designs. The case-only results confirmed findings from the case-control. The GSTM1 variant significantly modified the effect of aliphatic alcohols and aliphatic ketones on the risk of ALL. Modest protection was seen for exposures to chlorinated alkanes and chlorinated alkenes in individuals with the variant compared to those without. The effects were slightly different for the chemical families alkanes, MAH and mineral spirits, where harmful interactions were seen in the case-control, but protective interactions were seen in the case-only. For these families, none of the estimates in either design were significant, with wide confidence intervals.

Interaction estimates in all three study designs were relatively consistent for the *CYP1A1 *2A variant and occupational organic solvents*. The frequency of this variant allele in the study population was low resulting in unavailable interaction estimates in the case-parent and case-control analyses. However, the case-only design was able to estimate all G x E effects for the CYP1A1 *2A variant. The only difference between the study designs' results arose in the case-control estimate for the interaction between chlorinated alkanes and CYP1A2 *2A where the odds ratio was protective, but null in the case-only and case-parent. In all designs, the interactions varied from harmful in aliphatic alcohols, MAH, unleaded gasoline and chlorinated alkenes to protective in the mineral spirits, alkanes and aliphatic ketones. The confidence intervals for the interaction odds ratios were generally wide and inclusive for all study designs, though noticeably more narrow for the case-only. However, it appears CYP1A1 *2A interactions with organic solvents may be important, with the variant sometimes protecting and lowering the risk of ALL and other times increasing the risk, depending on the chemical family of the organic solvent exposure. The known substrates of CYP1A1 enzymes consist of aromatic amines, polycyclic aromatic hydrocarbons and constituents in cigarette smoke [3,37,95]. It is

biologically quite possible that many of the chemical families in this study include compounds that are actively metabolized in by this variant, however, adult metabolism differs from fetal metabolism and although this enzyme is present in the fetus, much less information is available on its activity [37].

The interaction trends for the *CYP2E1 *5 polymorphism and exposures to occupational organic solvents* were generally harmful and consistent across all three study designs. The analysis of interaction for aliphatic alcohols and MAHs in the case-control suggested harmful effects, but were much weaker in the case-only and case-parent designs. In addition, the G x E effect between aliphatic ketones and CYP2E1 *5 appeared protective in the case-control and case-parent, but was harmful in the case-only. Other G x E effect results between occupational organic solvent families and this polymorphism were generally consistent between the study designs. None of the G x E effects between this variant and exposures to maternal occupational organic solvents were significant and they had wide confidence intervals, remaining therefore inconclusive. CYP2E1 enzymes and their activity have been detected in fetal cells, though their activity remains somewhat elusive. The known substrates of the CYP2E1 enzymes are low molecular weight organic compounds such as benzene, ethanol, aromatic and halogenated solvents, alkanes, alkenes, *N*-nitrosamine and other organic solvents [102-105]. Therefore, it is biologically possible that having the variant leads to an increase in metabolism, increasing the level of ultimate carcinogens leading to potential DNA damage. Our results do suggest that having the variant and being exposed to organic solvents through breastfeeding or during pregnancy may confer an increased risk of ALL, but the results are not statistically significant. More information would be required on the activity of fetal CYP2E1 enzymes and larger sample sizes would also be required to isolate the G x E effects.

In regards to the interaction effects between *exposure to any solvent* and the polymorphisms, the interaction effects appear fairly consistent between the different study designs, with the exception of CYP2E1 *5, where the interaction effect appears quite harmful in the case-control (interaction OR 3.31 (0.66-16.49)), but null in the case-only and in the case-parent design. The case-parent trio found significant interactions

between CYP1A1 *2B and any organic solvent as well as close to significant interactions between CYP1A1 *2A and any organic solvent suggesting these variants increase the risk of ALL in exposed individuals. Grouping the organic solvents into one category is helpful by increasing exposure numbers and improving the power of detecting an effect, however, there are many substrates combined into one category. This grouping may hide effects between specific chemical substrates and CYP and GST variants and drive the odds ratio towards the null. Despite this, the effects suggest some potential interactions between occupational organic solvents and these various polymorphisms.

There were few important interaction effects between *organic solvents from household activities* and the candidate genes. In a few case-only analyses, particularly for activities involving electronics and CYP2E1 *5, there were some strong interactions, though no estimates were possible to confirm these findings in the case-parent and case-control designs. In addition, the confidence intervals were quite wide. There were some interactions in the case-only analyses between the CYP1A1 *2A variant and exposure to painting in breastfeeding mothers during the postnatal period, in particular to latex/acrylic paints, suggesting that this variant may modify the risk of painting and paint types on childhood ALL. This result was consistent with the case-control results, but not confirmed in the case-parent trio design. For this same variant there was a strong interaction with furniture stripping in the case-control, but these interaction results were less harmful in the case-only or case-parent designs. Finally, a harmful and significant interaction effect was found between GSTM1 null and exposures to organic solvents in furniture stripping in the case-only. A similarly harmful interaction effect was also found in the case-control, though it was weaker and not significant. These results suggest that having the GSTM1 null or the CYP1A1 2A variant and being exposed to organic solvents from furniture stripping during breastfeeding may confer an increased risk of developing ALL. Overall, these results point to the potential risks of exposures to organic solvents found in the household in breastfeeding mothers in addition with certain offspring genetic susceptibilities in CYP and GST xenobiotic metabolizing genes on the risk of childhood ALL. The CYP1A1 *2A, CYP2E1 *5 and GSTM1 null variants may differentially

biotransform organic solvents from breast milk in infants causing increased DNA damage and ALL risk.

This is the first study to look at the interactions between maternal occupational and household exposures to organic solvents and xenobiotic metabolizing genes in the offspring on the risk of childhood ALL. Similarly, a recent published study has found evidence that a CYP2E1 variant modified the effect of organic solvents in women on the risk of non-Hodgkin lymphoma [147]. As such, the results of this study remain exploratory and additional studies are needed to corroborate the study findings.

Study strengths and limitations

The data used for this study was from a well-designed population case-control study, as shown by the equal distribution of various measured confounders in the cases and controls (shown in Table 2). It is unlikely to be affected by important selection bias.

The effects of maternal exposures to organic solvents in the workplace have not commonly been studied. This study had a large sample of women and used the expert method to classify chemicals appropriately, which minimized misclassification of exposures, as previously suggested by several investigators [10,28,183,184]. In addition, interviewers were blinded to the study hypotheses, preventing differential ascertainment of exposures in cases and controls. Older studies used occupations as an exposure rather than specific chemical exposures. In such studies, maternal occupations where organic solvents were regularly used and which were held during pregnancy were found to be associated with the development of ALL (occupations included hydrogen related occupations, dry-cleaners, chemical processors and related workers (such as rubber and plastic products makers, leather workers, painters and chemical analysts), construction and porcelain industries) [12,15]. Assessing specific chemicals rather than occupation allows us to determine specific effects of certain chemicals and their carcinogenic potential. This stratification, however, leads to some categories of organic solvents having very few

exposed individuals thus increasing the width of confidence intervals. As such, statistical precision was lost.

The measurement of the household activities was fairly crude, with questions assessing any exposure versus none. The duration of exposure was not ascertained in the interview. A woman who frequently used large electronic tools could have been grouped with a woman who performed such activities only once or twice. In addition, the household activities studied here may involve several organic solvents and other chemical exposures. Separating the effects is difficult and such a grouping of exposure levels and different organic solvents may lead to an odds ratio that is attenuated. Furthermore, the time window for exposures to household activities in breastfeeding mothers was quite large and included one year before conception, pregnancy and all of the postnatal period until date of diagnosis. The preconception and pregnancy time windows may be important due to collection of organic solvents and accumulated damage to the breast tissue and breast milk. The inclusion of the entire postnatal period may be slightly too large. The effects are likely to have been attenuated due to the inclusion of data from such a large time window. The questionnaire was more specific for painting as it included specific information on the time period (either preconception, pregnancy or postnatal). Regardless of these limitations, some of the effects were nonetheless interesting and suggest that exposures in breastfeeding mother due to certain activities occurring before pregnancy to date of diagnosis in conjunction with child variants may lead to increased risks of ALL.

The sample size in this study can be considered large with 790 cases and 790 controls. Between 68% to 70% of the cases and 63% to 64% of the controls were genotyped for the CYP1A1, CYP2E1 and GSTM1 variants. This is unlikely to have generated a selection bias because providing samples or not was unlikely to have been related to any study exposure. The prevalence of exposure in the genotyped sample was not very different than that of the entire study population. A very large sample size is nonetheless required to estimate G x E interaction effect. The case-only and case-parent designs therefore had better power (the former in particular) to detect these interactions than the

case-control [5,169,175]. Nonetheless, the power remains an issue in the analysis with many of the interaction effects and main effects for occupational solvents during the breastfeeding period having very wide confidence intervals leading to inconclusive results. As such, the interaction results remain largely exploratory and need to be confirmed by additional studies with larger sample sizes.

Due to the large number of exposure categories and candidate variants, there were many interaction estimates. Multiple testing can lead to false positive associations and is often a problem with such studies [9]. Even though multiple tests were performed in our study, the hypotheses were predetermined and were founded by biological plausibility. Additionally, multiple study designs were used to confirm the results.

Limitations with many candidate gene studies involve small sample sizes as well as possible population stratification bias when case-control and case-only designs are used. In the candidate xenobiotic metabolizing gene and childhood ALL literature, several study populations were not homogeneous and included several ethnic backgrounds. Genotype frequencies can often vary in different population subgroups. This is an important bias that may invalidate results in both the case-parent and the case-only [168,172,175,185]. Unlike many candidate gene studies in ALL, this study had a large sample size. The results could have been biased by population stratification, though the majority of the study subjects were French Canadian, which are a homogenous founder population [146]. Moreover, analyses involving variants were adjusted for race. As such, population stratification is minimized yet remains a potential concern in the case-control and case-only analyses.

A strength of the study comes from the confirmation of our interaction case-control results with other study designs that have better power of detecting G x E effects [168,169,175,186]. An important assumption made in the case-only analysis is that there is a gene-exposure independence (i.e. in the population at large, having the variant does not predispose you to an exposure) [175,185]. This assumption was unlikely to have been violated because mothers were exposed and the investigated genes belonged to the fetus. The

case-parent and case-only analyses do not allow the estimation of main effects for environmental exposures; therefore we were unable to make comparisons with the case-control estimates. Finally, it is quite possible that the effect between the variants and ALL were not due to a casual association but rather linkage disequilibrium with a disease variant in a neighboring region [168] (see Appendix B for a complete list of the strengths and limitations of case-control, case-only and case-parent trio designs).

Although the effects of chemical exposures in the breast can be harmful, as shown in these analyses and in the literature, breastfeeding itself is protective against many diseases [47,72,187,188] and this protective effect was also seen in our results (OR in our analyses 0.79 (0.63-0.98)). Therefore the alternative is not to discontinue breastfeeding, but to increase awareness whereby women could be sensitized to the vulnerabilies of their newborns and prevent contamination, both at work and in the home [74,187]. The province of Quebec is quite advanced in this respect with laws in place that protect breastfeeding women in the workplace, providing them with the legal grounds to change posts if there is exposure to chemicals [73]. Our study does show that few Quebec women are exposed to occupational solvents during breastfeeding, though many were during pregnancy. There are seemingly some risks to the offspring of developing ALL during childhood when pregnant women are exposed to organic solvents.

Future studies

Additional research is required to support the evidence that the CYP1A1 *2A, *2B, *4, CYP2E1 *5 and GSTM1 null polymorphisms modify the effect of maternal occupational and household exposures to organic solvents during multiple times periods including pregnancy and breastfeeding on the risk of ALL development. In addition to xenobiotic metabolizing genes, DNA repair genes have also been associated with ALL [129] and future studies could analyze potential G x E effects with organic solvents. Furthermore, rather than study individual variants, where there is the potential of not being able to capture the variability in the entire candidate gene using haplotypes as a method of analysis to possibly capture more variability [189].

Conclusion

In addition to risks associated with maternal exposures to occupational organic solvents during pregnancy on childhood ALL, variants in offspring xenobiotic metabolizing genes (CYP1A1 *2A, *2B, *4 and GSTM1 null) were shown to modify these risks. The GSTM1 null variant was mostly protective whereas having the CYP variants often increased the risk of ALL. No significant effects were seen for household exposures to organic solvents in breastfeeding mothers on the risk of ALL, although the CYP1A1 *2A, CYP2E1*5 and GSTM1 null variants in the offspring did appear to significantly modify and increase the risks of some of these exposures (such as organic solvents from furniture stripping and activities involving electronics) on the development of childhood ALL. Despite limited statistical power, these results suggest potentially important interactions between xenobiotic metabolizing gene variants and household as well as occupational exposures to organic solvents in mothers during pregnancy and breastfeeding.

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Result Tables

Table 2. Distribution of demographic characteristics between cases and controls in the Quebec ALL study

Demographic Characteristics	Cases (n= 790)	Controls (n= 790)
Age of child at diagnosis		
< 1 yrs old	24 (3%)	25 (3%)
1-2 yrs old	69 (9%)	68 (9%)
2-5 yrs old	410 (52%)	413 (52%)
6-10 yrs old	246 (31%)	243 (31%)
11+ yrs old	41 (5%)	41 (5%)
Sex of child (Male)	457 (58%)	458 (58%)
Maternal education		
None of primary school	34 (4%)	25 (3%)
Secondary school	437 (55%)	436 (55%)
College or University	319 (40%)	328 (42%)
Maternal race		
White	748 (95%)	759 (96%)
Black	7 (0.9%)	15 (2%)
Hispanic	8 (1%)	5 (0.6%)
Amerindian	5 (0.6%)	3 (0.4%)
Asian	15 (2%)	6 (0.8%)
Indian	2 (0.3%)	0
Other	5 (0.6%)	2 (0.3%)
Maternal age at birth		
<35 years	721 (90%)	743 (94%)
≥35 years	69 (10%)	47 (6%)
Family income at diagnosis (Can $)		
< 10 000	42 (5%)	38 (5%)
10 000 – 39 000	427 (55%)	422 (55%)
≥ 40 000	312 (40%)	309 (40%)
Breastfeeding	381 (48%)	423 (54%)
Duration of breastfeeding		
< 1 month	163 (43%)	206 (49%)
>1-2 months	34 (9%)	25 (6%)
>2-4 months	72 (19%)	62 (15%)
>4-6 months	20 (5%)	35 (8%)
>6-8 months	20 (5%)	15 (4%)
> 8 months	72 (19%)	80 (19%)
Positive paternal family history of cancer	246 (32%)	231 (30%)
Positive maternal family history of cancer	259 (33%)	242 (31%)
Maternal history of smoking	468 (59%)	458 (58%)
Maternal smoking one month before or during pregnancy	322 (41%)	317 (40%)

Table 3. Crude and adjusted childhood ALL odds ratios for maternal exposure to occupational organic solvents during pregnancy and breastfeeding period

Organic solvents	**Pregnancy period Cases n=790, Controls n=790**			**Breastfeeding (early postnatal) period § Cases n=381, Controls n=423**		
Chemical families	**Ratio of discordant pairs (cases: controls)**	**Crude odds ratios**	**Adjusted odds ratios (95% CI) ***	**Ratio of discordant pairs (cases: controls)**	**Crude odds ratios**	**Adjusted odds ratios (95% CI) ****
Alkanes (C5-C17)	32:19	1.68 (0.95-2.97)	1.68 (0.95-2.96)	1:2	1.00 (0.06-15.99)	1.04 (0.06-16.88)
Aliphatic alcohols	84:94	0.89 (0.67-1.20)	0.91 (0.67-1.22)	10:3	4.00 (0.85-18.84)	3.57 (0.75-17.04)
Chlorinated alkanes	14:14	1.00 (0.48-2.10)	1.01 (0.48-2.12)	1:2	0.50 (0.05-5.51)	0.47 (0.04-5.22)
Chlorinated alkenes	8:9	0.89 (0.34-2.30)	0.86 (0.33-2.25)	0:1	----	----
Aliphatic ketones	18:12	1.50 (0.72-3.11)	1.50 (0.72-3.11))	0:2	----	----
MAH‡	48:29	1.66 (1.04-2.62)	1.65 (1.04-2.62)	2:3	1.00 (0.06-15.99)	1.04 (0.06-16.88)
Mixtures						
Mineral spirits (post-1970)	27: 14	1.93 (1.01-3.68)	1.91 (1.00-3.66)	1:2	1.00 (0.06-15.99)	1.04 (0.06-16.88)
Leaded gasoline	4:1	4.00 (0.45-35.79)	4.11 (0.46-35.88)	0:0	----	----
Unleaded gasoline	4: 5	0.80 (0.21-2.98)	0.80 (0.21-2.97)	0:0	----	----
All solvents	125:124	1.01 (0.79-1.29)	1.01 (0.79-1.30)	13:9	2.00 (0.68-5.85)	1.86 (0.63-5.47)

‡ MAH, Mononuclear aromatic hydrocarbons
* Odds ratios from conditional logistic regression, adjusted for maternal education
** Odds ratios from conditional logistic regression for breastfeeding period, adjusted for maternal age and maternal education
§ Among women who breastfed
---- Estimates were not possible due to the lack of exposed individuals or collinearity

Table 4. Effects of postnatal exposures to organic solvents from household activities (one year before pregnancy to date of diagnosis) and painting (birth of child to date of diagnosis) in breastfeeding mothers on the risk of childhood ALL (Cases=381, Controls=423)

Household activity	Ratio of discordant pairs (cases: controls)	OR (95% confidence interval)	Adjusted OR (95% confidence interval)*
Furniture stripping	47:52	0.89 (0.51-1.54)	0.88 (0.51-1.54)
Model building	6:7	1.50 (0.25-8.98)	1.38 (0.22-8.50)
Silkscreen printing	2:3	0.50 (0.05-5.51)	0.46 (0.04-5.24)
Electronic or radio operator amateur	10:4	4.00 (0.45-35.79)	4.97 (0.54-45.48)
Electronic equipment repair	3:2	1.00 (0.14-7.10)	1.19 (0.16-8.65)
Large electronic tool equipment	27:25	1.90 (0.88-4.09)	1.94 (0.90-4.22)
Sewing machine	107:119	1.15 (0.80-1.67)	1.14 (0.79-1.67)
Maintenance or repair of truck	5:7	----	----
Painting	97:100	1.27 (0.86-1.87)	1.33 (0.89-1.99)
Type of painting			
Latex / acrylic	77:82	1.20 (0.79-1.83)	1.25 (0.82-1.91)
Oil	19:25	1.44 (0.62-3.38)	1.55 (0.64-3.78)
Latex / acrylic and oil	37:36	0.68 (0.34-1.39)	0.69 (0.34-1.41)
Other	11:4	5.00 (0.58-42.80)	4.62 (0.53-39.85)
How paint was applied			
Jet	48:63	0.84 (0.50-1.41)	0.89 (0.52-1.51)
Brush	48:42	1.58 (0.89-2.81)	1.63 (0.91-2.91)
Roller	96:102	1.17 (0.79-1.72)	1.22 (0.83-1.81)

Large electronic tool equipment includes: table saw, band saw, circular saw and other; electronic equipment repair includes: TV, radio, stereo and other

* Odds ratios from conditional logistic regression, adjusted for maternal age and education

---- Estimates were not possible due to the lack of exposed individuals or collinearity

Table 5a. Risk of ALL associated with proband xenobiotic-metabolizing gene polymorphisms

Polymorphisms	No. Cases	No. Controls	Ratio of discordant pairs (cases: controls)	Adjusted Odds ratio (95% confidence interval) *
CYP1A1 *2A	551	504		
-/-	418 (76%)	403 (80%)		1.00 (reference)
-/+	125 (23%)	97 (19%)	106:77	1.34 (0.96-1.88)
+/+	8 (1%)	4 (0.8%)	19:19	1.81 (0.92-3.54)
-/+; +/+	133 (24%)	101 (20%)	112:80	1.34 (0.93-1.92)
CYP1A1 *2B	541	504		
-/-	494 (91%)	471 (93%)		1.00 (reference)
-/+	45 (8%)	31 (6%)	43:29	1.32 (0.80-2.19)
+/+	2 (0.4%)	2 (0.4%)	2:2	1.75 (0.64-4.81)
-/+; +/+	47 (8.7%)	33 (6.5%)	43:29	1.43 (0.83-2.46)
CYP1A1 *4	542	505		
-/-	516 (95%)	465 (92%)		1.00 (reference)
-/+	26 (5%)	40 (8%)	21:35	0.46 (0.24-0.87)
+/+	0	0	0:0	No individuals
CYP2E1 *5	541	498		
-/-	508 (94%)	466 (94%)		1.00 (reference)
+/-	33 (6%)	32 (6%)	32:31	0.91 (0.51-1.64)
+/+	0	0	0:0	No individuals
GSTM1	542	505		
Present	226 (42%)	227 (45%)		1.00 (reference)
null	316 (58%)	278 (55%)	188:150	1.23 (0.92-1.64)

For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model.
* Odds ratios from conditional logistic regression, adjusting for maternal race

Table 5b. Risk of childhood ALL for case xenobiotic metabolizing gene variants using a genotype relative risk model with case-parent trios

Gene (sample size)	Relative risk of gene (95% CI)
CYP1A1 2A (n=667)*	
-/-	1.00 (reference)
+/-	1.04 (0.78-1.38)
+/+	0.78 (0.33-1.85)
CYP1A1 2B (n=669)*	
-/-	1.00 (reference)
+/-	0.77 (0.49-1.19)
+/+	1.39 (0.18-10.71)
CYP1A1 4 (n=665)*	
-/-	1.00 (reference)
+/-	0.99 (0.55-1.77)
+/+	No individuals
CYP2E1 5 (n=667)*	
-/-	1.00 (reference)
+/-	0.82 (0.50-1.34)
+/+	No individuals
GSTM1 null (n=516) ‡	
+/+; +/-	1.00 (reference)
-/-	1.43 (1.20-1.70)

*For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants.

‡ For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-. The genetic model underlying the analysis is recessive (Relative risk (RR) of the homozygous variant is estimated; RR of one copy=1 is included in the present (+/+) category; the degrees of freedom=1).

Table 6. Interaction between occupational organic solvents during pregnancy and proband xenobiotic-metabolizing gene polymorphisms with case-control data

	GSTM1			CYP1A1 2A			CYP2E1 5		
Chemical Family	**OR (95% CI)* present**	**OR (95% CI)* null**	**LRT ****	**OR (95% CI)* (-/-)**	**OR (95% CI)* (+/-;+/+)**	**LRT ****	**OR (95% CI)* (-/-)**	**OR (95% CI)* (+/-)**	**LRT ****
Alkanes	1.23 (0.44-3.44)	2.07 (0.63-6.84)	0.52	----	----	----	1.60 (0.67-3.86)	2.69 (0.22-32.52)	0.70
Aliphatic alcohols	1.40 (0.79-2.47)	0.69 (0.40-1.22)	0.08	0.89 (0.56-1.41)	1.22 (0.51-2.95)	0.53	0.87 (0.56-1.34)	4.24 (0.69-26.08)	0.08
Chlorinated alkanes	1.16 (0.28-4.80)	0.94 (0.22-4.07)	0.84	1.02 (0.34-3.09)	0.34 (0.03-3.92)	0.38	----	----	----
Chlorinated alkenes	1.25 (0.17-9.41)	0.80 (0.11-5.78)	0.75	----	----	----	----	----	----
Aliphatic ketones	7.81 (0.95-64.32)	0.83 (0.26-2.69)	0.04	----	----	----	1.56 (0.56-4.36)	1.05 (0.06-18.30)	0.80
MAH	1.17 (0.48-2.83)	1.56 (0.66-3.71)	0.66	1.20 (0.62-2.29)	3.27 (0.36-30.12)	0.36	1.15 (0.59-2.27)	3.89 (0.37-41.18)	0.31
Mixture									
Mineral spirits (post-1970)	1.44 (0.49-4.21)	3.63 (0.75-17.46)	0.33	----	----	----	1.99 (0.75-5.30)	2.67 (0.22-32.22)	0.83

‡ MAH, Mononuclear aromatic hydrocarbons

For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model. For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-.

* Odds ratios from conditional logistic regression, adjusted for maternal race and education

** The likelihood ratio tests were performed comparing a model with the interaction variable to a model without the interaction variable (1 degree of freedom under chi-square)

----Estimates were not possible due to the lack of exposed individuals or collinearity

Table 7. Interactions between maternal exposures to occupational organic solvents during pregnancy on the risk of childhood ALL using case-parent trio and case only designs

	GSTM1 null	CYP1A1 2A				CYP2E1 5		
Organic Solvents	**Case-only**	**Case-parent**			**Case-only**	**Case-parent**		**Case-only**
Chemical Family	**Interaction OR (95% CI) ‡**	**Interaction RR for 1 copy (95% CI)***	**Interaction RR for 2 copies (95% CI)***	**LRT ****	**Interaction OR (95% CI) ‡**	**Interaction RR for 1 copy (95% CI)***	**LRT ****	**OR (95% CI) ‡**
Alkanes	0.62 (0.28-1.37)	1.81 (0.32-10.06)	----	0.18	0.80 (0.29-2.19)	1.11 (0.15-8.35)	0.99	1.26 (0.28-5.78)
Aliphatic alcohols	0.54 (0.32-0.90)	0.97 (0.47-2.01)	1.41 (0.11-18.72)	0.96	1.21 (0.69-2.13)	1.41 (0.38-5.20)	0.87	1.37 (0.53-3.53)
Chlorinated alkanes	0.85 (0.28-2.58)	0.96 (0.13-6.96)	----	0.81	0.99 (0.27-3.67)	----	----	----
Chlorinated alkenes	0.73 (0.14-3.68)	1.62 (0.15-17.69)	----	0.18	3.69 (0.72-18.86)	----	----	----
Aliphatic ketones	0.73 (0.25-2.11)	----	----	----	0.59 (0.13-2.71)	0.56 (0.05-6.51)	0.89	1.54 (0.19-12.47)
MAH ‡	0.81 (0.42-1.57)	2.48 (0.65-9.46)	5.93 (0.27-131.17)	0.29	1.26 (0.59-2.69)	1.66 (0.26-10.63)	0.86	1.53 (0.43-5.40)
Mixtures								
Mineral spirits (post-1970)	0.60 (0.25-1.41)	0.87 (0.12-6.23)	----	0.23	0.54 (0.16-1.85)	1.08 (0.14-8.12)	1.00	1.71 (0.37-7.93)
Unleaded gasoline	1.53 (0.14-17.06)	----	----	----	1.62 (0.14-18.25)	----	----	----
Leaded gasoline	0.74 (0.10-5.38)	----	----	----	1.14 (0.12-11.33)	-----	----	----

Relative risks (RR) and odds ratios (OR); ‡ MAH, Mononuclear aromatic hydrocarbons

For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model. For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-.

In the case-only design, the CYP polymorphisms were analysed using a dominant inheritance model. The GSTM1 variant was analysed as present versus null (recessive model). In the case-parent trio, the CYP polymorphisms were analysed using a genotype relative risk model.

‡ Interaction odds ratios from unconditional logistic regression, adjusted for maternal education, maternal race, age and sex of child

* Interaction relative risks from log-linear model, adjusted for maternally mediated genetic effects

** The likelihood ratio tests are reported as p-values and were performed comparing a model with the interaction variable to a model without the interaction variable (2 degree of freedom under chi-square).

---- Estimates were not possible due to the lack of exposed individuals or collinearity

Table 8. Interaction effects between maternal exposures to* any *occupational organic solvents and proband xenobiotic-metabolizing genes on the risk of childhood ALL, using case-control, case-parent trio and case-only designs during pregnancy

	Case-Control			Case-Parent Trio			Case-Only
Polymorphisms	**OR (95% CI)* Wild type**	**OR (95% CI)* Variant**	**LRT p-value ****	**Interaction relative risk for 1 copy (95% CI)†**	**Interaction relative risk for 2 copies (95% CI)†**	**LRT p-value ****	**Interaction odds ratio (95% CI) §**
GSTM1 null	1.26 (0.76-2.08)	0.81 (0.51-1.29)	0.20	----	----	----	0.65 (0.43-0.99)
CYP1A1 2A ‡	0.87 (0.59-1.29)	1.56 (0.72-3.42)	0.19	1.52 (0.79-2.95)	5.36 (0.76-37.41)	0.17	1.59 (1.00-2.53)
CYP1A1 2B ‡	0.97 (0.67-1.40)	1.03 (0.30-3.51)	0.93	1.57 (0.52-4.73)	----	0.03	1.28 (0.63-2.61)
CYP1A1 4 (-/+)	1.02 (0.71-1.46)	1.35 (0.40-4.51)	0.66	1.18 (0.35-4.01)	----	0.97	2.13 (0.91-4.96)
CYP2E1 5 (-/+)	0.91 (0.63-1.31)	3.01 (0.64-14.20)	0.13	0.93 (0.29-2.97)	----	0.99	0.98 (0.41-2.37)

For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model. For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-.

‡ For the case-control and case-only designs, the CYP1A1 2A and 2B polymorphisms were analysed using a dominant inheritance model. For the case-parent trio, the CYP polymorphisms were analysed using an genotype relative risk model.

* Odds ratios from conditional logistic regression, adjusted for maternal education and race

§ Interaction odds ratios from unconditional logistic regression, adjusted for maternal education, maternal race, age and sex of child

† Interaction relative risks from log-linear model, adjusted for maternally mediated effects

** The likelihood ratio tests are reported as p-values and were performed comparing a model with the interaction variable to a model without the interaction variable (2 degree of freedom under chi-square for case-parent and 1 degree of freedom under chi-square for case-control).

---- Estimates were not possible to calculate either because there were no individuals with that allele variant, or for GSTM1, no estimates were possible for case-parent trio

Table 9. Interaction between exposures to organic solvents from household activities (one year before pregnancy to date of diagnosis) and painting (birth of child to date of diagnosis) in breastfeeding mothers and proband xenobiotic-metabolizing gene polymorphisms with case-control data

	GSTM1			CYP1A1 2A			CYP2E1 5		
Household activities	**OR (95% CI)* present**	**OR (95% CI)* null**	**LRT ******	**OR (95% CI)* (-/-)**	**OR (95% CI)* (-/+; +/+)**	**LRT ******	**OR (95% CI)* (-/-)**	**OR (95% CI)* (-/+)**	**LRT ******
Furniture stripping	0.80 (0.40-1.60)	1.11 (0.67-1.85)	0.44	0.85 (0.54-1.34)	2.35 (0.88-6.26)	0.05	1.09 (0.70-1.71)	0.34 (0.07-1.59)	0.16
Model building	0.54 (0.10-2.96)	0.42 (0.08-2.22)	0.84	----	----	----	----	----	----
Electronic or radio operator amateur	2.88 (0.55-15.07)	2.12 (0.48-9.29)	0.79	3.97 (0.99-15.91)	1.80 (0.16-19.99)	0.59	----	----	----
Electronic equipment repair	----	----	----	1.00 (0.06-16.29)	0.77 (0.05-12.40)	0.90	----	----	----
Large electronic tool equipment	1.13 (0.46-2.75)	0.82 (0.40-1.66)	0.59	0.87 (0.49-1.56)	1.72 (0.31-9.45)	0.45	0.95 (0.54-1.69)	0.40 (0.04-4.25)	0.47
Sewing machine	0.85 (0.54-1.33)	0.68 (0.46-0.99)	0.45	0.77 (0.55-1.07)	0.81 (0.41-1.59)	0.90	0.74 (0.55-1.00)	0.77 (0.23-2.56)	0.95
Maintenance or repair of truck	----	----	----	0.60 (0.14-2.59)	2.79 (0.09-82.46)	0.41	----	----	----
Painting	1.00 (0.65-1.55)	1.25 (0.84-1.84)	0.46	1.06 (0.76-1.48)	1.31 (0.69-2.50)	0.56	1.13 (0.83-1.53)	0.98 (0.31-3.13)	0.82
Type of paint									
Latex / acrylic	0.92 (0.58-1.48)	1.14 (0.73-1.76)	0.52	0.94 (0.65-1.35)	1.33 (0.66-2.68)	0.38	1.03 (0.74-1.46)	0.77 (0.24-2.52)	0.64
Oil	1.52 (0.57-4.04)	0.69 (0.34-1.40)	0.21	0.99 (0.53-1.86)	0.47 (0.13-1.80)	0.32	0.81 (0.45-1.46)	1.58 (0.24-10.34)	0.51
Latex / acrylic and oil	0.75 (0.38-1.47)	1.41 (0.76-2.62)	0.17	1.22 (0.73-2.05)	0.90 (0.33-2.45)	0.60	1.10 (0.69-1.76)	1.39 (0.08-24.10)	0.88

Large electronic tool equipment includes: table saw, band saw, circular saw and otherelectronic equipment repair includes: TV, radio, stereo and other
For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model. For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-.
* Odds ratios from conditional logistic regression, adjusted for maternal age and education
** The likelihood ratio tests were performed comparing a model with the interaction variable to a model without the interaction variable (1 degree of freedom under chi-square)
----Estimates were not possible due to the lack of exposed individuals or collinearity

Table 10. Interactions between exposures to organic solvents from household activities (one year before pregnancy to date of diagnosis) and painting (birth of child to date of diagnosis) in breastfeeding mothers the risk of childhood ALL using case-parent trio and case only designs

	GSTM1 null	CYP1A1 2A			CYP2E1 5		
	Case-only	Case-parent		Case-only	Case-parent		Case-only
Household activities	**Interaction OR (95% CI)§**	**Interaction RR for 1 copy (95% CI)†**	**LRT ****	**Interaction OR (95% CI)§**	**Interaction RR for 1 copy (95% CI)†**	**LRT ****	**Interaction OR (95% CI)§**
Furniture stripping	1.84 (1.08-3.13)	1.37 (0.46-4.09)	0.67	1.28 (0.74-2.21)	----	----	0.75 (0.24-2.35)
Model Building	0.99 (0.22-4.50)	----	----	----	----	----	----
Electronic or radio operator amateur	0.96 (0.33-2.83)	----	----	0.93 (0.25-3.41)	----	----	4.16 (0.82-21.28)
Electronic equipment repair	0.22 (0.02-2.19)	----	----	1.19 (0.12-11.76)	----	----	18.28 (1.48-226.12)
Large electronic tool equipment	1.16 (0.57-2.33)	2.17 (0.51-9.30)	0.22	1.35 (0.63-2.90)	1.02 (0.06-17.47)	1.00	0.56 (0.07-4.35)
Sewing machine	0.90 (0.63-1.29)	0.85 (0.38-1.89)	0.85	0.91 (0.60-1.39)	1.56 (0.45-5.47)	0.78	0.74 (0.34-1.62)
Maintenance or repair of truck	2.28 (0.45-11.54)	----	----	0.43 (0.05-3.61)	----	----	----
Painting	1.11 (0.77-1.59)	0.86 (0.39-1.89)	0.33	1.37 (0.90-2.11)	1.97 (0.56-6.94)	0.57	1.26 (0.58-2.73)
Type of painting							
Latex / acrylic	1.01 (0.69-1.48)	0.85 (0.37-1.94)	0.65	1.54 (1.00-2.38)	2.28 (0.54-9.63)	0.52	1.51 (0.69-3.29)
Oil	0.99 (0.48-2.03)	3.10 (0.31-31.05)	0.56	0.89 (0.38-2.08)	3.54 (0.34-37.05)	0.53	2.37 (0.74-7.57)
Latex / acrylic and oil	1.44 (0.85-2.44)	0.88 (0.25-3.04)	0.78	0.80 (0.42-1.50)	0.48 (0.04-5.77)	0.84	0.47 (0.11-2.11)

Large electronic tool equipment includes: table saw, band saw, circular saw and other; electronic equipment repair includes: TV, radio, stereo and other

For the CYP genes, -/- represents the homozygous wild (reference category), -/+ heterozygous variants, and +/+ the homozygous variants; -/+; +/+ represents a combined category of homozygotes and heterozygotes together in a dominant inheritance model. For the GSTM1 deletion, the homozygous and heterozygous individuals indicated as +/+ and -/+ (present) are the referent category; the homozygous deleted individuals are indicated as -/-.

For the case-only design, the CYP polymorphisms were analysed using a dominant inheritance model. The GSTM1 variant was analysed as present versus null. For the case-parent trio, the CYP polymorphisms were analysed using an genotype relative risk model.

§ Interaction odds ratios from unconditional logistic regression, adjusted for maternal education, maternal race, age and sex of affected child

† Interaction relative risks from log-linear model, adjusted for maternally mediated effects. No interaction effects were possible for 2 copies of the CYP1A1 2A variant.

** The likelihood ratio tests are reported as p-values and were performed comparing a model with the interaction variable to a model without the interaction variable (2 degree of freedom under chi-square).

---- Estimates were not possible due to the lack of exposed individuals or collinearity

Appendix A

Table A1 Summary of Important Organic Solvents evaluated by the IARC

Compounds	Occupational Use	Cancers in humans	IARC evaluation of carcinogenesis
Benzene	gasoline / oil refining	leukemias	Sufficient evidence (1982)
Toluene	paints/printing/shoe industry	undetermined	Inadequate information (1999)
Xylene	paints	undetermined	Inadequate information (1999)
Styrene	lamination / plastic	lymphatic and haematopoietic neoplasms	Limited evidence (2002)
Morpholine	rubber production / optical brighterers /corrosion inhibitor / polishes and waxes	undetermined	Inadequate information (1989)
Cyclohexanone	production of nylon	undetermined	Inadequate information (1999)
Dimethylformamide	production of inks, adhesives, resins, fibres, pharmaceuticals and synthetic leather	undetermined	Inadequate information (1999)
Isopropanol	polypropylene production / pharmaceutical / cosmetic formulations	undetermined	Inadequate information (1999)
Chloroform	pharmaceuticals	undetermined	Inadequate information (1999)
Trichloroethylene	degreasing	liver and biliary tract cancers / non-Hodgkin's lymphoma	Limited evidence (1995)
Tetrachloroethylene	dry cleaning/degreasing	oesophageal / cervical cancer / non-Hodgkin's lymphoma	Limited evidence (1995)
Carbon tetrachloride	fluorocarbon production/solvent	undetermined	Inadequate information (1999)
Dichloromethane (Methylene Chloride)	pharmaceuticals/paint removal	undetermined	Inadequate information (1999)

1,1,1 - Trichloroethane	metal cleaning solvent	undetermined	Inadequate information (1999)
Formaldehyde	glues / varnishes / preservatives / textile agents	nasopharyngeal cancers	Limited evidence (1995)

A description of some widely used and studied organic solvents, their occupation use, the cancers in which there is evidence of their involvement as risk factors and the IARC evaluation of carcinogenesis [70,85,190-197]

Table A2: Table Summary of Important Organic Solvent Mixtures evaluated by the IARC

Mixtures	Components	Occupational Use	Cancers in humans	IARC evaluation of carcinogenesis
Gasoline	aromatic hydrocarbons / alkanes	Motor vehicle/service station	undetermined	Inadequate evidence (1989)
Fuel / Heating oils (e.g. kerosene)	alkanes / alkenes cycloalkanes / aromatic hydrocarbons	Heating equipment / cooking stoves	undetermined	Inadequate evidence (1989)
Petroleum solvents (e.g. mineral spirits)	various hydrocarbons	Painting / printing / adhesives / rubber processing and degreasing	undetermined	Inadequate evidence (1989)

A description of some widely used and studied organic solvent mixtures, some of their components, their occupation use, the cancers in which there is evidence of their involvement as risk factors and the IARC evaluation of carcinogenesis [70,192]

Appendix B: Advantages and disadvantages of the various study designs used in genetic epidemiology

Case-Control	Case-Only	Case-Parent
	Advantages	
Can assess main and joint effects	No selection of controls	No selection of controls
Common, widely used study design	Simple	Can estimate G x E interactions with increased validity
	Increased estimate precision	Can assess maternal genetic effects and child genetic effects independently
	Can estimate G x E interactions efficiently	No population stratification bias
		Can incorporate missing genotype data using expectation algorithms
	Disadvantages	
Population stratification bias	Assumption of genotype and exposure independence	Need parental data, which may be difficult for certain later onset diseases
Linkage disequilibrium rather than causal role	Main effects of genotype or exposure cannot be assessed	Main effects of exposure cannot be assessed
Assuming Mendelian transmission	Linkage disequilibrium rather than causal role	Within each parental mating time is the assumption of Mendelian transmission
Cannot assess maternal genetic effects and child genetic effects independently	Odds ratio obtained is only interpretable as a departure from multiplicative effects	Assumption of genotype and exposure independence
	Population stratification bias	

Information summarized from text (look in Interaction Methodology section for additional details)[154,167,168,172,175]

Appendix C: Child xenobiotic metabolizing genes of interest (CYP1A1, CYP2E1 and GSTM1) and their effects on the development of childhood ALL

Author and year	Study Design and Sample Size	Strengths / Limitations	Genotype	OR (95% confidence interval)
Yamaguti et al (2010)[133]	Case-Control 99 Caucasian patients with de novo childhood ALL (mean age 4) and 99 healthy blood donors (mean age 53) from Southeastern Brazil	**Strengths:** Hardy-Weinberg equilibrium tested in the controls; looked for combined gene effects **Limitations:** small sample size; no sample size calculations; mediocre reporting (no in depth literature review, limited detail on methods of study); no adjustment for population stratification bias	**CYP1A1 (T6235C)**	
			TT	1.0 (reference)
			TC	1.40 (0.76-2.59)
			CC	0.54 (0.13-2.26)
			TC / CC	1.25 (0.70-2.23)
			TT / TC	1.0 (reference)
			CC	0.48 (0.12-1.99)
			CYP1A1 (A4889G)	
			AA	1.00 (reference)
			AG	1.39 (0.76-2.53)
			GG	1.11 (0.21-5.73)
			AG / GG	1.36 (0.76-2.44)
			AA / AG	1.00 (reference)
			GG	1.00 (0.20-5.08)
Lee et al (2009)[101]	Case-Control 164 childhood leukemia cases (104 ALL cases) / 164 non-cancer controls from Seoul Korea	**Strength:** Looked at combined genetic effects; studied haplotypes **Limitations:** small sample size, there is also the likelihood of false positive findings; controls and cases potentially come from different study bases (controls recruited from 1 hospital, whereas the cases were recruited from 3)	**CYP1A1 (-17961 T>C)**	
			CC	1.0 (reference)
			CT	1.6 (0.9-2.8)
			TT	1.4 (0.6-3.1)
			CT/TT	1.6 (0.9-2.6)
			CYP1A1 (-9893 G>A)	
			GG	1.0 (reference)
			GA	0.8 (0.5-1.4)
			AA	1.0 (0.3-3.0)
			GA/AA	0.8 (0.5-1.4)
			CYP1A1 (Ex7+131A>G;	

			1462V)	
			AA	1.0 (reference)
			AG	0.9 (0.5 – 1.5)
			GG	0.9 (0.3-2.7)
			AG/GG	0.9 (0.5-1.5)
			CYP1A1 (1188 C >T)	
			TT	1.0 (reference)
			TC	1.1 (0.6-2.0)
			CC	0.6 (0.3-1.3)
			TC/CC	1.0 (0.6-1.7)
			CYP1A1 (11599 C>G)	
			CC	1.0 (reference)
			CG	1.8 (1.0-3.3)
			GG	1.3 (0.6-2.7)
			CG/GG	1.6 (0.9-2.8)
Suneetha et al (2008)[141]	Case-Control 92 ALL cases (includes individuals under 25 years old) / 150 cord blood sample controls from South India	**Strengths:** Combined genetic analysis; looked at Hardy-Weinberg equilibrium in the controls **Limitations:** Adults and children were grouped together; small sample size with no sample size calculation	**GSTM1**	
			present	1.0 (reference)
			null	1.96 (1.08-3.57)
Rimando et al (2008)[142]	Case-Control 60 pediatric ALL cases and 60 randomly selected normal children (all study subjects are less than 18 yrs old and are natural-born Filipino children)	**Strengths:** Genotyped in triplicate (control measure against genotyping error); used albumin gene as positive internal control; looked at Hardy-Weinberg equilibrium; performed a combined gene analysis; tried to remove bias from population stratification (limited patient selection to only natural born Filipino); **Limitations:** very small sample size; no sample size calculations	**GSTM1**	
			present	1.0 (reference)
			null	2.37 (1.11-5.04)

Bolufer et al (2007)[131]	Case-Control 141 cases of ALL (includes adults and children) and 454 controls from Spanish hospitals	**Strengths:** large sample size; healthy controls from the same study base as cases; sub-analyses by sex **Limitations:** adults and children were grouped together; no sample size calculation was provided	**CYP1A1*2A**	
			-/-	1.0 (reference)
			+/-	0.62 (0.32-1.17)
			+/+	0.87 (0.04-18.23)
			CYP2E1 *5B	
			-/-	1.0 (reference)
			-/+	0.86 (0.37-1.98)
			+/+	undefined
			GSTM1	
			present	1.0 (reference)
			null	0.79 (0.54-1.16)
Pigullo et al (2007)[137]	Case- Control 353 pediatric ALL cases / 384 hospital controls from Italy (including Sardinia)	**Strengths:** large sample size; they looked at combined genetic effects; matched their controls to cases based on geographical location to minimize population stratification bias; looked at Hardy-Weinberg equilibrium in controls **Limitations:** looked at few candidate genes	**GSTM1**	
			present	1.0 (reference)
			null	0.71 (0.47-1.07)
Ulusoy et al (2007)[107]	Case-Control 168 pediatric ALL cases / 207 healthy volunteer controls from Turkey	**Strengths:** large and healthy control group; they looked at combined genetic effect **Limitations:** control group includes adults – there could be different environmental exposures in this group as compared to the cases creating some bias; did not consider population stratification bias; no sample size calculations; did not assess Hardy-Weinberg equilibrium in controls	**CYP2E1 *5B**	
			-/-	1.0 (reference)
			-/+; +/+	1.9 (0.8-4.8)
Aydin-Sayitoglu et al (2006)[136]	Case-Control	**Strengths:** looked at multiple candidate genes; Hardy-Weinberg	**CYP1A1 *2A**	
			non *2A/non *2A	1.5 (0.9-2.7)

	119 pediatric cases of ALL / 140 healthy controls from Istanbul, Turkey	equilibrium in controls was assessed **Limitations:** small sample size; did not look at combined genetic effects; may be unknown confounding and/or population stratification bias; control group, especially for pediatric patients, was not representative (included adults, therefore there could be different environmental exposures in the controls as compared to the cases, creating some bias)	non *2A / *2A	1.7 (0.9-3.0)
			*2A / *2A	0.3 (0.03-3.0)
			CYP2E1 *5B	
			c1/c1	1.0 (reference)
			c1/c2	3.4 (1.3-9.1)
			c2/c2	No individuals
			GSTM1	
			Present	1.0 (reference)
			Null	1.5 (0.9-2.5)
Clavel et al (2005)[134]	Case-Control (hospital based) 240 ALL pediatric cases of acute leukemia and 288 matched hospital controls (mainly from orthopaedic department) from Paris, Lyon, Lille and Nancy	**Strengths:** large sample size; estimated gene-environment interactions (maternal tobacco, coffee and alcohol consumption during pregnancy); studied multiple candidate genes (xenobiotic and repair genes); used case-only analysis; tested for Hardy-Weinberg equilibrium **Limitations:** did not look at combined genetic effects; potential survivor bias	**CYP1A1**	
			*****1/*1 (wild-type)	1.0 (reference)
			*1/*2A	0.9 (0.5-1.6)
			*2A/*2A	Too few individuals
			GSTM1	
			present	1.0 (reference)
			null	1.2 (0.7-1.9)
Pakakasama et al (2005)[132]	Case Control 107 pediatric ALL cases / 320 healthy adult volunteers from Bangkok Thailand	**Strengths:** large healthy control group; looked at multiple candidate genes **Limitations:** small sample size for ALL group; used adult controls rather than children, may be different environmental exposures leading to bias; only one combined genetic effect; did not consider population stratification bias;	**CYP1A1**	
			*1/*1	1.0 (reference)
			*1/*2A	1.0 (0.5-1.9)
			*1/*2B	1.2 (0.7-2.0)
			*2A/*2A	3.4 (0.1-2.5)
			*2A/*2B	0.8 (0.4-1.8)
			*2A/*4	No individuals
			*2B/*2B	0.4 (0.1-1.4)
			*2B/*4	1.0 (0.1-9.7)

		Hardy-Weinberg equilibrium was not assessed	**GSTM1**	
			present	1.0 (reference)
			null	1.7 (1.0-2.7)
Barnette et al (2004)[182]	Case-Control 94 pediatric ALL cases and 340 randomly selected infant controls from the state of Utah	**Strengths:** high-throughput assays which do not require endonuclease digestion and can differentiate between non-null GSTM1 alleles, GSTM1 *A and GSTM1 *B; there may be population stratification bias, though they state that their population is homogenous (88% Caucasian of European descent) **Limitations:** small sample size, no sample size calculation; did not report variables included in logistic regression	**GSTM1**	
			null	1.0 (reference)
			*A /*0	5.66 (2.58-12.42)
			*B /*0	4.28 (1.80-10.20)
Canalle et al (2004)[102]	Case-Control 113 ALL pediatric cases / 221 Controls (general population from the same hospital) from Ribeirao Preto Brazil	**Strengths:** looked at combined genetic effects; looked at multiple candidate genes; subjects were matched on ethnicity (accounting for possible population stratification bias); assessed Hardy-Weinberg equilibrium in control group **Limitations**: small ALL group	**CYP1A1 *2**	
			-/-	1.0 (reference)
			-/+	1.0 (0.6-1.6)
			+/+	4.0 (1.0-16.6)
			CYP2E1 *5B	
			-/-	1.0 (reference)
			-/+	1.0 (0.6-2.5)
			+/+	Only 1 individual
			GSTM1	
			present	1.0 (reference)
			null	0.9 (0.5-1.4)
Joseph et al (2004)[144]	Case-Control 118 childhood ALL cases / 118 matched controls (sex and age) from Keralite population (South India)	**Strengths:** specific population, less likely to encounter population stratification bias; tested Hardy-Weinberg equilibrium in controls; performed analysis stratified by sex; performed combined gene analysis	**CYP1A1 m1**	
			+/+	1.0 (reference)
			-/-	6.22 (1.30–29.71)*
			-/+	2.58 (1.41 – 4.72)*
			CYP1A1 m2	
			+/+	1.0 (reference)
			-/-	4.28 (1.14–16.11)*

		Limitations: Small sample size / may be population stratification bias	-/+	2.18 (1.16 – 4.10)*
			GSTM1	
			present	1.0 (reference)
			deleted	2.10 (1.21 – 3.67)
Wang et al (2004)[140]	Case-Control 67 pediatric ALL cases / 146 healthy controls from China	**Strengths**: combined gene analysis **Limitations:** very small sample size; no sample size calculation; they studied few candidate genes; controls included adults, may be different environmental exposures leading to bias; limited reporting on patients and methods making it difficult to assess their strengths and limitations	**GSTM1**	
			present	1.0 (reference)
			null	2.86 (1.49 – 5.47)
Balta et al (2003)[130]	Case-Control 144 ALL pediatric cases / 185 healthy pediatric controls from Ankara, Turkey	**Strengths:** analyzed several candidate genes; looked at combined gene effects, though the results were not reported; assessed genetic frequencies across sex and ALL subgroup (B-cell versus non B-cell) strata; tested Hardy-Weinberg equilibrium in controls **Limitations:** small sample size; could be some population stratification bias	**CYP1A1 *2A**	
			-/-	1.00 (reference)
			+/-	1.37 (0.78-2.4)
			+/+	0.21 (0.03-1.72)
			+/+; +/-	0.99 (0.64-1.55)
			GSTM1	
			present	1.0 (reference)
			null	1.03 (0.66-1.61)
Alves et al (2002)[143]	Case-Control 47 pediatric ALL cases and 107 geographically matched, healthy controls from North Portugal	**Strengths:** Tested for Hardy-Weinberg equilibrium; looked at combined effects of the candidate genes; summarized past studies on GSTM1; used recent genotyping procedure of GSTM1 whereby heterozygotes can be identified; looked at phenotype (present or deleted) and genotype effects **Limitations:** small sample size; could be some population stratifications bias	**GSTM1 - phenotype**	
			+/-; +/+	1.0 (reference)
			-/-	2.2 (p-value=0.035)
			GSTM1 - genotype	
			+/+	reference
			+/-	p-value = 0.09
			-/-	

Davies et al (2002)[139]	Case-Control 651 pediatric ALL cases (616 whites and 35 blacks) / 733 normal blood donor controls (532 whites and 201 blacks) from Minnesota and California (Los Angeles and Arcadia)	**Strengths:** large sample size; good reporting of patient characteristics and methods; combined gene effects **Limitations:** no effect measures for ALL susceptibility (only p-values)	**GSTM1 - whites**	
			null	No odds ratio reported; 54% null variants in controls vs 54% in cases (p-value 1.0)
			GSTM1 - blacks	
			null	No odds ratio reported; 32% null variants in controls vs 40% in cases (p-value 0.45)
Krajinovic et al (2002)[103]	Case-Control 174 pediatric ALL cases/337 controls from Quebec Canada	**Strengths:** studied various candidate genes; homogenous population (limiting population stratification bias); combined genetic effects; assessed gene-gene interaction effects **Limitations**: small ALL group	**CYP2E1 *5**	
			-/-	1.0 (reference)
			+/-	2.8 (1.2-6.7)
Saadat et al (2000)[181]	Case-Control 38 pediatric ALL cases / 75 healthy donor controls, all were Iranian Muslims	**Strengths:** homogenous population, limiting population stratification bias **Limitations:** no effect measures reported (limited analyses); small sample size; limited reporting of patient selection and study methods	**GSTM1**	
			null	No odds ratio reported, but there was a significant difference between cases and controls using chi-square (χ^2= 5.76; P<0.025)
Krajinovic et al (1999)[135]	Case Control 177 pediatric ALL cases / 304 controls from the same hospital from Montreal Quebec	**Strengths:** studied various candidate genes; looked at combined genetic effects; restricted to one ethnicity (limiting population stratification bias) **Limitations:** small ALL group	**CYP1A1 *2A**	
			-/-	1.0 (reference)
			-/+; +/+	1.8 (1.1-3.1)
			CYP1A1 *2B	
			-/-	1.0 (reference)
			-/+; +/+	0.9 (0.4-1.8)
			CYP1A1 *4	
			-/-	1.0 (reference)
			-/+; +/+	0.6 (0.3-1.2)
			GSTM1	

			present	1.0 (reference)
			null	1.8 (1.2-2.6)

Notations and Polymorphism definitions:

CYP1A1 in the table primarily consists of three mutations named m1 (T6235C), m2 (A4889C) and m4 (C4887A) [102,103,156]

CYP1A1*2A (presence of m1 only), ***2B** (both m1 and m2), and ***4** (m4 only) [156]

GSTM1 null a homozygous deletion (null allele) caused by homologous recombination, rendering the enzyme inactive [118]

GSTM1 *A creates Lyn at codon 173 AAG) and **GSTM1 *B** creates Asn at codon 173 (AAC) [182]

CYP2E1 *5B is the variant C to T transversion at position 1019 [107]

CYP2E1 *5 is the variant G to C transversion at position -1295 [103]

CYP2E1 *6 is a *Dra*I RFLP in intron 6 [107]

CYP2E1 *7B is the variant *Dde*I RFLP in the promoter region [107]

CYP1A1 *2 is a variant at base pair 6235 in the 3 -flanking region, resulting in a new recognition, sequence for the restriction enzyme *Msp*I [102]

NQO1 *2 (C609T) and ***3** (C465T); **MPO *2** (G463A) [103]

Appendix D: Combined effects of xenobiotic metabolizing genes (includes CYP1A1, CYP2E1 and GSTM1) on the risk of developing childhood ALL

Author and year	Study Design and Sample Size	Strengths / Limitations	Genotypes	OR (95% confidence interval)
Yamaguti et al (2010)[133]	Case-Control 99 Caucasian patients with de novo childhood ALL (mean age 4) and 99 healthy blood donors (mean age 53) from Southeastern Brazil	**Strengths:** Hardy-Weinberg equilibrium tested in the controls; looked for combined gene effects **Limitations:** small sample size; no sample size calculations; mediocre reporting (no in depth literature review, limited detail on methods of study); no adjustment for population stratification bias	**CYP1A1 (T6235C) + CYP1A1 (A4889G)**	
			No genotype at risk TT + AA	1.00 (reference)
			Two genotypes at risk TC / CC + AG / GG	approx 1.00 (no CI provided)
			CYP1A1 (T6235C) + NQO1 (C609T)	
			No genotype at risk TT + CC	1.00 (reference)
			Two genotypes at risk TC / CC + CT / TT	5.77 (1.76-19.00)
			CYP1A1 (A4889G) + NQO1 (C609T)	
			No genotype at risk AA+ CC	1.00 (reference)
			Two genotypes at risk AG / GG + CT / TT	3.10 (1.24-7.74)
			CYP1A1 (T6235C) + CYP1A1 (A4889G) + NQO1 (C609T)	
			No genotype at risk TT + AA+ CC	1.00 (reference)
			Two genotypes at risk TC / CC + AG / GG + CT / TT	10.71 (1.20-95.46)
Rimando et al (2008)[142]	Case-Control 60 pediatric ALL cases and 60 randomly selected normal children (all study subjects are less than 18 yrs old and are natural-born Filipino children)	**Strengths:** Genotyped in triplicate (control measure against genotyping error); used albumin gene as positive internal control; looked at Hardy-Weinberg equilibrium; performed a combined gene analysis; tried to remove bias from population stratification (limited patient selection to only natural born Filipino);	**GSTM1 + NQO1**	
			No genotype at risk GSTM1 positive + NQO1 C/T	1.00 (reference)
			One genotype at risk GSTM1 null + NQO1 C/T	2.31 (0.77-6.88)
			Two genotypes at risk GSTM1 null + NQO1 T/T	11.9 (3.45-41.09)

		Limitations: very small sample size; no sample size calculations		
Suneetha et al (2008)[141]	Case-Control 92 ALL cases (includes individuals under 25 years old) / 150 cord blood sample controls from South India	**Strengths:** combined genetic analysis; looked at Hardy-Weinberg equilibrium in the controls **Limitations:** Adults and children were grouped together; small sample size with no sample size calculation	**GSTM1 and GSTP1**	
			No genotype at risk GSTM1 (present) and GSTP1 (Ile/Ile)	1.00 (reference)
			One genotype at risk GSTM1 (null)	1.69 (0.71-4.03)
			Two genotypes at risk GSTM1 (null) and GSTP1 (Ile/Val; Val/Val)	2.78 (1.16-6.69)
Pigullo et al (2007)[137]	Case- Control 323 pediatric ALL cases / 384 hospital controls from Italy (including Sardinia)	**Strengths:** large sample size; they looked at combined genetic effects; matched their controls to cases based on geographical location to minimize population stratification bias; looked at Hardy-Weinberg equilibrium in controls **Limitations:** looked at few candidate genes	**GSTM1, GSTT1 and GSTP1**	
			No genotype at risk GSTM1 (present), GSTT1 (present) and GSTP1 (Ile/Ile)	1.0 (reference)
			One genotype at risk	
			GSTM1 (null)	0.87 (0.54-1.41)
			Two genotypes at risk	
			GSTM1 (null) and GSTT1 (null)	0.61 (0.25-1.43)
			GSTM1 (null) and GSTP1 (Ile/Val; Val/Val)	1.05 (0.65-1.70)
			Three genotypes at risk	
			GSTM1 (null), GSTT1 (null) and GSTP1 (Ile/Val; Val/Val)	0.87 (0.36-2.10)
Ulusoy et al (2007)[107]	Case-Control 168 pediatric ALL cases / 207 healthy volunteer controls from Turkey	**Strengths:** large and healthy control group; they looked at combined genetic effect **Limitations:** control group includes adults – there could be different environmental exposures in this group as compared to the cases creating some bias; did not consider population stratification	**CYP2E1*5B, *6 and *7B**	
			No genotype at risk CYP2E1*5B (-/-),*6 (-/-) and *7B (-/-)	1.0 (reference)
			One genotype at risk CYP2E1*5B (-/+; +/+)	No individuals
			Two genotypes at risk CYP2E1*5B (-/+; +/+), *6 (-/+; +/+)	2.9 (1.0-8.5)
			Three genotypes at risk CYP2E1*5B (-/+; +/+), *6 (-/+; +/+), *7B (-/+; +/+)	3.9 (1.4-11.0)

		bias; no sample size calculations; did not assess Hardy-Weinberg equilibrium in controls		
Pakakasama et al (2005)[132]	Case Control 107 pediatric ALL cases / 320 healthy adult volunteers from Bangkok Thailand	**Strengths:** large healthy control group; looked at multiple candidate genes **Limitations:** small sample size for ALL group; used adult controls rather than children, may be different environmental exposures leading to bias; only one combined genetic effect; did not consider population stratification bias; Hardy-Weinberg equilibrium was not assessed	**GSTM1 and GSST1**	
			present / present	1.0 (reference)
			null / null	1.7 (1.1-2.9)
Joseph et al (2004)[144]	118 cases / 118 controls	**Strengths:** specific population, less likely to encounter population stratification bias; tested Hardy-Weinberg equilibrium in controls; performed analysis stratified by sex; performed combined gene analysis **Limitations:** Small sample size / may be population stratification bias	**CYP1A1 m2 and CYP1A1 m1**	
			m1- /m2-	5.67 (2.11-15.27)*
			m1 - / m2 +	3.08 (1.55 – 6.15)*
			m1+ /m2-	2.63 (1.28 – 5.41)*
			CYP1A1 m1 and GSTM1	
			m1 -/- and deleted	9.68 (1.13 – 83.05)
			m1 -/- and present	4.84 (0.49 – 48.01)
			m1 -/+ and deleted	5.81 (2.01 – 16.76)
			m1 -/+ and present	2.18 (1.05 – 4.54)
			m1 +/+ and deleted	1.68 (0.85 – 3.34)
Canalle et al (2004)[102]	Case-Control 113 ALL pediatric cases / 221 Controls (general population from the same hospital)	**Strengths:** looked at combined genetic effects; looked at multiple candidate genes; subjects were matched on ethnicity (accounting for possible population stratification	**GSTM1, GSTT1, GSTP1, CYP1A1 *2, CYP2E1*5B**	
			No genotypes at risk GSTMI (present), GSTT1 (present), GSTP1 (Ile/Ile), CYP1A1 *2 (-/-) and CYP2E1 *5B (-/-)	1.0 (reference)
			One genotype at risk	

	from Ribeirao Preto Brazil	bias); assessed Hardy-Weinberg equilibrium in control group **Limitations**: small ALL group	GSTM1 (null)	1.3 (0.4-4.0)
			CYP1A1 (-/+; +/+)	2.1 (0.7-6.7)
			CYP2E1 *5B (-/+; +/+)	2.3 (0.3-15.9)
			Two genotypes at risk	
			GSTM1 (null) and GSTT1 (null)	1.9 (0.5-7.2)
			GSTM1 (null) and GSTP1 (Ile/Val; Val/Val)	1.3 (0.5-3.6)
			GSTM1 (null) and CYP1A1 2 (-/+;+/+)	3.1 (0.9-10.2)
			GSTM1 (null) and CYP2E1 *5B (-/+;+/+)	No individuals
			CYP1A1 2 (-/+;+/+) and GSTT1 (null)	3.4 (0.4-28.0)
			CYP1A1 2 (-/+;+/+) and GSTP1 (Ile/Val; Val/Val)	1.5 (0.5-4.7)
			CYP2E1 2 (-/+;+/+) and GSTT1 (null)	No individuals
			CYP2E1 2 (-/+;+/+) and GSTP1 (Ile/Val; Val/Val)	1.4 (0.2-8.3)
			CYP2E1 2 (-/+;+/+) and CYP1A1 2 (-/+;+/+)	No individuals
			Three genotypes at risk	
			GSTMI (null), GSTT1 (null), GSTP1 (Ile/Val; Val/Val)	1.7 (0.3-11.0)
			GSTMI (null), GSTT1 (null), CYP1A1 *2 (-/+;+/+)	1.4 (0.2-8.3)
			GSTMI (null), GSTT1 (null), CYP2E1 *5B (-/+;+/+)	No individuals
			GSTMI (null), GSTP1 (Ile/Val; Val/Val) and CYP1A1 *2 (-/+;+/+)	1.6 (0.4-5.7)
			GSTMI (null), GSTP1 (Ile/Val; Val/Val) and CYP2E1 *5B (-/+;+/+)	2.1 (0.4-10.4)
			GSTMI (null), CYP1A1 *2 (-/+;+/+) and CYP2E1 *5B (-/+;+/+)	No individuals
			CYP1A1 *2 (-/+;+/+), GSTT1 (null) and GSTP1 (Ile/Val; Val/Val)	1.1 (0.1-12.4)
			CYP2E1 *5B (-/+;+/+),GSTT1 (null) and GSTP1 (Ile/Val; Val/Val)	No individuals
			CYP1A1 *2 (-/+;+/+), GSTT1 (null) and CYP2E1 *5B (-/+;+/+)	No individuals
			CYP1A1 *2 (-/+;+/+), GSTP1 (Ile/Val; Val/Val) and CYP2E1 *5B (-/+;+/+)	1.1 (0.1-12.4)

			Four genotypes at risk	
			GSTM1 (null), GSTT1 (null), GSTP1 (Ile/Val; Val/Val), CYP1A1 *2 (-/+;+/+)	1.7 (0.3-11.0)
			GSTM1 (null), GSTP1 (Ile/Val; Val/Val), CYP1A1 *2 (-/+;+/+) and CYP2E1 *5B (-/+;+/+)	10.3 (1.0-111.8)
			GSTM1 (null), GSTT1 (null), GSTP1 (Ile/Val; Val/Val) and CYP2E1 *5B (-/+;+/+)	No individuals
			GSTT1 (null), GSTP1 (Ile/Val; Val/Val), CYP1A1 *2 (-/+;+/+) and CYP2E1 *5B (-/+;+/+)	No individuals
			Five genotypes at risk	
			GSTM1 (null), GSTT1 (null), GSTP1 (Ile/Val; Val/Val), CYP1A1 *2 (-/+;+/+) and CYP2E1 *5B (-/+;+/+)	No individuals
Wang et al (2004)[140]	Case-Control 67 pediatric ALL cases / 146 healthy controls from China	**Strengths**: combined gene analysis **Limitations:** very small sample size; no sample size calculation; they studied few candidate genes; controls included adults, may be different environmental exposures leading to bias; limited reporting on patients and methods making it difficult to assess their strengths and limitations	**GSTM1 and GSTT1**	
			No genotypes at risk GSTM1 (present) an GSTT1 (present)	1.0 (reference)
			Two genotypes at risk GSTM1 (null) and GSTT1 (null)	3.15 (1.71 – 5.79)
Davies et al (2002)[139]	Case-Control 651 pediatric ALL cases (616 whites and 35 blacks) / 733 normal blood donor controls (532 whites and 201 blacks)	**Strengths:** large sample size; good reporting of patient characteristics and methods; combined gene effects **Limitations:** no effect measures for ALL susceptibility (only p-values)	**GSTM1 and GSTT1 in whites**	
			Two genotypes at risk GSTM1 (null) and GSTT1 (null)	No odds ratio reported; 10% null /null variants in controls vs 9.7% in cases (p-value 1.0)
			GSTM1 and GSTT1 in blacks	
			GSTM1 (null) and GSTT1 (null)	No odds ratio reported; 10% null /null variants in controls vs 8.6% in cases

				(p-value 1.0)
Krajinovic et al (2002)[103]	Case-Control 174 cases/337 controls from Quebec Canada	**Strengths:** studied various candidate genes; homogenous population (limiting population stratification bias); combined genetic effects; assessed gene-gene interaction effects **Limitations**: small ALL group	**CYP2E1 *5 and MPO *2 and NQO1 *2 or *3**	
			No genotype at risk CYP2E1 (-/-), NQO1 (-/-) and MPO (+/+; -/+)	1.0 (reference)
			One genotype at risk CYP2E1 (+/+; -/+)	3.6 (0.7-17.3)
			Two genotypes at risk CYP2E1 (+/+; -/+) and MPO (-/-)	1.3 (0.1-15.6)
			Two genotypes at risk CYP2E1 (+/+; -/+) and NQO1 (+/+; -/+)	2.7 (0.2-44.9)
			Three genotypes at risk CYP2E1 (+/+; -/+), NQO1 (+/+; -/+) and MP0 (-/-)	5.4 (1.2-23.4)
Krajinovic et al (2000)[146]	Case-Control 174 cases/285 controls from Quebec Canada	**Strengths:** assessed gene-gene combined effects with polymorphisms previously found to be associated with increased risk of ALL; homogenous population (limiting population stratification bias); tested for linkage disequilibrium between NAT1 and NAT2 polymorphisms **Limitations:** small ALL group	**NAT2 slow[a], GSTM1 null, and CYP1A1 *2A**	
			No genotype at risk NAT2 (rapid,) GSTM1 (present), CYP1A1 *2A (-/-)	1.0 (reference)
			One genotype at risk	
			GSTM1 (null)	1.3 (0.7-2.7)
			CYP1A1 *2A (-/+; +/+)	0.6 (0.1-3.1)
			Two genotypes at risk	
			NAT2 slow, GSTM1 (null)	2.4 (1.3-4.5)
			NAT2 slow, CYP1A1 *2A (-/+; +/+)	2.5 (0.9-7.1)
			GSTM1 (null), CYP1A1 *2A (-/+; +/+)	6.1 (1.9-20.1)
			Three genotypes at risk	
			NAT2 slow, GSTM1 (null), CYP1A1 *2A (-/+; +/+)	3.1 (1.1-8.4)
Krajinovic et al (1999)[135]	Case-Control 177 pediatric ALL cases / 304 controls from the same hospital from Montreal Quebec	**Strengths:** studied various candidate genes; looked at combined genetic effects; restricted to one ethnicity (limiting population stratification bias) **Limitations:** small ALL	**GSTM1 and CYP1A1 *2A**	
			No genotype at risk GSTM1 (present), CYP1A1 *2A (-/-)	1.0 (reference)
			One genotype at risk	
			GSTM1 (null)	1.6 (1.0-2.4)
			CYP1A1 *2A (-/+; +/+)	1.6 (0.7-3.6)
			Two genotypes at risk	

		group	GSTM1 (null); CYP1A1 *2A (-/+; +/+)	3.3 (1.6-6.9)
Chen et al (1997)[138]	Case-Control 34 black pediatric ALL patients and 163 white pediatric ALL cases / 203 healthy black adult controls and 213 healthy white adult controls	**Strengths:** large sample size; odds ratios were adjusted for race (consideration of population stratification bias); gene-gene combined effects **Limitations:** no sample size calculations; small sample size for the black analysis (only 34 cases); for the cases, the genotyped patients had better prognoses than the non genotyped individuals (potential survivor bias)	**GSTM1 and GSTT1 in Blacks and Whites**	
			No genotypes at risk GSTM1 (present) and GSTT1 (present)	1.0 (reference)
			One genotype at risk GSTM1 (null)	1.20 (0.87 CI lower bound; 0.2 SE)
			GSTM1 and GSTT1 in Blacks	
			No genotypes at risk GSMT1 (present) and GSTT1 (present)	1.0 (reference)
			Two genotypes at risk GSTM1 (null) and GSTT1 (null)	7.36 (2.61 CI lower bound; 1.71 SE)
			GSTM1 and GSTT1 in Whites	
			No genotypes at risk GSMT1 (present) and GSTT1 (present)	1.0 (reference)
			Two genotypes at risk GSTM1 (null) and GSTT1 (null)	0.75 (0.35 CI lower bound ; 0.36 SE)

Notations and Polymorphism definitions:
CYP1A1 in the table primarily consists of three mutations named m1 (T6235C), m2 (A4889C) and m4 (C4887A) [102,103,156]
CYP1A1*2A (presence of m1 only), ***2B** (both m1 and m2), and ***4** (m4 only) [156]
GSTM1 null a homozygous deletion (null allele) caused by homologous recombination, rendering the enzyme inactive [118]
GSTM1 *A creates Lyn at codon 173 AAG) and **GSTM1 *B** creates Asn at codon 173 (AAC) [182]
CYP2E1 *5B is the variant C to T transversion at position 1019 [107] and **CYP2E1 *5** is the variant G to C transversion at position -1295 [103]
CYP2E1 *6 is a *Dra*I RFLP in intron 6 [107]
CYP2E1 *7B is the variant *Dde*I RFLP in the promoter region [107]
CYP1A1 *2 is a variant at base pair 6235 in the 3 -flanking region, resulting in a new recognition, sequence for the restriction enzyme *Msp*I [102]
NQO1 *2 (C609T) and ***3** (C465T); **MPO *2** (G463A) [108]
NAT1 and NAT2 polymorphisms in these genes are correlated with rapid and slow acetylation phenotypes[146]
GSTT1 null is a homozygous deletion (null allele) rendering the enzyme inactive [138]
GSTP1: polymorphism *B is a A→G transition of nucleotide 313 in exon 5 whereas polymorphism *C is a G→T transversion of nucleotide 341 in exon 6 (results in a substitution of Ile→Val and Val→Ala)[102]

Appendix E: Effects of organic solvent exposure on the risk of developing childhood ALL (exposures also include occupations where organic solvents are regularly used)

Reference	Study Design and Sample Size	Strengths and Limitations	Exposure information (Interviewee / window and occurrence)	Organic Solvent Exposure	Exposure to Mother, father or offspring	Odds ratio and 95% confidence interval
Scélo et al (2009)[22]	550 ALL pediatric cases and 550 randomly selected population controls from California, United States	**Strengths:** performed analyses on frequency of use, performed analyses for cytogenetic subtypes and for different age groups (not reported here), clearly defined time windows, large sample size **Limitations:** many exposures grouped into two broad categories of chemicals, self reported exposures (potential for misclassification bias)	In home personal interviews about household exposures to paints (paints, stains or lacquers) and solvents (adhesives, petroleum products such as pain thinners, spot remover, paint remover, glue, solvents, gasoline, kerosene or lubricating oil) **during preconception, pregnancy and early childhood**	**Paint**		
				overall	mother / father / offspring	1.65 (1.26-2.15)
				preconception	mother/father	1.10 (0.71-1.69)
				pregnancy	mother	1.21 (0.88-1.67)
				After birth	offspring	1.39 (1.07-1.81)
				Any time	mother	1.19 (0.89-1.58)
				Any time	father	1.44 (1.08-1.91)
				Any time	others	1.63 (1.17-2.26)
				Frequent users	mother/father/ offspring	1.74 (1.25-2.43)
				Rare users	mother/father/ offspring	1.28 (0.92-1.78)
				Solvents		
				overall	mother/father/ offspring	1.15 (0.87-1.51)
				Before birth	mother / father	1.19 (0.83-1.71)
				After birth	offspring	1.06 (0.76-1.50)
				Any time	mother	1.08 (0.77-1.51)
				Any time	father	1.17 (0.88-1.56)
				Any time	others	1.16 (0.73-1.86)
				Frequent users	mother/father/ offspring	1.31 (0.95-1.81)
				Rare users	mother/father/ offspring	1.12 (0.75-1.67)

McKinney et al (2008)[21]	Case-control (Refining the exposure assessments of a population based case-control study – results using this data was published by McKinney et al in 2000 and 2003) 1881 pediatric leukemia and lymphoma cases (between 0 and 14) and 3742 matched controls from the United Kingdom	**Strengths:** large sample size; used a novel method to recode their occupational exposures using better techniques – resulted in the reclassification of many exposures; new assessment was done with personnel blinded to case-control status; face to face personal interviews were conducted; gathered information on specific chemicals; assessed frequency and level of exposure; external validation was done on a random sample of the subjects by an expert hygienist; data quality was assessed; assessed through diagnostic group analyses whether cases were overreporting; diagnostic subgroup analyses were performed **Limitations:** possible selection bias; possible differential recall bias (differential underreporting was not verified)	Complete occupational history of all parental occupations held for over 6 months after leaving full-time education until the date of diagnosis. Time windows for analysis: **preconception, pregnancy and postnatal**	**ALL subgroup**		
				Preconception	Mothers	
				Solvents		1.4 (0.9-1.9)
				Petrol		0.8 (0.4-1.4)
				Pregnancy		
				Solvents		2.7 (1.6-4.6)
				Petrol		0.9 (0.2-3.3)
				Postnatal		
				Solvents		1.9 (1.1-3.3)
				Petrol		2.4 (0.8-6.9)
Abadi-Korek et al (2006)[18]	Case-control 112 ALL pediatric cases / 112 randomly selected hospital controls from same department in Petach-Tikva, central district in Israel	**Strengths:** Very good reporting of results; adjusted odds ratio for confounders **Limitations:** small sample size; analyses for organic solvents were not extensive enough (no sub-analysis by parent or time window was reported); no sample size calculation; controls were also afflicted with	Detailed questions on occupational exposures to parents during **preconception, pregnancy and the postnatal period**	Parental exposure to any organic solvent, during any period	Mother or Father	2.1 (1.1-4.2)

		hematologic diseases, possible similar etiologies affected OR				
Infante-Rivard et al (2005)[10]	Case-Control 790 ALL pediatric cases / 790 randomly selected population control children from Montreal Canada	**Strengths:** large sample size; detailed exposure ascertainment; exposure coding was done by trained chemists and industrial hygienists who were blinded to case/control status; did an analysis with level of exposure (not reported here) **Limitations:** some chemicals had very small numbers of people exposed, limiting the interpretation; no paternal data	Occupational exposures of organic solvents to mothers **2 years before pregnancy up to birth and during pregnancy**	**2 years before pregnancy up to birth**	**Mother**	
				Methanol		0.77 (0.41-1.47)
				Ethanol		1.22 (0.66-2.25)
				Isopropanol		0.96 (0.71-1.29)
				Chloroform		0.25 (0.05-1.17)
				Methylene chloride		1.34 (0.54-3.34)
				1,1,1-Trichloroethane		7.55 (0.92-61.97)
				Perchloroethylene		0.96 (0.41-2.25)
				Acetone		1.05 (0.53-2.08)
				Benzene		0.82 (0.22-3.06)
				Toluene		1.88 (1.01-3.47)
				Diethyl ether		0.50 (0.17-1.48)
				Turpentine		1.76 (0.42-7.42)
				Mineral spirits, post-1970		1.82 (1.05-3.14)
				Leaded gasoline		5.09 (0.59-43.65)
				Unleaded gasoline		0.90 (0.30-2.71)
				Alkanes (C5–C17)		1.78 (1.11-2.86)
				Aliphatic alcohols		0.90 (0.68-1.18)
				Chlorinated alkanes		1.33 (0.68-2.61)
				Chlorinated alkenes		0.97 (0.43-2.17)
				Aliphatic ketones		1.30 (0.68-2.50)
				Mononuclear aromatic hydrocarbons		1.64 (1.12-2.41)
				Any solvent		1.09 (0.87-1.38)
				During pregnancy	**Mother**	

				Methanol		0.78 (0.39-1.55)
				Ethanol		1.06 (0.55-2.03)
				Isopropanol		0.95 (0.69-1.31)
				Chloroform		0.25 (0.05-1.17)
				Methylene chloride		1.25 (0.46-3.35)
				1,1,1-Trichloroethane		4.07 (0.45-36.7)
				Perchloroethylene		0.84 (0.30-2.34)
				Acetone		1.13 (0.52-2.44)
				Benzene		1.39 (0.31-6.25)
				Toluene		2.25 (1.02-4.95)
				Diethyl ether		0.63 (0.20-1.93)
				Turpentine		1.76 (0.42-7.42)
				Mineral spirits, post-1970		1.66 (0.86-3.22)
				Leaded gasoline		4.14 (0.46-37.16)
				Unleaded gasoline		0.83 (0.22-3.10)
				Alkanes (C5–C17)		1.72 (0.98-3.03)
				Aliphatic alcohols		0.89 (0.66-1.20)
				Chlorinated alkanes		1.05 (0.50-2.19)
				Chlorinated alkenes		0.86 (0.33-2.25)
				Aliphatic ketones		1.46 (0.70-3.03)
				Mononuclear aromatic hydrocarbons		1.68 (1.06-2.67)
				Any solvent		1.00 (0.78-1.28)
Ali et al (2004)[23]	Case-Control 103 leukemia cases (under the age of 30) and 417 randomly	**Strengths:** used a standard classification system for industry and occupation; trained interviewers and used detailed questionnaires (in person or telephone	Occupations held by subjects since the age of 16 and parents, during **preconception** (1 yr before birth of child), **perinatal period** (one	**Building finishers and related trades workers**	**Father**	
				All times		4.08 (1.13-14.82)
				Preconception		4.08 (1.13-14.82)
				Perinatal		4.51 (1.04-19.57)

	selected and healthy, matched controls from Kaohsiung, Southern Taiwan	interviewers); used clear time windows **Limitations:** adult and pediatric cancers were grouped – different biology and possible risk factors; all leukemia outcomes were grouped into one category; small sample size; tested hundreds of occupations and listed only positive results; potential selection bias due to high refusal rates	yr prior to birth and birth) and **postnatal period** (after child's birth)	**Wood treaters**	**Father**	
				All times		16.03 (1.77-145.49)
				Preconception		12.17 (1.36-109.21)
				Perinatal		13.08 (1.38-125.50)
				Electronic equipment assemblers	**Father**	
				Preconception		4.57 (1.05-19.90)
				Other assemblers	**Father**	
				Preconception		10.24 (1.02-102.57)
Steffen et al (2004)[81]	Case-control 280 pediatric acute leukemia cases (includes 240 pediatric ALL cases) and 287 frequency-matched hospital controls (age, sex, centre and ethnic origin) hospitalized for acute pathologies. Subjects are from Paris, Lyon, Lille and Nancy (France)	**Strengths:** face to face interviews using standardized questionnaire by specially trained medical doctors; did an effect size calculation for 80% power; looked at confounding between socioeconomic status and proximity to repair garage or petrol stations – found no association; performed a sensitivity analysis of the impact of missing values on the odds ratios; performed sub-analyses on ALL and acute non-lymphocytic leukemia **Limitations:** exposures were self-reported, potential for some misclassification bias (possible differential misclassification if case mothers overreported)	Maternal occupational exposures to hydrocarbons during pregnancy as well as environmental exposures to hydrocarbons during pregnancy or childhood (primarily interested in benzene, unleaded fuel or other hydrocarbons found in air) *reference category is no neighboring business	***ALL subgroup***		
				Childhood		
				Exposure to a neighboring repair garage or petrol station	Child	3.6 (1.3-9.9)
				Other neighboring business	Child	1.5 (0.5-4.6)
				All acute leukemias		
				During pregnancy		
				Exposure to a neighboring repair garage or petrol station	Mothers	2.2 (0.9-5.7)
				Other neighboring business	Mothers	0.8 (0.3-2.4)
				Exposures to glues, paints or varnishes containing organic solvents	Mothers	1.7 (0.5-6.0)
				During childhood		
				Exposure to a neighboring repair garage or petrol station	Child	4.0 (1.5-10.3)
				Other neighboring business	Child	1.4 (0.5-4.1)

				Exposure to neighboring repair garage or petrol station between 1-35 months (compared to 0)	Child	3.4 (0.9-12.4)
				" " over 36 months (compared to 0)	Child	4.7 (1.2-18.5)
				For every month of exposure to neighboring repair garage or petrol station	Child	1.03 (1.01-1.05) (p trend <0.05)
McKinney et al. (2003)[20]	Case-control 1461 ALL pediatric cases / 7629 control children from the United Kingdom	**Strengths:** used classification systems for occupational coding and reliability of the coding was regularly verified; used experts for occupational groups; large sample size; looked at both parents **Limitations:** Self reported exposures (possible recall or reporting bias); understanding of biological mechanism limited; more surrogate reporting for control fathers than case fathers; could not test for specific chemicals	Occupational exposures to parents during **periconcepion** (includes jobs held 6 months from leaving full time education until the time of the interview)	Organic solvents	Mothers	1.00 (0.66-1.51)
					Fathers	1.05 (0.79-1.37)
				Dermal hydrocarbons	Mothers	2.16 (1.16-4.02)
					Fathers	0.96 (0.76-1.21)
				Inhaled particulate hydrocarbons	Mothers	2.26 (0.79-6.45)
					Fathers	1.41 (1.11-1.79)
				Exhaust fumes	Mothers	1.68 (0.76-3.74)
					Fathers	1.26 (1.02-1.56)
				Ceramics / glass	Mothers	1.29 (0.27-6.10)
					Fathers	1.51 (0.47-4.27)
				Leather workers	Mothers	1.31 (0.37-4.63)
					Fathers	1.23 (0.26-5.80)
				Medical / health care	Mothers	0.79 (0.61-1.02)
					Fathers	0.66 (0.38-1.13)
				Metal	Mothers	3.91 (1.64-9.32)
					Fathers	0.95 (0.78-1.16)
				Paints	Mothers	Not enough individuals
					Fathers	1.22 (0.73-1.91)
				Plastic	Mothers	3.26 (0.78-13.75)
					Fathers	1.52 (0.55-4.14)
				Printing	Mothers	1.32 (0.58-3.03)

					Fathers	0.81 (0.41-1.57)
				Rubber manufacturing	Mothers	Not enough individuals
					Fathers	4.80 (1.20-19.28)
Costas et al (2002)[14]	Case-Control 19 pediatric leukemia cases (17 ALL, 3 acute myelocytic leukemia and 1 chronic myelocytic leukemia) and 37 healthy children, randomly selected population controls from Worbun Massachusetts, United States	**Strengths**: dose dependent analyses; **Limitations**: self reported exposures (mother first respondent for entire family); small sample size (limited power for analyses); recall bias from exposure frequency (community awareness of well water contamination)	Household exposures to **contaminated municipal drinking water (exposure to arsenic, trichloroethylene, tetrachloroethylene, chloroform, and low levels of other organic compounds)** to parents and affected offspring. The time windows are: **from 2 years before conception to case diagnosis (full etiologic period); preconception (2 years); pregnancy and postnatal (until diagnosis)**	**Entire etiologic period**	**Parents/affected offspring**	
				Ever		2.39 (0.54-10.59)
				Least		5.00(0.75-33.50)
				Most		3.56 (0.51-24.78)
				Preconception	**Mother/father**	
				Ever		2.61 (0.47-14.37)
				Least		2.48 (0.42-15.22)
				Most		2.82 (0.30-26.42)
				Pregnancy	**Mother**	
				Ever		8.33 (0.73-94.67)
				Least		3.53 (0.22-58.14)
				Most		14.30 (0.92-224.52)
				Postnatal	**Affected offspring**	
				Ever		1.18 (0.28-5.05)
				Least		1.82 (0.31-10.84)
				Most		0.90 (0.18-4.56)
Feychting et al (2001)[24]	Cohort 161 cases of childhood leukemia and 235 635 children in the study base from Sweden	**Strengths:** no selection or recall bias, large sample size, used senior occupational hygienists, reported relative risk estimates for chemicals and occupations (not reported here) **Limitations:** broad disease category**,** did not look at subtypes, did not interview the fathers for exposure assessment (assessment was based on occupational title)	Paternal occupational exposures to chemicals **before conception**. Paternal occupational information was liked with a job-exposure matrix constructed for the study	**Preconception**	**Father**	Relative risks instead of odds ratios
				Solvents		1.25 (0.80-1.95)
				Benzene		1.23 (0.39-3.85)

Freedman et al (2001)[84]	Case-Control 640 pediatric ALL cases and 640 controls (random digit dialing control acquisition) from 9 midwestern and mid-Atlantic states	**Strengths:** did analyses using frequency and duration of exposure **Limitations:** self reported exposure frequency and duration, potential for differential recall bias	Household exposures to selected chemicals during childhood and to indoor house painting during **preconception, pregnancy and childhood**	**Childhood**	**Affected offspring**	
				Model Building		
				Ever		1.1 (0.8-1.5)
				Low		0.9 (0.6-1.3)
				Medium		1.5 (0.8-2.8)
				High		1.9 (0.7-5.8)
				Artwork (using solvents)		
				Ever		1.3 (0.9-1.8)
				Low		1.1 (0.7-1.8)
				Medium		1.2 (0.7-2.0)
				High		4.1 (1.1-15.1)
				Furniture stripping		
				Ever		1.1 (0.8-1.6)
				Low		0.9 (0.6-1.5)
				Medium		1.8 (0.9-3.6)
				High		1.0 (0.4-2.7)
				Auto/truck maintenance		
				Ever		0.9 (0.7-1.2)
				Low		0.9 (0.7-1.2)
				Medium		0.9 (0.6-1.2)
				High		1.5 (0.8-2.7)
				Electronic repair		
				Ever		1.4 (0.8-2.4)
				Low		1.5 (0.7-3.0)
				Medium		2.7 (1.0-7.7)
				High		0.3 (0.1-1.5)
				Painting during preconception	**Mother / father / other**	
				Ever		1.2 (0.9-1.5)

				1-2 rooms		1.0 (0.8-1.3)
				3-4 rooms		1.4 (0.9-2.1)
				Over 4 rooms		1.7 (1.1-2.7)
				Were not at home during painting		2.3 (0.6-8.9)
				Were home during painting		1.9 (0.6-6.4)
				Mother painted	Mother only	1.1 (0.9-1.5)
				Other individual painted	Father / other only	1.3 (0.9-1.7)
Schuz et al (2000)[11]	Case-Control 1138 pediatric cancer cases selected from German Childhood Cancer Registry and 2962 matched population controls selected from population registration files Subjects are all from Germany.	**Strengths:** large population based study; looked at exposure prevalence in controls and cases alongside time to interview (time lag) in an attempt to assess differential recall; assessed exposures in both parents; used clearly defined time windows **Limitations:** self administered questionnaire, potential for differential misclassification (overreporting) – no classification system for the chemicals was used, nor were experts used to assess occupational exposures; possible selection bias due to nonparticipation; broad exposure categories	Parental occupational exposures were assessed in three time windows: **during the year before conception, during pregnancy and after birth**	Any time window		
				Solvents	Mothers	1.1 (0.8-1.6)
					Fathers	1.0 (0.8-1.3)
				Paints or lacquers	Mothers	1.8 (1.2-2.6)
					Fathers	1.1 (0.9-1.4)
				Oil products	Mothers	1.3 (0.8-2.2)
					Fathers	1.1 (0.9-1.3)
				Preconception		
				Solvents	Mothers	1.2 (0.9-1.7)
					Fathers	1.0 (0.8-1.2)
				Paints or lacquers	Mothers	1.6 (1.1-2.4)
					Fathers	1.1 (0.9-1.4)
				Oil Products	Mothers	1.5 (0.9-2.5)
					Fathers	1.1 (0.9-1.3)
				During pregnancy		
				Solvents	Mothers	1.3 (0.8-1.9)
					Fathers	1.0 (0.8-1.3)
				Paints or lacquers	Mothers	2.0 (1.2-3.3)
					Fathers	1.1 (0.8-1.4)
				Oil Products	Mothers	1.6 (0.8-2.9)
					Fathers	1.2 (0.9-1.5)

				Postnatal		
				Solvents	Mothers	1.1 (0.6-1.8)
					Fathers	1.0 (0.8-1.2)
				Paints or lacquers	Mothers	1.0 (0.6-1.8)
					Fathers	1.0 (0.8-1.3)
				Oil Products	Mothers	1.1 (0.5-2.4)
					Fathers	1.0 (0.8-1.3)
Shu et al (1999)[9]	Case-Control 1842 pediatric ALL case-control pairs where the controls were randomly selected population controls from the United States	**Strengths:** large sample size; randomly selected population controls (random digit dialing); used industrial hygienist to aid classification of chemical exposures; used both parents; did duration of exposure analyses (not reported here); studied many time windows **Limitations:** used self-reported exposure information; multiple testing (increased risk of false positives); no data on the level or intensity of exposure; some surrogate responses were required for parents unable to attend interview	Occupational exposures to **parents during preconception, gestation and postnatal periods**	**Exposure at any time period**		
				Freon	Mothers	2.0 (1.0-4.1)
				Any solvent, degreaser or cleaning agent	Mothers	1.3 (1.0-1.7)
				Any plastic material	Mothers	2.3 (1.2-4.4)
				Fuels	Fathers	1.5 (1.0-2.4)
				Preconception		
				Organic (non-chlorinated) solvents	Mothers	2.0 (1.0-4.2)
				Possible organic solvents	Mothers	2.0 (1.2-3.5)
				Any solvent, degreaser or cleaning agent	Mothers	1.8 (1.3-2.5)
				Polystyrene	Fathers	2.4 (1.2-4.8)
				Any plastic material	Fathers	1.4 (1.0-1.9)
				Paint remover	Mothers	2.5 (1.0-5.9)
				Paint thinner	Mothers	1.9 (1.0-3.7)
				Paints (not spray)	Mothers	1.9 (1.2-3.1)
				Any paint or thinner	Mothers	1.6 (1.2-2.2)
				Glues	Fathers	1.2 (1.0-1.6)
				During Pregnancy		
				Possible organic solvents	Mothers	1.9 (1.0-3.6)
				Any solvent, degreaser or cleaning agent	Mothers	1.6 (1.1-2.3)
				Turpentine	Mothers	3.5 (1.3-10.0)

				Turpentine	Fathers	1.7 (1.1-2.8)
				Paint remover	Mothers	5.2 (1.7-15.8)
				Paint thinner	Mothers	3.3 (1.5-7.1)
				Paints (not spray)	Mothers	2.0 (1.2-3.5)
				Any paint or thinner	Mothers	1.7 (1.2-2.3)
				During Postnatal period		
				Any plastic material	Mothers	2.2 (1.0-4.47)
				Turpentine	Fathers	1.5 (1.0-2.2)
Smulevich et al (1999)[176]	Case-Control 593 pediatric cancer cases from the Moscow Central Cancer Dispensary and 1181 healthy, matched and randomly selected controls from local pediatric polyclinics in Moscow	**Strengths:** large sample size; detailed questionnaires on jobs held for both parents, including detailed information of military service; job exposure ascertainment from job-matrices created by occupational hygienists; performed some sub-analyses on cancer sub-types; studied occupations and exposures in both parents **Limitations:** many exposures were grouped into broad categories; self reported job details covering a wide time window (any job before conception)	Occupational history and exposures for every job held in both parents Parents were considered exposed to organic solvents if they worked in the following trades: motor-vehicle drivers or mechanics, boot-and-shoe plants, as painters, printing workers or furniture workers, in some cases laboratory assistants, workers in chemical production lines or dry cleaners or workers with cutting oil	**Leukemias and exposure to organic solvents**		
				Any time before conception	Fathers	1.4 (0.95-2.1)
				Any time during job history	Mothers	3.1 (1.5-6.3)
				Any cancer and exposure to paints		
				Any time before conception	Fathers	2.0 (p-value between 0.05 and 0.1)
					Mothers	1.9 (p value over 0.1)
				2 months before conception	Fathers	1.6 (p-value between 0.05 and 0.1)
					Mothers	1.6 (p-value between 0.05 and 0.1)
Cocco et al	Case-Control	**Strengths:** assessed multiple	Occupational exposures	Workplace exposure to	Fathers	1.5 (0.3-8.0) (crude)

(1996)[34]	9 cases of childhood ALL and 36 controls selected from a birth register from the municipality of Carbonia in Sardinia, Italy	exposures and parental characteristics; authors were conservative in their conclusions **Limitations:** very small sample size for determining etiologic causes; time period of exposure assessment unclear; potential differential recall bias from self-reported exposures; no details on paternal occupations; did not report any confounders	to solvents in fathers assessed using a job/exposure matrix	solvents		
Feingold et al (1992)[28]	Case-Control 243 pediatric cancer cases (under 14) and 212 control randomly selected healthy controls from the Denver Standard Metropolitan Statistical Area	**Strengths:** used job-exposure matrix, which allowed examinations of specific chemicals; looked at level of exposures; used a cancer registry and hospital record; trained interviewers and usually interviewed in the home; conducted sub-analyses on different cancer sub-types; low response rate in cases and more so in controls **Limitations:** small sample size, possible exposure misclassification due to data imputation; potential non-differential misclassification from surrogate information	Occupational history: looked at jobs held for 6 months longer between the year prior to the child's birth and yr of diagnosis Parents considered exposed had medium or high level exposures to specific chemicals under question	**ALL sub-analyses during the year prior to child's birth**		
				Any chemical	Father	1.2 (0.5-2.7)
				Any hydrocarbon		1.3 (0.6-3.0)
				Aromatic hydrocarbon		1.2 (0.5-5.8)
				Benzene		1.6 (0.5-5.8)
				Solvents		1.7 (0.4-8.2)
				Acetamide		1.6 (0.5-5.1)
				Diethylene glycol		1.4 (0.4-4.5)
Infante-Rivard et al (1991)[82]	Case-Control 128 pediatric cases of ALL and 128 randomly	**Strengths:** good selection of controls; precise outcome specification; used classification system for occupational data; in depth	Maternal occupations and substance exposures at work and in the home during **pregnancy**	Exposure to solvents (occupational and household)	Mothers	Relative Risk 0.62 (0.20-1.91)

	selected controls from census data living in one of the following Spanish provinces: Castellon, Valencia, Alicante, Murcia, Madrid and Albacete	face-to-face interviews; industrial hygienists aided with the identification of substances; studied household exposures in addition to occupational exposures **Limitations:** small sample size; many selected controls were unavailable or refused; controls outside the census were missed (potential for selection bias if mobility is associated with exposures); interviewers were not blind; possible recall bias				
McKinney et al (1991)[19]	Case-Control 109 pediatric leukemia or non-Hodgkin's lymphoma cases and 206 matched controls from birth registers Subjects are from three areas in northern England	**Strengths:** trained interviewers and face to face interviews with parents; other adults living in the same household other than parents were also interviewed; used a coding classification for occupations; hobby and household exposures were also measured **Limitations:** broad outcome categories; self reported exposures – potential for misclassification bias (recall bias); small sample size	Parental occupations and occupational exposures during **preconception, pregnancy and the postnatal period** (the adult must be living with the family during these time periods)	Chemical Exposures	Preconception	
				Carbon tetrachloride	Mothers	2.98 (0.50-24.19)
					Fathers	2.90 (1.14-7.36)
				Trichlororethene	Mothers	1.16 (0.13-7.91)
					Fathers	2.27 (0.84-6.16)
				Xylene	Fathers	6.86 (0.90-168.29)
				Benzene	Mothers	4.00 (0.30-117.99)
					Fathers	5.81 (1.67-26.44)
				Chemical Exposures or Occupation	Periconceptional and gestational	
				Carbon tetrachloride	Fathers	2.16 (0.54-9.14)
				Trichlororethene	Fathers	4.40 (1.15-21.01)
				Xylene	Fathers	3.24 (0.24-98.20)
				Benzene	Fathers	2.98 (0.50-24.19)
				Catering, cleaning and hairdressing	Mothers	3.12 (1.12-8.65)
				Chemical Exposures	Posnatal	
				Carbon tetrachloride	Fathers	3.48 (0.86-17.22)
				Trichlororethene	Fathers	2.66 (0.82-9.19)

				Xylene	Fathers	3.24 (0.23-98.20)
				Benzene	Fathers	1.39 (0.38-4.87)
Olsen et al (1991)[80]	Case-Control 1742 cases of childhood cancers (less than 15 yrs old) selected from the Danish Cancer registry and 8630 randomly selected matched population controls (selected from the Danish Central Population Register) (603 leukemia case mothers and	**Strengths:** large sample size; assessment of exposures in both parents; time window clearly defined; no recall bias, exposures obtained from a database (The Pension Fund) which classifies occupations based on the International Standard Classification; performed subanalyses for different diagnostic groups **Limitations:** no parental interviews; only presented positive associations (did not include all analyses in tables); potential for misclassiciation	Parental occupations at the **time of conception** (traced occupations up to 270 days before birth of the child) * reporting only occupations were exposure to organic solvents is likely	**Industry of employment** (Leukemia cases only)		OR (p-value)
				Rubber industry	Father	5.8 (p-value<0.01)
				Porcelain industry	Mother	14.5 (p-value<0.01)
				Manufacture of office machine	Father	7.0 (p-value<0.05)
				Machine repair workshops	Father	2.8 (p-value<0.05)
				Other manufacture of communication material	Mother	14.5 (p-value<0.01)
				Construction industry	Mother	3.1 (p-value<0.05)
				Wholesale in agricultural machinery	Father	4.6 (p-value<0.05)
				Refuse removal and cleaning	Father	4.6 (p-value<0.05)
				Job titles (all childhood cancers)		
				Machinists	Father	1.6 (p-value<0.01)
				Smiths	Father	1.5 (p-value<0.05)
				Laundry/dry cleaner owners	Mother	3.7 (p-value<0.01)
Shu et al (1988)[15]	Case-Control 309 pediatric leukemia cases from a long-standing population-based cancer registry and 618 healthy matched and randomly selected	**Strengths:** in person interviews; parental occupations and occupational exposures were grouped according to a coding system; gathered extensive demographic data; performed subtype disease analyses for mothers; used clear time windows; good reporting of methods and results	Parental occupations and occupational exposures during **pregnancy** (coding based on the Chinese Third National Population Census)	**Occupations**		
				Chemical processors and related workers, rubber and plastic products makers, leather workers, painters, chemical analysts	Fathers	1.1 (0.5-2.7)
					Mothers	3.3 (1.6-6.8)
					Mothers (only ALL subtype)	3.2 (1.5-7.0)
				Transportation equipment operator	Fathers	1.2 (0.6-2.3)
					Mothers	1.4 (0.4-5.0)
				Metal refining and	Fathers	0.8 (0.3-1.9)

	population controls Subjects are from Shanghai, China	**Limitations:** no disease sub-type analyses for fathers; potential for differential recall bias; controls were selected between 1985 to 1986 whereas cases were selected between 1974 to 1986; interviewers were not blinded to case/control status; more mothers were interviewed than fathers; list of chemical exposures being assessed were all already suspected carcinogens, potentially biasing parental responses; no sample size calculations		processing workers	Mothers	2.6 (0.9-7.7)
					Mothers (only ALL subtype)	1.0 (0.2-4.9)
				Blacksmiths, tool makers and machine assemblers and precise instrument makers	Mothers	1.1 (0.6-2.0)
					Fathers	0.9 (0.5-1.4)
				Electrical fitters, related electrical and electronic workers	Mothers	1.0 (0.5-2.0)
					Fathers	1.0 (0.5-2.1)

				Occupational Exposures		
				Benzene	Mothers	2.0 (0.9-4.3)
					Mothers (only ALL subtype)	1.3 (0.5-3.0)
				Gasoline	Mothers	1.6 (0.8-3.1)
					Mothers (only ALL subtype)	1.7 (1.0-3.0)
				Toluene	Mothers	1.5 (0.6-3.4)
					Mothers (only ALL subtype)	1.2 (0.5-2.7)
				Other organic solvents	Mothers	0.5 (0.2-1.0)
				Kerosene	Mothers	1.4 (0.6-3.1)
					Mothers (only ALL subtype)	1.5 (0.6-3.4)
				Diesel oil	Mothers	1.4 (0.6-3.3)
Lowengart et al (1987)[16]	Case-Control 123 pediatric leukemia case-control pairs from Los Angeles population based cancer registry; matched controls were selected through friends of the family or from random digit dialing (107 cases had ALL)	**Strengths:** looked at both parents; used classification for occupational coding; gathered both occupational and household exposures; looked at frequency of exposure; had clearly defined time windows of exposures **Limitations:** self-reported occupational histories; non-random controls (friends of cases); many proxy respondents for fathers; interviewers were not blinded ; possible recall bias leading to misclassification of exposures	Occupational exposures to parents from 1 year before conception until shortly before diagnosis of childhood leukemia in offspring	**One year before pregnancy**		OR (p-value)
				All chlorinated solvents		2.2 (0.09)
				Carbon tetrachloride		0.7 (0.33)
				Trichloroethylene		2.0 (0.16)
				Perchloroethylene		∞ (0.89) (1 case, 0 control)
				Methyl ethyl ketone		1.7 (0.24)
				During pregnancy		
				All chlorinated solvents		2.2 (0.09)
				Carbon tetrachloride		0.7 (0.33)
				Trichloroethylene		2.0 (0.16)
				Perchloroethylene		∞ (0.89) (1 case, 0 controls)
				Methyl ethyl ketone		1.7 (0.24)
				After delivery	Fathers	OR (confidence interval)
				All chlorinated solvents		3.5 (1.10-14.60)
				Low (<50/yr) High (≥ 50/yr)		1.7 8.0 (p-value of trend: 0.03)

				Carbon tetrachloride		1.7 (0.32-10.73)
				Trichloroethylene		2.7 (0.64-15.60)
				Perchloroethylene		∞ (0.19-∞) (2 cases, 0 controls)
				Methyl ethyl ketone		3.0 (0.75-17.23)
				Low (<50/yr) High (≥ 50/yr)		1.0 7.0 (p-value of trend: 0.03)
				Household exposures (Mothers – during pregnancy or nursing Father- during pregnancy)		OR (p-value)
				Paint, lacquer	Mothers	1.8 (0.03)
					Fathers	1.2 (0.31)
				Petroleum products	Mothers	2.0 (0.07)
					Fathers	1.1 (0.40)
Van Steensel-Moll et al (1985)[12]	Case-Control 519 childhood ALL cases and 507 randomly selected matched controls from census lists in the Netherlands	**Strengths:** large sample size; used a coding classification system for the occupational exposures; used precise outcome definition **Limitations:** exposures were assessed using mailed questionnaires to parents, potential for misclassification; high non response rate in the controls- potential for selection bias	Ascertainment of occupation history and occupational chemical exposures in both parents **during pregnancy and during the year preceding date of diagnosis**	**Occupations with potential for solvent exposure Pregnancy**		Relative Risk (reference category for fathers: administration and education and for mothers: no occupation outside home)
				Manual and mechanical skills	Mothers	2.0 (0.8-4.7)
					Fathers	1.0 (0.7-1.3)
				Scientific and artistic	Mothers	1.0 (0.2-7.5)
					Fathers	0.8 (0.4-1.8)
				All occupations	Mothers	1.3 (1.0-1.7)
				Auto mechanic, machinist, gas station attendant, and miner	Fathers	0.8 (0.4-1.5)
				Painter, cleaner, and dyer	Fathers	1.6 (0.5-5.0)
				Petroleum and chemical industry worker	Fathers	1.2 (0.3-4.8)
				Pharmacist, printer, and chemical analyst	Fathers	1.5 (0.4-5.4)
				All hydrocarbon-related	Fathers	1.0 (0.6-1.7)

				occupations	Mothers	2.5 (0.7-9.4)
				All hydrocarbon-related occupations	Mothers	(with referent category other occupations outside home) 2.0 (0.5-7.6)
				All hydrocarbon-related occupations	Mothers	(with referent category other occupations without hydrocarbon exposure) 2.0 (1.0-4.2)
				One year before diagnosis		Relative Risk
				Manual and mechanical skills	Mothers	0.5 (0.2-1.9)
					Fathers	1.1 (0.8-1.5)
				Scientific and artistic	Mothers	0.6 (0.1-3.7)
					Fathers	0.9 (0.4-1.9)
				All occupations	Mothers	0.8 (0.6-1.2)
				Auto mechanic, machinist, gas station attendant, and miner	Fathers	0.8 (0.4-1.7)
				Painter, cleaner, and dyer	Fathers	1.3 (0.4-4.0)
				Petroleum and chemical industry worker	Fathers	2.0 (0.5-8.0)
				Pharmacist, printer, and chemical analyst	Fathers	2.0 (0.5-8.0)
				Hydrocarbon-related occupations	Fathers	1.2 (0.7-2.0)
				Hydrocarbon-related occupations	Mothers	1.0 (0.2-4.7)
				Occupational exposures which may include solvents Pregnancy		Relative Risk
				Pigments (dyes)	Mothers	1.8 (0.9-3.6)
					Fathers	1.6 (0.8-3.3)
				Chemicals	Mothers	2.4 (1.2-4.6)
					Fathers	1.2 (0.8-1.7)

				Plastic or rubber	Fathers	2.0 (0.9-4.0)
				Cleaning products	Mothers	1.9 (0.6-5.8)
					Fathers	1.4 (0.7-2.8)
Shaw et al (1984)[32]	Case-Control 255 pediatric leukemia cases from the California Tumor Registry and 510 matched controls from California, United States	**Strengths:** large sample size; no recall bias leading to differential misclassification; tested several potential childhood leukemia risk factors **Limitations:** did not interview the parents; potential selection bias; broad occupational categories**;** did not look at maternal occupations or exposures (lacked data); time windows are not well defined	Paternal occupations with exposures obtained from birth certificates and the occupational classification by the National Occupational Hazard Survey	Exposure to benzene	Fathers	Percentage exposed Cases: 0.76 % Controls: 0.75 % (p value: 0.75)
				Occupation: Precision production and craft repair	Fathers	Cases: 19.2% Controls: 18.2% (p value>0.05)
				Occupation: Operators, fabricators, and laborers	Fathers	Cases: 22.4% Controls: 21.0% (p value>0.05)
Vianna et al (1984)[27]	Case-Control 60 infant acute leukemia cases (diagnosed before the age of 1) selected from the Tumor Registry of the New York State Health Department and 120 matched controls from birth certificate information All subjects are from New York State (excluding New York City)	**Strengths:** trained interviewers and face to face interviews; precise outcome definition, limited to a narrow leukemia subtype; good characterization of exposure – separated by dosage; good time window specfication **Limitations:** very small sample size; no specific chemical exposures, only occupations; no maternal occupational data; potential recall bias leading to differential misclassification of exposures; proxy respondents for fathers; unclear if there was blinding to outcome	Paternal occupational information: full-time work in a specific occupation for **at least one year prior to the year of birth** of the affected offspring Two occupational groups, one of which is relevant here because these occupations also lead to exposures to many organic solvents 1) **heavily exposed to exhaust fumes (gas station attendants, automobile or truck repairmen, aircraft maintenance personnel)**	Heavily exposed group	Father	2.43 (binomial probability 0.032)

Hemminki et al (1981)[25]	Case-Control 1959-65: 829 case-mothers and 1459 referents 1969-75: 700 case-fathers and 1182 referents Cases were children diagnosed with childhood cancers and healthy controls were selected from within the same maternity welfare district in Finland	**Strengths:** no differential recall bias due to exposure status recorded before outcome; first study to examine maternal occupations and childhood cancers **Limitations:** no specific information on chemicals; heterogeneous occupational groups; unclear reporting for sample size	Occupations of parents at the time of **pregnancy,** taken through records of the welfare centers attended by mothers. Case children are divided into 3 time periods (from 1959 to 68, 1969 to 75 and overall 1959 to 75)	Offspring 1959-1968		Odds ratio for all **childhood cancers**
				Technical, scientific, humanistic work	Mothers	1.05 (p>0.10)
					Fathers	1.34 (p>0.10)
				Agriculture, gardening, forestry	Mothers	0.80 (p>0.10)
					Fathers	1.42 (p<0.01)
				Industrial, construction work	Mothers	1.18 (p>0.10)
					Fathers	0.71 (p<0.01)
				Mining	Fathers	3.00 (p>0.10)
				Women on farms	Mothers	1.32 (p<0.05)
				Offspring 1969-1975		Odds ratio for all **childhood cancers**
				Technical, scientific, humanistic work	Mothers	0.94 (p>0.10)
					Fathers	0.89 (p>0.10)
				Agriculture, gardening, forestry	Mothers	2.16 (p<0.10, >0.05)
					Fathers	1.05 (p>0.10)
				Industrial, construction work	Mothers	0.93 (p>0.10)
					Fathers	0.96 (p>0.10)
				Mining	Fathers	
				Women on farms	Mothers	1.00 (p>0.10)
				Offspring 1959-1975		Odds ratio for all **childhood cancers**
				Technical, scientific, humanistic work	Mothers	0.97 (p>0.10)
					Fathers	1.00 (p>0.10)
				Agriculture, gardening, forestry	Mothers	1.73 (p>0.10)
					Fathers	1.19 (p>0.10)
				Industrial, construction work	Mothers	1.03 (p>0.10)
					Fathers	0.85 (p<0.05)
				Mining	Fathers	0.84 (p>0.10)
				Women on farms	Mothers	1.05 (p>0.10)

				Offspring 1969-1975		Odds ratio for **leukemias**
				Motor vehicle drivers	Fathers	1.90 (p<0.05)
				Painters	Fathers	2.67 (p>0.10)
				Factory worker	Mothers	1.33 (p>0.10)
				Offspring 1959-1975		Odds ratio for **leukemias**
				Farmers	Fathers	1.26 (p>0.10)
				Motor vehicle drivers	Fathers	1.50 (p<0.10, p>0.05)
				Machine repair men	Fathers	0.25 (p<0.05)
				Painters	Fathers	1.50 (p>0.10)
Sanders et al (1981)[31]	In total there were 4395 and 2525 children dying from neoplasms in England and Wales during two time periods 1) 1959-62 2) 1970-72, respectively and 112 840 and 54 806 children dying from all causes in the same time periods	**Strengths:** many subjects for every occupational group; looked at hydrocarbon related occupations; no differential recall bias due to objective exposure measurement **Limitations:** no face to face interviews; time windows unclear; potential for misclassification and selection bias; difficult to compare their association measure to other published results; outcomes are quite broad and limited to dying children, do not include survivors	Paternal occupations recorded on death certificates of offspring – reflecting postnatal exposures *Only reporting the occupations where organic solvent exposure is more likely	Deaths from neoplasms between 1959 and 1963	Fathers	Estimates measured in **PMR** (>100 = association). See paper for additional details. †: significant result
				Gas, coke and chemical		143 (p-value<0.05) †
				Glass and ceramics		102 (p-value>0.05)
				Woodworkers		96 (p-value>0.05)
				Leather workers		87 (p-value>0.05)
				Paper workers		119 (p-value>0.05)
				Painters and decorators		97 (p-value>0.05)
				Professional, technical workers, artists		141 (p-value<0.05) †
				Leukemia outcome between 1959 -1963		
				All hydrocarbon related occupations		95 (p-value>0.05)
				Deaths from neoplasms between 1970 and 1972		
				Gas, coke and chemical		87 (p-value>0.05)
				Glass and ceramics		98 (p-value>0.05)
				Woodworkers		94 (p-value>0.05)
				Leather workers		71 (p-value>0.05)
				Paper workers		99 (p-value>0.05)
				Painters and decorators		74(p-value>0.05)

				Professional, technical workers, artists		137 (p-value<0.05) †
				Leukemia outcome between 1970 -1972		
				All hydrocarbon related occupations		92 (p-value>0.05)
Zack et al (1980)[33]	Case-Control 296 pediatric cancer cancer cases, 296 fathers and 298 mothers (includes step-parents) 283 hospital control fathers and 282 hospital control mothers, 413 uncles and 425 aunts of the cases, and finally 228 neighbor fathers and 237 neighbor mothers from Texas, United States	**Strengths:** used face-to-face or telephone interviews; had many controls**;** performed some analyses on cancer sub-types; collected data in both parents **Limitations:** small sample size; looked at broad categories of exposures; potential for misclassification bias; for some occupational analyses there were no reported p-values or confidence intervals	Occupational history was collected a year before the birth of the affected child to a year before the child's diagnosis There were three different control groups used for paternal analyses: (1) uncles of cases (2) male neighbors of cases (3) fathers of controls ** narrow definition of hydrocarbon exposures includes the following occupations: machinist and apprentice, automobile mechanic and apprentice, painter and apprentice, dyer, gas station attendant, laundry operative, mine operative and lumberman. * broad definition adds on top of what is mentioned above, other vehicle mechanics, other painters and all other persons classified	**Leukemias**		
				Motor vehicle mechanic, service station attendant		
				Control Group 1		0.71
				Control Group 2		0.75
				Machinist, miner, lumberman		
				Control Group 1		0.81
				Control Group 2		1.51
				Other Blue Collar		
				Control Group 1		1.21
				Control Group 2		1.32
				All cancers		
				Hydrocarbon related occupations (broad definition)*		
				Control group 1		1.10 (0.70-1.73)
				Control group 2		1.08 (0.64-1.82)
				Control group 3		0.98 (0.61-1.58)
				Hydrocarbon related occupations (narrow definition)**		
				Control group 1		0.93 (0.44-1.96)
				Control group 2		1.33 (0.52-3.44)
				Control group 3		0.50 (0.25-1.02)
				Petroleum exposure		
				Control group 1		1.58 (0.85-2.94)

			under mining, lumber, wood products, laundering and gasoline service stations	Control group 2		0.72 (0.39-1.32)
				Control group 3		0.87 (0.47-1.58)
				Chemical exposure		
				Control group 1		0.95 (0.50-1.79)
				Control group 2		1.01 (0.48-2.12)
				Control group 3		1.09 (0.53-2.22)
				Both petroleum and chemical		
				Control group 1		1.24 (0.79-1.96)
				Control group 2		0.81 (0.50-1.32)
				Control group 3		0.95 (0.59-1.53)
Hakulinen et al (1976)[29]	Case-Control 339 pediatric cases of leukemias and lymphomas and 339 matched controls from Finland between 1959 and 1968	**Strengths:** large sample size with patients selected from a large study base (Finnish Cancer Registry); used incident cases of cancer; exposure assessment done before diagnosis, eliminating recall bias **Limitations:** outcomes and occupations grouped into broad categories; no specific information on chemical exposures; selection bias due to incomplete records of older patients	Paternal occupations during the **first trimester of pregnancy / preconception** **Group 1 occupations**: motorvehicle mechanics, machinists and miners **Group 2 occupations:** painters, dyers and printers **Group 3 occupations:** motorvehicle mechanics and motorvehicle drivers	Children less than 5 yrs old	Fathers	Risk Ratios
				group 1 and group 2	0.33	(0.01-4.2)
				group 3	0.74	(0.34-1.6)
				group 1, 2 and 3	0.68	(0.33-1.4)
				Children less than 15 yrs old	Fathers	Risk Ratios
				group 1 and group 2	0.50	(0.11-1.9)
				group 3	1.06	(0.63-1.8)
				group 1, 2 and 3	0.95	(0.57-1.6)