The etiology of hematologic malignancies in children and adolescents: Pre- and postnatal factors

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Amy Marie DeVries Linabery

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Julie A. Ross

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Dedication

This dissertation is dedicated to all of the infants, children, and adolescents who are dealt the blow of a cancer diagnosis and to their families and caretakers. May you prevail and find some comfort in knowing that others are working to identify the causes of and ultimately to prevent these devastating and debilitating diseases.

Abstract

Little is known about the etiology of most pediatric hematologic malignancies, although there is evidence for prenatal initiation of leukemogenesis for many cases. The current body of research, a series of three complementary studies, evaluated the potential for unbiased measurement of prenatal exposures through retrieval of existing biospecimens and examined associations between pre- and postnatal exposures and pediatric/adolescent leukemia.

The first study assessed the feasibility of retrospective collection of residual neonatal blood spots (NBS) for 947 childhood/adolescent leukemia and lymphoma cases from state newborn screening programs nationwide. Biological mothers were also asked to complete self-administered questionnaires regarding prenatal exposures, personal and family history of atopic disease, and selected demographic factors. Overall, 37% of families provided consent for NBS release and 41% of mothers completed questionnaires. Consenting cases were born in 39 states and 46 NBS were obtained from 5 states (CA, NY, MI, TX, and WA). NBS storage/release policies are rapidly evolving; requests are pending in states involved in litigation (MN), reviewing policies (NJ), and reviewing this study (MA). Currently, population-based NBS studies can be conducted in a limited number of states; fortunately, most of these have large populations to provide reasonable pediatric case and control groups.

In the second study, the largest of its kind, the association between self-reported prenatal vitamin supplementation and infant leukemia was examined, since folic acid is postulated to play a preventative role in the pathogenesis of childhood leukemia,

particularly among ALL cases. After adjustment for race/ethnicity and income, there was little evidence supporting associations between periconceptional vitamin use (OR = 0.89, 95% CI: 0.64-1.24), use after knowledge of pregnancy (OR = 0.78, 95% CI: 0.48-1.28), or use in all periods (OR = 0.84, 95% CI: 0.62-1.14) and infant leukemia. These results may be attributable to high rates of folic acid supplementation in the study population, including personal vitamin use and national folic acid fortification programs implemented in the U.S. and Canada early in the study period.

Atopic disease is hypothesized to be protective for several malignancies. In the third study, meta-analysis was performed to summarize and quantify the risk of acute leukemia associated with atopic disease in children and adolescents and to identify sources of heterogeneity in the existing literature. Inverse associations were observed for ALL and atopy overall (OR = 0.69, 95% CI: 0.54-0.89)), and for asthma (OR = 0.79, 95% CI: 0.61-1.02), eczema (OR = 0.74, 95% CI: 0.58-0.96), and hay fever (OR = 0.55, 95% CI: 0.46-0.66) examined separately. ORs for ALL differed across strata of study design, exposure data source, and latency period, indicating these factors impact study results. Although these results should be interpreted cautiously given the modest number of studies, substantial heterogeneity, and potential exposure misclassification, they are useful in designing future research.

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List of Abbreviations

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
ANLL	acute non-lymphoblastic leukemia
APC	average annual percent change
ART	assisted reproductive technology
BDNF	brain-derived neurotrophic factor
BHMT	betaine-homocysteine methyltransferase
BMI	body mass index
bp	base pair
cALL	common acute lymphoblastic leukemia
CBS	cystathionine-β-synthase
CCG	Children's Cancer Group
CCRN	Childhood Cancer Research Network
CDC	Centers for Disease Control and Prevention
CI	confidence interval
COG	Children's Oncology Group
CPRC	Cancer Protocol Review Committee
CRP	C-reactive protein
CSLI	Clinical and Laboratory Standards Institute
DAG	directed acyclic graph
DHFR	dihydrofolate reductase
DHMH	Department of Health and Mental Hygiene
DNA	Deoxyribonucleic acid
DSB	double strand break
dUMP	deoxyuridine monophosphate
EBV	Epstein-Barr Virus
FE	fixed effects
FISH	fluorescent in situ hybridization

FROGS	Feasibility of Retrospectively Obtaining Guthrie Spots
HAMP	hepcidin antimicrobial peptide
HFE	hereditary hemochromatosis gene
HL	Hodgkin lymphoma
HLA	human leukocyte antigen
HMO	health maintenance organization
HR	homologous recombination
ICD-O	International Classification of Diseases for Oncology
IFN	interferon
IgE	immunoglobulin E
IGF	insulin-like growth factor
IL	interleukin
IRB	institutional review board
IRR	incidence rate ratio
ISAAC	International Study of Asthma and Allergies in Childhood
MeSH	medical subject headings
MLL	mixed lineage leukemia
MMP	matrix metalloproteinase
MTHFD1	5,10-methylenetetrahydrofolate dehydrogenase
MTHFR	5,10-methylenetetrahydrofolate reductase
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
MTRR	5-methylenetetrahydrafolate-homocysteine methyltransferase reductase
NA	not available
NBS	neonatal blood spot
NCCLS	National Committee on Clinical Laboratory Standards
NHANES	National Health and Nutrition Examination Survey
NHEJ	non-homologous end joining
NHIS	National Health Interview Survey
NHL	non-Hodgkin lymphoma

NIH	National Institutes of Health
NNMT	nicotinamide N-methyltransferase
NNSIS	National Newborn Screening Information System
NT	neurotrophin
NTD	neural tube defect
OR	odds ratio
PKU	phenylketonuria
PON1	paraoxonase 1
PRAMS	Pregnancy Risk Assessment Monitoring System
RDD	random digit dialing
RE	random effects
RFC1	reduced folate carrier
RR	rate ratio
RT-PCR	reverse transcription polymerase chain reaction
SAM	S-adenosylmethionine
SE	standard error
SEER	Surveillance, Epidemiology, and End Results
SES	socioeconomic status
SHMT1	hydroxymethyltransferase
SLC19A1	solute carrier family 19 member 1
SNP	single nucleotide polymorphism
SSB	single strand break
TCN2	transcobalamin II
Th1	T helper 1 cell
Th2	T helper 2 cell
TMP	thymidine monophosphate
TNF	tumor necrosis factor
TREM	triggering receptor expressed on myeloid cells
TSH	thyroid stimulating hormone

TYMS	thymidylate synthase
UKCCS	United Kingdom Childhood Cancer Study
UTR	untranslated region

Chapter 1: Overview of hematologic malignancies in children/adolescents

Childhood cancers are the leading cause of disease-related mortality among those aged 1-19 years.[1] The American Cancer Society estimated that 10,730 U.S. children ages 0-14 years would develop cancer in 2009 and 1,380 children would die of the disease.[2] Leukemia is the most common childhood malignancy, with an estimated annual incidence of 42 cases per 1,000,000 persons for those diagnosed at ages 0-19 years; the incidence has been increasing in recent years (average annual percent change 1992-2004: 0.7%; 95% confidence interval: -0.1-1.5%)[3] Childhood lymphomas are the third most common malignancy (22 cases/1,000,000 persons)[3] following tumors of the central nervous system; however, relatively little research has been dedicated to the study of the determinants of childhood lymphoma. These hematologic cancers may share similar etiologic factors, particularly T-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL), which have identical histologies.[4]

There is important evidence in support of an *in utero* initiation of most childhood leukemias. One significant line of evidence establishing the prenatal origins of leukemia has been the detection of common chromosomal translocations present in neonatal blood spots (NBS) collected at birth from affected children, including t(4;11) (MLL-AF4) in infant ALL cases,[5] t(12;21) (TEL-AML1) in ALL in children diagnosed at 2-5 years, and t(8;21) (AML1-ETO) in childhood acute myeloid leukemia (AML) cases.[6] These translocations may constitute a first genetic hit in a multistep pathway,[6] which may involve subsequent hits resulting from exposure to endogenous or exogeneous factors. Of note, TEL-AML1 translocations have been reported at a rate of 1% in a series of cord

blood samples, which is 200-fold greater than the rate of leukemia in the general U.K. population,[7] supporting a multifactorial model of causation. The researchers further estimate that if all pediatric preleukemic genetic insults were surveyed, they would cumulatively be detected in approximately 5% of children.[7] Other compelling evidence includes the results of twin studies, indicating nearly complete concordance for infant leukemia among monochorionic monozygotic twins (~100%) and a concordance rate of ~10% among older children and adolescents,[8] which is much greater than would be expected by chance.

Knudson originally presented the concept of a two-step model for carcinogenesis with respect to childhood retinoblastoma in 1971.[9] Greaves adapted this model to pediatric leukemia and suggested a minimum of two hits, one occurring prenatally and the other after birth, are required for leukemic transformation.[8] A proposed multi-step model for pediatric leukemogenesis is depicted in Figure 1-1 below. An *in utero* genetic event, such as an *MLL* rearrangement in infant leukemia, another chromosomal translocation (t(12;21) or t(8;21), for example), or hyperdiploidy may constitute the first hit.[10] In the case of a two-hit model, only one additional mutation in a hematopoietic cell is required for leukemia development. Environmental exposures (yet to be identified, see below) may play a role in leukemogenesis as additional 'hits'.

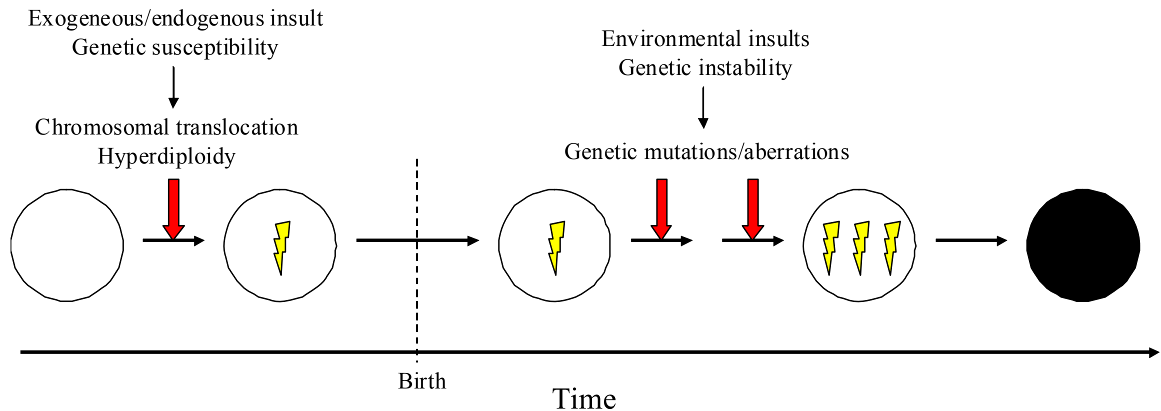

Figure 1-1. Theoretical model for the development of childhood hematologic

The etiology of acute leukemia is likely attributable to an underlying genetic susceptibility coupled with endogenous or exogenous exposures. Greaves' Hypothesis, for example, postulates that at least 2 spontaneous mutations are involved in leukemogenesis of common B-cell ALL occurring in early childhood.[11] According to Greaves, the first genetic mutation occurs randomly *in utero* as a result of the high rate of precursor B cell proliferation and the second occurs postnatally, due to an abnormal response to delayed exposure to a common infectious agent resulting in increased precursor B cell proliferation.[11, 12] He suggests that other immune-related factors, such as breastfeeding, exposure to infectious agents during infancy (via older siblings, crowding, daycare attendance, or lack of hygiene), vaccinations, and HLA type (or other genetic factors affecting susceptibility), may modulate this relationship [11, 12]

There are few established causal factors for pediatric hematologic malignancies.[4] Risk factors for childhood leukemia include predisposing genetic conditions, such as Down syndrome (OR ~10-20),[13] neurofibromatosis type I,[14] and Fanconi anemia;[15] exposure to *in utero* (RR ~ 1.4)[16] or postnatal therapeutic irradiation;[17-19] and exposure to

chemotherapeutic agents, such as alkylating agents[20] or epidopophyllotoxins.[21, 22] There is also reasonable evidence for an association with high birthweight,[23] although the underlying mechanism is yet to be unveiled.

Relatively little research has been dedicated to the study of the determinants of childhood lymphoma; the only established risk factor for Hodgkin lymphoma (HL) is a family history of HL[24, 25] and for NHL, the known risk factor is immune deficiency, such as that due to therapeutic immune suppression,[26] acquired immune deficiency syndrome (AIDS),[27] or congenital immunodeficiency syndromes (e.g., ataxia telangiectasia).[28] There is convincing evidence for infection with Epstein-Barr virus (EBV) as an underlying cause of both diseases,[29, 30] although EBV infection likely accounts for a small proportion of cases in the United States.

Factors warranting further investigation include *in utero* exposure to vitamins (folic acid),[31] alcohol,[32] and tobacco smoke,[32] maternal use of fertility or other hormone treatments,[33] and a personal or family history of atopic disease.[34-38] These exposures were explored in the current body of research, a series of three complementary studies. The first study examined the feasibility of retrospective collection of residual neonatal blood spots for childhood cancer patients on a nationwide basis. These blood spots are of interest because they may provide a unique opportunity for the unbiased measurement of *in utero* exposures. In the second study, the association between prenatal vitamin supplementation, as measured via maternal self-report, and infant leukemia was examined, since folic acid is postulated to play a preventative role in the pathogenesis of childhood leukemia, particularly among ALL cases.[39-42] The third study is a meta-

analysis with the purpose of summarizing and quantifying the risk of acute childhood leukemia associated with atopic disease. Five of six previously identified studies suggested an inverse association between any atopy and childhood ALL, for example.[34-38, 43]

Chapter 2: Review of the literature for the Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study

Descriptive epidemiology

Pediatric hematologic malignancies, although rare, comprise a substantial proportion of childhood morbidity and mortality. Childhood leukemia is the most common malignancy among children ages 0-19 years, with an estimated annual incidence of 42 cases per 1,000,000 persons; the incidence has been increasing in recent years (average annual percent change $(APC)_{1992\text{-}2004}$: 0.7%, 95% confidence interval (CI): -0.1-1.5%).[3] Childhood lymphomas, including Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), collectively represent the third most common malignancy (11.7 cases/1,000,000 persons and 10.4 cases/1,000,000 persons, respectively); the rate of NHL also appears to be rising over time ($APC_{1992\text{-}2004}$: 1.4%, 95% CI: -0.1-3.0%).[3] These hematologic cancers may share similar etiologic factors, particularly T-cell acute lymphoblastic leukemia (ALL) and NHL, which have identical histologies.[4]

Children's Oncology Group

The rarity of childhood cancers necessitates a cooperative, multi-center approach to research. The Children's Oncology Group (COG) is a cooperative trials group consisting of 220 institutions in the U.S., Canada, Australia, New Zealand, Switzerland, and the Netherlands; the majority (87%) of these are U.S. Institutions. An estimated 85% of childhood and 44% of adolescent leukemia cases and 70% of childhood and 27% of adolescent lymphoma patients are registered with COG in the U.S.[44] COG and its

predecessors (Children's Cancer Group (CCG) and Pediatric Oncology Group (POG)) have been credited with many therapeutic advances in pediatric oncology, owing to the conduct of Phase III randomized trials, which allow the safety and efficacy of novel therapeutic modalities to be investigated in reference to standard treatments on a larger scale than would otherwise be feasible. Oversight of these studies permits gatekeeping, such that successful therapies are retained and those deemed ineffective or harmful are abandoned, ultimately leading to better outcomes for affected patient populations.[45]

The COG Epidemiology Steering Committee is responsible for the conduct of many of the largest etiologic studies of pediatric malignancies in North America.[46] The committee was also instrumental in the implementation of the Childhood Cancer Research Network (CCRN) on December 24, 2007, which should prove a nearly population-based childhood cancer registry in the U.S.[47] Participating COG institutions are required to approach/inform all parents of childhood cancer patients about the CCRN, regardless of their participation in clinical trials. Parents (and children ages ≥18 years) are asked to choose one of three participation levels: (1) no participation (information compliant with the Health Insurance Portability and Accountability Act is collected), (2) registration with CCRN, including personal identifiers, but no future contact regarding future COG-approved non-therapeutic studies, or (3) registration with CCRN and agreement to future contact.

The current study utilized the dataset assembled in COG AADM01P1 - Protocol for Registration and Consent to the Childhood Cancer Research Network (CCRN): A Limited Institution Pilot (Principal Investigator: J. Ross) for identification of potential

participants. Through supplemental funds to the COG Chairman's grant, this pilot protocol (AADM01P1) was developed and activated at a 10% (n = 23; see Appendix A) random sample of COG institutions in North America.[47] The protocol was opened on May 1, 2001, and by March 2002 all 23 institutions had obtained IRB approval to enroll patients. The protocol involved two upfront consents for parents (and children, if age eligible). The first consent allowed name and contact information to be released to the COG registration system. In addition to this first consent, the second consent involved agreement to be potentially contacted in the future to consider taking part in a non-therapeutic study. These future studies would then be separately consented by the investigators conducting the study. As of January 19, 2007 (pilot study close), 2,233 individuals among the 23 institutions were approached. Of these, 2,136 (96%) agreed to both levels of consent, while 70 (3%) agreed to release of name and contact information only. Only 27 (1%) refused both consent levels.

What are neonatal blood spots (NBS)?

The concept of neonatal blood spot screening was introduced by Dr. Robert Guthrie in the 1960s.[48] Dr. Guthrie advocated that neonatal blood could be collected from heelsticks, spotted uniformly onto heavy, absorbent filter paper, dried, and subsequently tested for phenylketonuria (PKU), the most well-known of the inborn errors of metabolism, on a population scale.[48] The filter paper forms currently used in the collection of dried blood spots are commonly called "Guthrie cards" and the NBS are also termed "Guthrie spots" in recognition of their earliest proponent. A diagram of a Guthrie card used by the state of Michigan is shown in Figure 2-1 below.

Figure 2-1. Guthrie card used by the Michigan Department of Community Health newborn screening program.[49]

Today, over 40 years after the initial introduction by Guthrie, blood spots are collected from a vast majority of neonates (>95%)[50] in the U.S. according to a standard protocol[51] to test for a panel of inborn errors of metabolism. The former National Committee on Clinical Laboratory Standards (NCCLS, now the Clinical and Laboratory Standards Institute, CLSI) developed the widely accepted standard procedure for the collection of NBS.[51] The protocol is summarized in Appendix B. In 2008, approximately half of the NBS samples were obtained within 24-48 hours of birth, while an additional 34% were tested between days 3-7, and 3% were tested after day 7.[52] (The timeframe for testing was unknown for 13% of samples in the National Newborn Screening Information

System Database.[52] Of note, these data are incomplete and there are some redundancies because some states require multiple draws per individual.)

Screening programs have historically fallen under the jurisdiction of the individual U.S. states, resulting in a great deal of variability in the implementation of screening programs nationwide. Each state selects the tests to be performed, with a range of 28 blood tests mandated in Arizona to 54 tests in South Dakota as of December 2009.[53] Infants in all 50 states and the District of Columbia are tested for PKU as well as 21 other disorders (i.e., amino acid, endocrine, fatty acid, hemoglobin, and organic acid disorders, cystic fibrosis, transferase deficient galactosemia).[53] The consent process also varies by state. The majority of screening programs employ "opt out" or implied parental consent, while Maryland, Wyoming, and the District of Columbia use "opt in" consent.[54] Thirty-three states allow parents to refuse newborn screening for religious reasons and an additional 12 allow parents to refuse for religious or other reasons, while 5 states do not allow parents to opt out and 1 state (New Hampshire) does not have a statute governing refusal.[54] After testing, each state determines the fate of the unused NBS; as of 2006, 24 states choose to store the spots ≤6 months, 6 store them for 1 year, 7 reported storage of 2-7 years, 6 store them for 21-23 years, and 8 states store them indefinitely.[54] The NBS storage methods differ widely by state as well, as discussed below.

A federal newborn screening law (The Newborn Screening Saves Lives Act of 2007)[55] was passed on April 24, 2008, which includes provisions to standardize the tests performed nationwide and to conduct epidemiologic research on effective interventions

to prevent adverse effects of selected heritable disorders. This law should eradicate much of the interstate variation in screening in the future.

The COG Epidemiology Committee conducted a preliminary survey (led by L. Spector) of 48 state health departments (or equivalent) in 2006; results of this survey indicated that 12 states retained NBS for more than 5 years and were willing/able to release them for research purposes with written parental consent. These states included: California, Iowa, Maine, Michigan, Minnesota, New Mexico, North Carolina, North Dakota, New Jersey, Ohio, South Carolina and Washington. Current state storage and release policies are described in Table 3-3 of this document.

Significance of NBS

Although the etiology of the majority of childhood hematologic malignancies remains unknown, there is important evidence in support of prenatal origins. One significant line of evidence establishing the prenatal origins of leukemia has been the detection of common translocations present in NBS collected at birth from affected children, including t(4;11) (MLL-AF4) in infant ALL cases, t(12;21) (TEL-AML1) in ALL in children diagnosed at 2-5 years, and t(8;21) (AML1-ETO) in childhood AML cases, via polymerase chain reaction (PCR) technology.[6] These translocations may constitute a first genetic hit in a multistep pathway,[6] which may involve subsequent hits resulting from exposure to endogenous or exogenous factors. It is therefore of keen interest to further elucidate the potential role of *in utero* exposures in carcinogenesis.

Theoretically, any substrate that can be measured in whole blood, serum, or plasma can also be analyzed in NBS.[50] Accordingly, a long list of analytes have been

successfully extracted from blood spots and evaluated, such as amino acids, enzymes, human and viral DNA, antibodies, markers of inflammation, steroids, metals, protein adducts, and pesticides.[50, 56-58] A list of specific substrates that have been analyzed is provided in Appendix C.

Analysis of NBS for analyte levels, such as insulin-like growth factor I (IGF-I),[59] folate,[60] DNA adducts as markers of *in utero* exposure to alcohol or tobacco smoke,[61] or reproductive hormones,[62] would represent important methodological advancement in the study of childhood cancers. While analysis of IGF, folate, and gonadal hormones have been previously undertaken in blood spots obtained from other populations,[59, 60, 62] the analysis of DNA adducts has not been previously reported, to our knowledge. We are currently working in collaboration with Stephen S. Hecht, Ph.D., Program Leader of the Carcinogenesis and Chemoprevention Research Program at the University of Minnesota Cancer Center, to develop an assay for this purpose.[63-65] Further, in collaboration with Dr. Hecht, we have completed an analysis of cotinine levels in NBS, which provide a measure of tobacco exposure.[66]

Factors influencing NBS retrieval

In addition to state retention and release policies, other factors have been associated with the storage, release, and/or acquisition of NBS. Tarini *et al* conducted a nationally representative survey of 3,047 parents of children <18 years and observed that 78% of respondents would authorize NBS storage by state newborn screening programs for a period of time, 38% would agree to indefinite storage, and 76% would be willing to release their children's NBS for research if their permission was sought.[67] Only 10%

were very unwilling to release NBS for research. Respondents were considerably less amenable to research conducted without parental permission (28% were willing). The authors indicate that the definition of parental permission needs to be explored further; their results do not necessarily implicate a formal written consent process for each individual research project. They also reported that parents with children with very good to excellent health and those with a high school education or less were less likely to agree to NBS storage. Similarly, older parents and those with healthy children were less likely to permit future use in research. Notably, this survey was designed to assess parental attitudes and was hypothetical in that no consent forms or NBS were collected.

Loffredo and Ewing conducted a sub-study of the Baltimore-Washington Infant Study, in which they attempted to retrieve NBS from the Maryland Department of Health and Mental Hygeine (DHMH) from 522 cases with congenital heart defects and 1,645 representative controls born during the period 1981-1989.[68] Retrieval rates were significantly lower among cases (65%) and varied by type of cardiac abnormality (range: 26% of those with truncus arteriosus to 76% of those with atrial septal defects) compared with controls (84%). Among controls, 10% had no DHMH laboratory number available to link the participant with his/her blood spot and 5% had a lab number, but the blood spot was not located. Rates of retrieval were significantly lower among low birthweight infants (64% among those with a birthweight of <2500g vs. 85% among those with a birthweight of ≥ 2500g) and among those born before 38 weeks gestation (69% vs. 86% for ≥ 38 weeks). There were no significant differences in retrieval rates for other infant (race, gender, county of residence, household income) or maternal (age, parity, marital

status, educational attainment) characteristics examined. Of note, Maryland is one of the few states employing opt-in consent for screening.

Two small studies of childhood cancer conducted in New Jersey[69] and Washington state[70] have reported using the name of the mother (and the child in NJ), the child's date of birth, and the hospital of birth as matching variables in locating the NBS of childhood cancer cases and matched controls. One of these, a case-control study of childhood brain tumors, examined factors influencing the NBS retrieval; the value of these results is limited, however, given the small sample size in the unretrieved group (n = 13).[70]

NBS storage conditions

An important area of concern to researchers interested in the retrieval and analysis of NBS is the varied storage methodology employed by different states.[56] As recommended in the CSLI /NCCLS protocol, ideal storage conditions for NBS involve individual storage (along with a desiccant and humidity indicator card) at -20°C in sealed bags with low gas permeability; humidity should be maintained at <30%.[51] Maintaining these ideal conditions can be costly,[71] especially in states with high birth rates. Some states report storage at ambient conditions, in which temperature and humidity can fluctuate with seasons, while others report storage in a refrigerator (2-8°C) or freezer (-20 to -30°C), with or without desiccant.[71, 72] Storage in ambient conditions could result in denatured DNA and/or the breakdown of other analytes over time. Similarly, some states package the NBS individually, while other states store them in bulk, with adjacent Guthrie cards touching;[71] a concern of bulk storage is the potential for cross-

contamination across specimens.[73] Table 3-3 of this document lists current storage conditions.

In a series of two papers, Skogstrand *et al* investigated the effects of various NBS storage conditions on a panel of 25 markers of inflammation. In the first study, they observed that NBS stored at -24°C for 23 years had generally similar levels of cytokines as those stored 1 month or 3 years, respectively, with the exception of diminished levels of IL-1β, IL-8, sIL-6rα, MMP-9, TREM-1, CRP, BDNF, and NT-4.[74] The authors provide three possible explanations for the observed decreases, including (1) the analytes degraded over time, (2) the analytes were not extracted as efficiently from the older cards, or (3) secular increases in population levels of these analytes over time. In the second study, they compared storage at 4°C, room temperature, and 35°C for up to 30 days and found that storage at lower temperatures yielded better results overall.[58] Although there was some variation, cytokine levels were well preserved at the higher temperatures for ≤7 days as compared with those stored at -20°C immediately upon collection. Levels of IL-2 and IL-18 were greater in those stored at room temperature and 35°C, while reduced concentrations of IL-1B, IL-6, TNF-B and BDNF were measured in those stored at ≥4°C.

With respect to genotyping, Klotz *et al* reported that the ability to amplify and genotype DNA was robust to "simple storage" (storage in boxes without separators), but was not robust to extreme temperatures, as occurred in NBS collected in New Jersey prior to 1983.[69] They were able to genotype DNA from 97% of NBS stored after 1982 compared with 3% of those stored from 1979-1982. Conversely, Searles Nielsen *et al*

obtained genotype data from all stored NBS in Washington state, although some specimens had been stored in bulk up to 25 years without climate control.[70] Furthermore, they did not find evidence of cross-contamination.

Finally, McDade *et al* provided a summary of protocols for 45 NBS analytes most applicable to population-based health research, including the duration of stability for 31 substrates at both 4°C and room temperature.[57] They reported a stability range of 1 day to >3 months for both temperatures.

To investigate these storage concerns further, investigators in the University of Minnesota Department of Pediatrics, Division of Epidemiology and Clinical Research (L. Spector, S. Ognjanovic, and J. Ross) are currently establishing a pregnancy cohort (n = 500 women) in Minneapolis, Minnesota with the aim of testing different storage conditions reported by various U.S. states. Results from this comparative study will aid in the design of analytic methods of NBS collected in the current study.

Etiologic factors of interest

The relevant literature regarding etiologic factors of primary interest is summarized below. The summaries are not intended to provide an exhaustive review of the literature, but rather to justify the inclusion of related items in the questionnaire. As noted above, few etiologic studies of childhood lymphomas have been conducted, thereby limiting the summaries to leukemias for some factors.

<u>Birthweight.</u> High birthweight has been fairly well established as a risk factor for childhood leukemia. Upon pooling the results from 23 studies published through 2008,

Caughey and Michels reported a modestly higher risk of ALL associated with high birthweight (generally classified as birthweight >4000g) compared with normal birthweight (OR = 1.23, 95% CI: 1.15-1.32) and an OR of 1.18 (95% CI: 1.12-1.23) associated with each 1000g increment in birthweight.[75] The summary odds of AML from 7 studies for those with a high birthweight were 1.40-fold greater than those with a normal birthweight (95% CI: 1.11-1.76).[75] Insulin-like growth factor I (IGF-I) [76] and thyroid stimulating hormone (TSH)[77] have been suggested as possible mediators of the observed association.

The majority of studies evaluating birthweight and childhood leukemias and lymphomas, lymphomas, or HL or NHL examined separately have found no evidence of an association.[78-82] Studies conducted in the U.S. and Greece reported increased risk associated with higher birthweight for NHL (US: $OR_{>4000g}$ = 1.5, 95% CI: 1.0-2.4; Greece: $OR_{500g\ increment}$ = 1.42, 95% CI: 1.04-1.92), but found no association with respect to HL.[83, 84] Conversely, a German study observed an elevated OR for NHL for <u>low</u> birthweight ($OR_{<2500g}$ = 2.3, 95% CI: 1.2-4.3), but no association with high birthweight.[85]

<u>Maternal pre-pregnancy weight/weight gain during pregnancy.</u> Hematologic malignancies are thought to be initiated *in utero*, implicating the maternal environment in etiology. A case-cohort analysis of leukemia and perinatal factors from the state of New York reported complex results.[86] Maternal pre-pregnancy weight was not statistically significantly associated with ALL in offspring, although there was evidence of a monotonic dose response overall (p_{trend} = 0.03) and among those <5 years at diagnosis

(p_{trend} = 0.002). A pre-pregnancy body mass index (BMI) of >25 kg/m^2 (compared to BMIs of 20-24 kg/m^2) was associated with 44% increased odds of ALL diagnosed at <5 years (95% CI: 3-101%). There was evidence of an interaction between maternal pre-pregnancy weight and offspring birthweight, such that the presence of either high birthweight or high pre-pregnancy weight, but not both, increased the risk of ALL by 26% or 72%, respectively. High maternal weight gain (>14 kg) during pregnancy was associated with a 40% increased risk of ALL ($OR_{14.1\text{-}18.1\ kg}$ = 1.42, 95% CI: 1.04-1.94; $OR_{\geq 18.6\ kg}$ = 1.38, 95% CI: 0.99-1.94). For AML, there was no association observed for maternal pre-pregnancy weight or elevated weight gain during pregnancy.[86] Conversely, a case-control study in Washington state found little evidence of an association between childhood leukemia and maternal pre-pregnancy weight or pregnancy weight gain, respectively.[87]

Maternal exposure to fertility/hormone treatments. This exposure is of interest due to the rapid secular increases in use of assisted reproductive technologies (ART) over the past decade.[88] A meta-analysis examining the incidence of childhood cancers following ART did not provide evidence of an association upon pooling of 11 cohort studies (OR = 1.33, 95% CI: 0.62-2.85).[89] In the initial report on this topic, von Steensel-Moll *et al* found a suggested increased risk of ALL associated with a history of fertility problems (OR = 6.0, 95% CI: 0.9-38.2) and a positive association with use of drugs to maintain pregnancy (OR = 1.9, 95% CI: 1.0-3.5).[90] Roman *et al* observed a nonsignificant increased odds of childhood leukemias for mothers ever treated hormonally for infertility (OR = 2.5, 95%

CI: 0.7-9.3) and for those treated with the index pregnancy (OR = 2.7, 95% CI: 0.6-11.9).[81] Schüz *et al* also reported an increased risk of acute leukemia (OR = 1.6, 95% CI: 1.0-2.5), but not NHL (OR = 0.9, 95% CI: 0.4-1.9), associated with maternal hormonal treatment for infertility.[85] Finally, Kobayashi *et al* found a statistically significantly higher number of lymphoma cases (compared with other cancers) in mothers undergoing ovulation induction (2/517, p = 0.013).[91] Of note, all studies conducted to date are limited by small numbers of cases.

Maternal vitamin supplementation. Of the 7 studies examining maternal vitamin supplementation and childhood ALL, 4 reported significant inverse effects in the range $0.40 \leq OR \leq 0.84$,[39-42] while another reported a nonsignificant inverse association[92] and 2 reported associations near the null.[93, 94] Upon pooling of 3 studies, the fixed effects summary odds ratio computed by Goh *et al* indicated a significant reduction associated with childhood ALL (OR = 0.61, 95% CI: 0.50-0.74).[31] Dockerty *et al* did not find evidence of an association with ALL in pooling results from 3 different studies (OR = 0.9, 95% CI: 0.8-1.1).[93] For AML (and acute non-lymphocytic leukemia, ANLL), each of the 3 identified studies failed to find evidence of an association with maternal prenatal vitamin supplementation.[39, 40, 95] (A more thorough literature review is provided in Chapter 4 and Appendix H of this document.)

Schüz *et al* reported an inverse association between maternal vitamin, folate and/or iron supplement use during pregnancy and NHL in offspring (OR = 0.68, 95% CI: 0.48-0.97).[39]

Maternal prenatal alcohol exposure. The literature on the association between maternal prenatal alcohol use and childhood leukemias and lymphomas has been inconsistent. Of the 11 studies evaluating maternal prenatal alcohol consumption and leukemia or ALL, 5 observed no evidence of an association,[38, 85, 90, 96, 97] and 3 found significantly increased risk associated with any use during pregnancy, with ORs in the range of 1.4-2.0.[98-100] A fourth case-control study conducted in France reported a nonsignificant association for any alcohol use during pregnancy (OR = 1.3, 95% CI: 0.8-2.0), but observed a positive association with >1 glass/day (OR = 2.8, 95% CI: 1.3-5.9).[101] Conversely, 2 other studies observed inverse ORs of 0.57 (95% CI: 0.34-0.95)[102] and 0.7 (95% CI: 0.5-0.9),[103] respectively. Of note, Infante-Rivard reported inverse associations with each of the individual exposures examined, including stratification by trimester, type of alcohol (wine, beer, spirits) and frequency of use (<1 drink/day, ≥1 drink/day).[103]

Of the 7 studies investigating the association with AML, 3 failed to find evidence of an association,[97, 99, 100] while 3 found evidence of a sizable positive association with ORs of 2.4 (95% CI: 1.3-4.5)[96], 1.9 (95% CI: 1.0-3.6),[98] and 2.6 (95% CI: 1.2-5.8),[101] respectively, and a fourth reported a nonsignificant positive association.[104] The positive associations were observed consistently upon stratification by frequency of use in one study,[96] by trimester, type of alcohol (wine, beer, liquor), and frequency of use in the second,[98] and by rate of daily use in the third.[101]

Two studies evaluating maternal alcohol consumption during pregnancy with respect to leukemia and lymphomas combined did not find evidence of an association.[78, 105] A study of NHL, however, reported a nonsignificant inverse association for exposure

to 1-7 glasses per week of beer, wine or strong liquor during pregnancy (OR = 0.8, 95% CI: 0.6-1.2).[85]

Maternal prenatal tobacco smoke exposure. A meta-analysis of studies exploring the association between maternal tobacco smoking and childhood hematologic malignancies was published in 2000.[106] The pooled ORs calculated in that meta-analysis did not provide evidence of increased risk of leukemia and lymphomas combined (9 studies, OR = 1.03, 95% CI: 0.90-1.17), leukemias, acute leukemias, and ALL combined (8 studies, OR = 1.05, 95% CI: 0.82-1.34) or lymphomas and NHL combined (6 studies, OR = 1.13, 95% CI: 0.85-1.49). Several studies have been published since that meta-analysis was conducted. For acute leukemia, an additional 10 studies found no association,[85, 99-101, 107-112] while a large cohort study in Sweden computed an inverse association between maternal smoking in the first trimester and ALL (HR = 0.73, 95% CI: 0.58-0.91), but reported a nonsignificant positive association with AML (HR = 1.41, 95% CI: 0.74-2.67).[113] The association with AML was attributable to those smoking ≥10 cigarettes per day (HR = 2.20, 95% CI: 1.00-4.83).

For lymphomas, there was one additional report of no association [113] and one study reporting a positive association between maternal consumption of >20 cigarettes per day during pregnancy and childhood NHL (OR = 5.2, 95% CI: 1.2-22.4).[85] The latter estimate was based on small numbers; no association was observed for lower rates of maternal smoking in that study.

Atopy. Six case-control studies have explored the possible relationship between atopic disease (i.e., asthma, eczema, hay fever, hives) and the development of childhood ALL; five of these reported inverse relationships with ORs in the range of 0.3-0.7, [34-38] while one found a 2.2-fold increased risk associated with atopy.[43] Two studies reporting on AML failed to find an association; these studies included very small numbers of cases.[34, 36] (A more thorough literature review is provided in Chapter 6 of this document and a meta-analysis is provided in Chapter 7.)

Significance of the research

The primary aim of the current study is to assess the feasibility of obtaining NBS from childhood cancer cases on a nationwide basis. NBS are a rich resource, in that they are collected uniformly from nearly all children in the United States at birth for the purpose of testing for inborn errors of metabolism and other disorders,[50, 51, 114] and therefore, constitute the only consistent source of prediagnostic biological specimens existing for children with cancer. A significant limitation faced by pediatric cancer epidemiologists conducting case-control studies is the issue of recall bias. NBS may represent an unbiased record of an individual's *in utero* biology, including exposure to endogenous and exogenous factors, since they are collected at the time of birth. Some states retain residual blood spots for a period of 5 years or more, potentially permitting their use in etiologic research.[71]

To achieve the study aims, mothers were asked to complete a self-administered paper questionnaire regarding prenatal exposures and birth characteristics of their children, and all parents were asked to consider providing written permission for

researchers to obtain their child's NBS from state newborn screening programs. If a child had reached the age of majority (≥18 years) at the time of contact, he/she was asked to provide release of his/her NBS instead. State newborn screening programs were then contacted to request the NBS. Etiologic hypotheses of interest involve the index child's birthweight,[23] maternal weight gain during pregnancy,[86] maternal vitamin supplementation with a focus on folate consumption,[31] maternal alcohol and tobacco smoke exposure[32] and maternal exposure to fertility or other hormone treatments.[33] Factors related to the immune system are also of interest.[35]

Chapter 3: Feasibility of obtaining neonatal blood spots for childhood cancer cases: A Children's Oncology Group study

Although the etiology of most pediatric hematologic malignancies is unknown, there is evidence supporting prenatal origins. Neonatal blood spots (NBS) are collected uniformly from newborns in the U.S. to test for metabolic disorders. NBS therefore constitute the only consistent source of prediagnostic specimens for children with cancer and may provide an unbiased record of prenatal exposures. Some states retain NBS for ≥5 years, potentially permitting their use in case-control studies. The primary aim of this study was to assess the feasibility of obtaining NBS from pediatric/adolescent cancer cases nationwide. A secondary aim was to assess study participation 2-8 years after agreeing to future contact. Biological mothers of hematological cancer cases diagnosed at 19 U.S. Children's Oncology Group institutions in 2001-2007 were asked to complete questionnaires regarding prenatal exposures (n = 887). All parents/guardians and cases aged ≥18 years were asked to release their child's/their NBS (n = 947). NBS were then requested from state newborn screening programs. Overall, 41% of mothers completed questionnaires and 37% of families provided consent for NBS release. Notably, 82% of those completing questionnaires also provided consent. Consenting cases were born in 39 states and 46 NBS were obtained from 5 (CA, NY, MI, TX, WA); 124 NBS were unobtainable because cases were born prior to the dates of state retention. NBS storage/release policies are rapidly evolving; requests are pending in states involved in litigation (MN), reviewing policies (NJ), and reviewing this study (MA). Currently,

population-based NBS studies can be conducted in a limited number of states; fortunately, many have large populations to provide reasonably sized pediatric case and control groups.

Introduction

Although the etiology of most childhood hematologic malignancies remains unknown,[4] there is important evidence in support of prenatal origins. One significant line of evidence is the detection of chromosomal translocations present in neonatal blood spots (NBS) collected at birth from acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) cases.[5, 6] These translocations may constitute a first genetic hit in a multistep pathway,[6] with subsequent hits from endogenous or exogenous factors. It is therefore of keen interest to elucidate the role of *in utero* exposures in leukemogenesis.

NBS are collected from nearly all neonates (>95%)[50] in the U.S. per a standard protocol[51] to test for metabolic and other disorders.[53] Theoretically, any substrate that can be measured in whole blood, serum, or plasma can also be analyzed in NBS.[50] Accordingly, several analytes have been evaluated in NBS, such as amino acids, enzymes, human and viral DNA, antibodies, markers of inflammation, steroids, metals, protein adducts, pesticides and cotinine.[50, 56-58, 66]

Newborn screening programs are mandated by U.S. states, resulting in considerable variability in implementation nationally.[54] Each state selects tests to be

performed, establishes consent and refusal processes, and determines the fate of residual NBS.[a]

Recall bias is a potentially serious limitation of retrospective case-control studies of rare pediatric/adolescent disorders. NBS constitute the only consistent source of prediagnostic biospecimens for children with cancer and in theory would provide an unbiased record of *in utero* exposures, since they are collected at birth. Some states retain residual NBS for ≥5 years, potentially permitting their use in etiologic research.[71] The primary aim of this study was to assess the feasibility of obtaining NBS from childhood cancer cases with written consent on a nationwide basis. A secondary aim was to assess subject willingness to participate in a study years after consenting to future contact.

Methods

Eligible subjects were identified via Children's Oncology Group (COG) protocol AADM01P1 - Protocol for Registration and Consent to the Childhood Cancer Research Network (CCRN): A Limited Institution Pilot, which was activated at a 10% random sample of COG institutions in North America (n = 23).[47] The protocol involved two levels of consent for parents/guardians and children ages ≥18 years. The first level of consent allowed name and contact information to be released to the COG registration system. The second level involved agreement for future contact regarding COG-approved non-therapeutic studies. Institutions were to approach all newly diagnosed cancer cases.

[a] Of note, The Newborn Screening Saves Lives Act (2007) passed into law in April 2008 includes provisions to standardize the tests performed nationwide (The Newborn Screening Saves Lives Act of 2007, Pub. L. No. 110-204, 122 Stat. 705-712 (April 24, 2008)).

Between May 2001 and January 2007, 2,233 families were approached and 2,136 (96%) agreed to both consent levels.

Cases were eligible for this study (COG AEPI08N1) if they were diagnosed with leukemia or lymphoma between the ages of 0-21 years, were born in the U.S. (Canada has no provision to provide NBS), and they or their parent/guardian participated in AADM01P1, agreed to future contact, and spoke English or Spanish. Deceased cases were eligible.

COG diagnosing institutions were provided with study summaries and were asked to supply contact information for family members providing AADM01P1 consent or any reasons why families should not be contacted (e.g., child recently died). Subjects were contacted in a series of mailings (introductory letter, study packet, reminder/thank you postcard) over a period of 4 weeks, followed by a phone call and/or a final mailing (second study packet).[b] Spanish translations were sent to known Spanish speakers.

Biological mothers, if available, were asked to complete self-administered questionnaires regarding prenatal exposures and birth characteristics.[c] All parents/guardians were asked to consider providing three levels of written consent, including release of their child's NBS to study investigators, long-term storage of NBS by investigators, and future contact regarding this study. All parents/guardians were asked to

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[b] Study packets contained letters, questionnaires, and consent and assent forms, as applicable, for mothers; letters, consent and assent forms, or adult contact information forms, as applicable, for parents/guardians; and letters and consent forms for adult children. The self-administered paper questionnaire and samples of other study materials are provided in Appendices D and E. Questionnaire content was restricted due to a mandate from the COG Scientific Council that the questionnaire require 20 minutes or less for completion in order to minimize respondent burden for a feasibility study.

[c] Items focused on exposures during the index pregnancy, such as maternal reproductive history, weight gain, vitamin supplementation, and alcohol and tobacco exposure, as well as the child's birthweight and length and personal and family history of atopy.

provide contact information for children ≥18 years in the questionnaire or contact information form, as applicable, so that adult children could be asked to provide written release for their own NBS.

Due to lower than anticipated response rates, non-respondents were traced via telephone and reverse directories and on-line methods. Multiple attempts were made to reach non-respondents via telephone at different times of day/days of the week; voicemail/answering machine messages were left on third or subsequent failed attempts. Families who did not respond via mail, who were not reached via telephone, and whose outgoing message did not clearly identify them were classified as "contact unknown."

State newborn screening programs were provided with signed consent/assent forms and asked to release NBS. States were also asked to provide their NBS retention, storage, and release policies.[d] States of birth were contacted for all participating cases regardless of prior responses to surveys,[54, 71] as policies are evolving.

The protocol was approved by the University of Minnesota Institutional Review Board (IRB) and IRBs of states releasing NBS, as needed.[e]

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[d] Some of this information is available and updated regularly on the National Newborn Screening Information System (NNSIS) website (National Newborn Screening Information System. Laboratory Specimen Information for Newborn Screening in the U.S. in 2010. [Last accessed January 27, 2010]. http://www2.uthscsa.edu/nnsis/).

[e] The COG Scientific Council and the University of Minnesota Cancer Protocol Review Committee (CPRC) provided approvals prior to seeking IRB approvals. IRB applications were submitted to the University of Minnesota, Minnesota Department of Health, State of New York Department of Health, Washington State Department of Health, Michigan Department of Community Health, Texas Department of State Health Services, and New Jersey Department of Health and Senior Services. COG and CPRC approval letters and IRB response letters are shown in Appendix F; we currently await a response from New Jersey.

Statistical analysis

Univariate unconditional logistic regression (SAS v9.2, SAS Institute, Cary, North Carolina) was performed to assess whether baseline participant characteristics (type of respondent initially contacted, language, age group at diagnosis, year of birth, time since diagnosis, last known vital status) were statistically significant predictors of questionnaire completion, consent, assent, provision of adult child contact information, or NBS retrieval; odds ratios (ORs) and 95% confidence intervals (CIs) were produced. Multivariable logistic regression was also performed to determine the significance of predictors after adjustment for all other factors.

Results

Nineteen U.S. COG institutions provided updated contact information and reasons not to contact subjects, while 2 refused and 2 Canadian institutions were excluded (Canada has no provision to provide NBS).[f] Of 1,006 hematologic cases in the AADM01P1 dataset, contact was attempted for 947 in the current study, while the remainder were not contacted due to institutional refusal to provide name and contact information (n = 25), subjects retracting consent for future contact (n = 11), institutional losses to follow up (n = 7), parent's language (n = 6), subject's place of birth outside of U.S. (n = 5), biological mother unavailable (n = 3), and patient's poor health (n = 2).

For the vast majority of the 947 families, we initiated contact with mothers (94%) and native English speakers (93%), as these individuals had provided permission for future contact in the AADM01P1 study (Table 3-1). Forty-one percent of cases were

[f] COG institutions are listed in Appendix A.

diagnosed at <5 years and approximately 20% were diagnosed in each of the other age categories. Half were born in 1997 or thereafter (48%), while 59% were diagnosed ≥5 years prior to contact (median = 5 years). Most cases were last known to be alive (89%).

Of the 947 families, 385 participated in some manner, resulting in an overall response rate of 41%, while 27 actively refused (3%) and materials from 56 were returned to sender (6%). An additional 165 were passive refusals (17%), meaning contact was confirmed via telephone. It was unclear if contact was made with the remaining 314 (33%). Frequencies, response rates, and results of univariate regression prediction models are provided in Table 3-2.[g]

The distribution of questionnaire responses was very similar to those described above for overall response; 41% of mothers returned completed questionnaires. Mothers of cases diagnosed at 15-20 years and mothers of cases born in 1987-1991 were approximately 2 times more likely to complete questionnaires than mothers of cases aged 0-4 years (OR = 2.05, 95% CI: 1.41-2.98) and those born in 1997-2001 (OR = 1.85, 95% CI: 1.29-2.66), respectively. Spanish speakers were significantly less likely to return completed questionnaires than English speakers (OR = 0.39, 95% CI: 0.19-0.79). Upon adjustment for all factors, only Spanish language remained a significant predictor of non-response (OR = 0.38, 95% CI: 0.19-0.79).

Fifty-four percent of parents/guardians with children ≥18 years at the time of contact provided names and addresses; 132 adult children were therefore asked to provide consent for release of NBS.

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[g] A full listing of the response rates for the questionnaire, written consent and assent, and adult contact information forms, and NBS retrieval rates overall and by age at diagnosis, year of birth, and years since diagnosis are shown in Appendix G.

The consent response distribution was also similar to that for any participation; 37% of families returned signed consent forms, including 228 mothers (34%), 7 parent/guardians (21%), and 75 adult children contacted upon receipt of information from parents/guardians (57%). Of those providing consent, 304 agreed to NBS release, NBS storage, and future contact, while 2 agreed to release and storage, 2 agreed to release and future contact, and 2 agreed to release only. Notably, 82% of families providing completed questionnaires also returned signed consents and 1 family provided consent but declined the questionnaire (the contact was a step-mother and was ineligible for the questionnaire). One consent was inadvertently signed by a minor and written consent could not be obtained from that parent/guardian. Similar to questionnaire response, diagnosis at ages 15-20 years (OR = 1.95, 95% CI: 1.28-2.97), birth in the years 1987-1991 (OR = 2.08, 95% CI: 1.38-3.15), and Spanish language (OR = 0.33, 95% CI: 0.14-0.75) were significant predictors of consent. Spanish language was the only significant predictor after adjustment for the other factors (OR = 0.32, 95% CI: 0.14-0.74).

Thirty-two percent of children ages 8-17 years returned signed assent forms. In addition, 7 were signed by parents/guardians and signed assents were not obtained from these children.

Respondents were born in 42 U.S. states[h] and those providing consent for NBS release were born in 39 of these.[i] NBS were requested for 299 cases (and were not requested for 7 missing signed assents and 4 not born in the U.S.). Of 39 states queried, 5

[h] States of birth for respondents included: AK, AZ, CA, CT, DE, FL, GA, HI, IA, ID, IL, IN, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, SD, TN, TX, UT, VA, VT, WA, WI, and WY.

[i] States of birth for those providing consent included the states listed above less HI, NH, and KY.

(CA, MI, NY, TX, WA) released 46 NBS. These states were unable to provide NBS for 12 "age-ineligible" cases (those born prior to the oldest date of state retention) and were unable to locate spots for 2 age-eligible cases based on the information provided (child's last name, date of birth, mother's full name, hospital name and city). NBS retrieval rates and results from prediction models are listed in Table 3-2. None of the factors examined was a significant predictor of NBS retrieval, although this may be attributable to the small number of retrieved spots.

NBS were unobtainable for 112 cases because states store spots for <1 year (n = 72, AZ, GA, IL, LA, NE, OK, PA, UT, VA, WY), 1-<5 years (n = 32, AK, DE, ID, MT, NM, NV, OH, TN, WI) or ≥5 years (n = 7, IA, IN, MD, ME, MO, NC) and cases were age-ineligible. Of note, state policies may not allow for NBS retrieval in 5 of these (GA, IL, NM, NV, WY). Policies in 6 states (CT, FL, MS, OR, SD, VT) do not allow for release of identifiable NBS for research (n = 60). Requests for NBS are pending in 3 states (n = 67), including one involved in litigation unrelated to the current study (MN), a second currently reviewing its storage/release policies (NJ), and a third performing a protocol review (MA). Information regarding length of retention, willingness to release spots with written consent, and storage conditions for the 39 queried states (provided in written personal communications with newborn screening program contacts listed on the NNSIS website,[115] or their designates) is listed in Table 3-3.

Table 3-1. Characteristics of 947 eligible pediatric hematologic cancer cases.

Characteristic	N (%)
Initial contact	
Mother	887 (93.7)
Parent/Guardian	57 (6.0)
Child ≥18 years at diagnosis	3 (0.3)
Language	
English	877 (92.6)
Spanish	46 (4.9)
Unknown	24 (2.5)
Age at diagnosis	
0-4 years	375 (39.6)
5-9 years	204 (21.5)
10-14 years	181 (19.1)
15-20 years	187 (19.8)
Year of birth	
1982-1986	86 (9.1)
1987-1991	208 (22.0)
1992-1996	197 (20.8)
1997-2001	326 (34.4)
2002-2006	130 (13.7)
Years since diagnosis	
2-4 years	386 (40.8)
5-10 years	561 (59.2)
Vital status	
Alive	844 (89.1)
Deceased	83 (8.8)
Unknown	20 (2.1)

Table 3-2. Frequencies, response rates, and results from univariate logistic regression models for the questionnaire, consent, assent, child contact information request, and NBS retrieval, overall and by initial contact, language, age at diagnosis, year of birth, years since diagnosis and vital status.

	Questionnaire			**Consent**			**Assent**		
	N (%)	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**
Overall	366 (41.3)			310 (37.1)			148 (32.0)		
Initial contact									
Mother	366 (41.3)	1.00		299 (37.9)	1.00		144 (32.4)	1.00	
Guardian	NA	NA		11 (26.2)	0.58	0.29 - 1.18	4 (22.2)	0.60	0.19 - 1.85
Adult child	0 (0.0)	--		0 (0.0)	--		NA	NA	
Language									
English	355 (42.6)	1.00		298 (38.5)	1.00		145 (33.1)	1.00	
Spanish	10 (22.2)	**0.39**	**0.19 - 0.79**	7 (17.1)	**0.33**	**0.14 - 0.75**	2 (11.1)	0.25	0.06 - 1.11
Unknown	1 (11.11)	0.17	0.02 - 1.35	5 (26.3)	0.57	0.20 - 1.60	1 (14.3)	0.34	0.04 - 2.82
Age at diagnosis									
0-4 years	138 (38.6)	1.00		136 (36.3)	1.00		70 (36.3)	1.00	
5-9 years	65 (33.7)	0.81	0.56 - 1.17	62 (30.5)	0.77	0.54 - 1.11	56 (29.6)	0.74	0.48 - 1.14
10-14 years	69 (40.8)	1.10	0.76 - 1.60	51 (36.2)	1.00	0.67 - 1.49	21 (26.3)	0.63	0.35 - 1.12
15-20 years	94 (56.3)	**2.05**	**1.41 - 2.98**	61 (52.6)	**1.95**	**1.28 - 2.97**	1 (100)	--	
Year of birth									
1982-1986	36 (50.7)	1.61	0.96 - 2.70	23 (43.4)	1.39	0.77 - 2.50	NA	NA	
1987-1991	104 (54.2)	**1.85**	**1.29 - 2.66**	69 (53.5)	**2.08**	**1.38 - 3.15**	3 (27.3)	0.71	0.18 - 2.74
1992-1996	57 (30.5)	0.69	0.47 - 1.01	55 (27.9)	0.70	0.48 - 1.03	51 (28.3)	0.75	0.50 - 1.13
1997-2001	122 (39.0)	1.00		116 (35.6)	1.00		94 (34.6)	1.00	
2002-2006	47 (37.9)	0.96	0.62 - 1.47	47 (36.2)	1.03	0.67 - 1.57	NA	NA	

	Questionnaire			Consent			Assent		
	N (%)	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**
Years since diagnosis									
2-4 years	158 (42.5)	1.09	0.83 - 1.43	131 (36.8)	0.98	0.74 - 1.30	44 (28.4)	0.78	0.51 - 1.19
5-10 years	208 (40.4)	1.00		179 (37.4)	1.00		104 (33.8)	1.00	
Last known vital status									
Alive	338 (42.1)	1.00		280 (38.0)	1.00		148 (32.5)	1.00	
Deceased	28 (36.4)	0.79	0.48 - 1.28	26 (31.3)	0.74	0.46 - 1.21	NA	NA	
Unknown	0 (0.0)	--		4 (26.7)	0.59	0.19 - 1.88	0 (0.0)	--	

OR = odds ratio; CI = confidence interval

Table 3-2 (Continued). Frequencies, response rates, and results from the univariate logistic regression models for the questionnaire, consent, assent, child contact information request, and NBS retrieval, overall and by initial contact, language, age at diagnosis, year of birth, years since diagnosis and vital status.

	Adult Child Contact Information			NBS Retrieved		
	N (%)	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**
Overall	132 (54.1)			46 (15.4)		
Initial contact						
Mother	123 (55.9)	1.00		45 (15.6)	1.00	
Guardian	9 (37.5)	0.47	0.20 - 1.13	1 (9.1)	0.54	0.07 - 4.32
Adult child	NA	NA		0 (0.0)	--	
Language						
English	125 (55.1)	1.00		46 (15.9)	1.00	
Spanish	3 (37.5)	0.49	0.11 - 2.10	0 (0.0)	--	
Unknown	4 (44.4)	0.65	0.17 - 2.49	0 (0.0)	--	
Age at diagnosis						
0-4 years	NA	NA		23 (17.6)	1.00	
5-9 years	0 (0.0)	--		10 (17.0)	0.96	0.42 - 2.17
10-14 years	39 (49.4)	0.74	0.43 - 1.28	5 (10.4)	0.55	0.20 - 1.53
15-20 years	93 (56.7)	1.00		8 (13.1)	0.71	0.30 - 1.69
Year of birth						
1982-1986	38 (53.5)	0.97	0.56 - 1.68	3 (13.0)	0.86	0.23 - 3.24
1987-1991	94 (54.3)	1.00		7 (10.6)	0.68	0.27 - 1.76
1992-1996	NA	NA		9 (16.4)	1.13	0.46 - 2.74
1997-2001	NA	NA		16 (14.8)	1.00	
2002-2006	NA	NA		11 (23.4)	1.76	0.74 - 4.15

	Adult Child Contact Information			NBS Retrieved		
	N (%)	**OR**	**95% CI**	**N (%)**	**OR**	**95% CI**
Years since diagnosis						
2-4 years	50 (62.5)	1.67	0.97 - 2.88	25 (20.0)	1.82	0.97 - 3.43
5-10 years	82 (50.0)	1.00		21 (12.1)	1.00	
Last known vital status						
Alive	128 (54.5)	1.00		41 (15.2)	1.00	
Deceased	NA	NA		5 (19.2)	1.32	0.47 - 3.71
Unknown	4 (44.4)	0.67	0.18 - 2.55	0 (0.0)	--	

OR = odds ratio; CI = confidence interval

Table 3-3. Neonatal dried blood spot retention and release policies for queried states.*

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
AK	3 years	2007	Yes, as long as not tested for anything already screened	No -- not age eligible	Stored at room temperature in sealed boxes without desiccant.	
AZ	90 days	2009	Yes	No -- not age eligible	Stored at room temperature in bulk in plastic boxes without desiccant.	
CA	Indefinitely	October 1980 (but only indexed electronically since March 1982)	Yes	Yes	Stored at -20°C in plastic bags in cardboard boxes.	IRB approval is required. Researchers may encounter a delay due to backlog and may be assessed a fee.
CT	2 years	2008	No	No	Stored at -20°C in sealed bags with desiccant.	
DE	3 years (beginning in 2010)	2009	Yes	No -- not age eligible	Stored at -20°C in sealed bags with desiccant.	A notarized original consent form is required; no copies or faxes.
FL	Indefinitely	June 2005	No	No	Stored at room temperature.	Currently in the process of writing new release policies.
GA	6 weeks	2009	Maybe	No -- not age eligible	Stored in refrigerator in gas-permeable bags without desiccant.	No written policy exists, but is currently being drafted.

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
IA	5 years	2005	Yes	No -- not age eligible	Stored at -70°C for 1 year in non-permeable bags with desiccant, then at room temperature for 4 years.	Approval is required from the Center for Congenital & Inherited Disorders (Iowa Department of Health)
ID	1 year	2009	Yes	No -- not age eligible	Stored at room temperature in boxes without desiccant.	
IL	3 months, unless a positive test	2009	Maybe	No	Negatives stored at room temperature in lab drawer. Positives stored in batches at -30°C or -80°C (depending on disorder) in closed plastic bins without desiccant.	Could submit application to Illinois Department of Public Health Data Release and Research Committee, but a very small chance of success is expected.
IN	21 years	1991	Yes	No -- not age eligible	Stored at room temperature in boxes.	
LA	30 days	2010	Yes	No -- not age eligible	Stored at 2-8°C in gas permeable bags with desiccant.	Written release form must be signed by parents and reviewed by state's legal department.
MA	≥10 years	1991	Yes	Pending	Stored at -20°C with desiccant.	IRB approval is required.

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
MD	Indefinitely	2004	Yes	No -- not age eligible	Stored at -20°C for 6 months.	IRB approval is required. At time of publication, state reviewing/updating policies.
ME	Indefinitely	1998	Yes	No -- not age eligible	Stored at -20°C with desiccant.	
MI	Indefinitely	1985	Yes	Yes	Stored in a warehouse with no temperature or humidity controls.	IRB approval is required. May need to sign a materials transfer agreement and pay a fee.
MN	Indefinitely	1997	Pending	Pending	Since 2005, stored at -20°C in plastic containers. Prior to 2005, stored at room temperature in cardboard boxes. All are in stacks of ~1000 with no desiccant.	At time of publication, state involved in litigation.
MO	5 years (beginning in Fall 2010)	2010	Yes	No -- not age eligible	Stored in bulk in sealed bags with desiccant in the refrigerator for one month and at -20°C for 5 years.	
MS	1 year	2009	No	No	Stored at room temperature in boxes without desiccant.	

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
MT	1 year	2009	Yes	No -- not age eligible	Stored at -20°C in biohazard bags without desiccant.	Must be approved by the State Medical Officer and Office of Legal Affairs.
NC	Indefinitely	2002	Yes	No -- not age eligible	Stored at room temperature in boxes without desiccant.	IRB approval is required. Policies may be changing soon.
NE	90-120 days	2009	Yes	No -- not age eligible	Stored in the refrigerator in sealed bags of low gas permeability.	Must submit an application to the Chief Medical Officer and the Newborn Screening Advisory Committee for approval.
NJ	23 years	1987	Pending	Pending	Stored at ambient temperature and humidity; bundled together in cardboard boxes.	IRB approval is required. At time of publication, state reviewing/updating policies.
NM	1 year	2009	Will not release NBS to researchers, but will consider requests from parents	No	Stored at room temperature in boxes without desiccant.	
NV	1 year	2009	Maybe	No -- not age eligible	Stored at room temperature in sealed boxes without desiccant.	

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
NY	27 years	1997	Yes	Yes	Stored at 4°C; some may have spent time in -20°C.	IRB approval is required. Can only release specimens if 1 full spot remains.
OH	2 years	2008	Yes	No -- not age eligible	Stored at room temperature in cardboard boxes.	
OK	42 days maximum	2010	Yes	No -- not age eligible	Bundled and placed in low gas-permeable, sealed bags with desiccant and humidity indicator cards. Stored at 2-8°C and <30% humidity.	
OR	1 year	2009	No, can only release NBS to primary physician	No	Stored at room temperature in boxes without desiccant.	
PA	8 months, unless a positive test	2009	Yes	No -- not age eligible	Stored at -20°C in low gas-permeable bags with desiccant.	A state-specific release form must be signed by parents.
SD	30 days	2010	No	No	Stored at room temperature in cardboard boxes with no desiccant.	
TN	1 year, unless a positive test	2009	Yes	No -- not age eligible	Normal samples stored at room temperature for 1 year. Confirmed positives stored at -20°C with desiccant indefinitely.	

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
TX	Indefinitely	May 2009	Yes	Yes	Stored at ambient temperature ~88 bundled together in cardboard boxes without desiccant. Humidity controlled via air conditioning.	IRB approval is required. Per lawsuit settlement, state must destroy spots stored without consent between July 2002 and May 2009 by April 2010. NBS collected after consent process implemented (on May 27, 2009) available for research.
UT	90 days	2009	Yes	No	Stored at room temperature for 3 days and in refrigerator for remaining days; placed in boxes inside bags without desiccant.	
VA	6 months, unless a positive test	2009	Yes	No -- not age eligible	Stored at room temperature in cardboard boxes with no desiccant.	Notarized written request from parents is required.
VT	Indefinitely	Unknown	No	No	Spots collected prior to 2003 are stored in boxes at room temperature. Spots collected after 2003 stored in freezer conditions.	

State	Length of retention	Oldest year retained	Willing to release spots with written parental consent?	Spots received?	Storage conditions	Notes
WA	21 years	1988	Yes	Yes	Stored at ambient room temperature.	IRB approval is required. IRB Authorization Agreement form must also be signed by parent.
WI	1 year	2009	Yes	No -- not age eligible	Stored at 4°C in plastic bags (76 per bag) without desiccant.	
WY	6 months	2009	Maybe	No -- not age eligible	Stored at room temperature.	No official release policy exists.

[*] Reported by state newborn screening program contacts provided by the National Newborn Screening Information System[115] or their designate.

Discussion

Forty-one percent of subjects recontacted 2-8 years after consenting to COG protocol AADM01P1 participated in the current COG feasibility study, including 41% of mothers completing questionnaires and 37% of families contributing signed consents. Accurate cooperation rates cannot be calculated, since we are unable to determine if one-third of subjects were reached. Among families in which mothers provided completed questionnaires, however, a strong majority (82%) were willing to release their/their child's NBS, indicating a high level of participation in NBS research if families are reached and engaged in the study. Nevertheless, due to limited state retention and release practices, NBS were retrieved for only a small fraction of those requested (15%). Notably, many states queried were willing/able to release NBS with subject consent, however, NBS were not stored long enough to permit their release.

Prior studies assessing NBS retrieval have been conducted within single states; these studies also differed from the current study in that investigators did not acquire subject consent prior to specimen retrieval.[68-70] Our results are consistent with those from a nationally representative survey of 3,047 parents of children <18 years, in which 78% of respondents indicated they would authorize state storage of their children's NBS and 76% would be willing to release NBS for research if their permission was sought.[67] Only 10% were "very unwilling" to allow NBS release. Respondents were considerably less amenable to research conducted without parental permission (28% were willing). The authors indicate that the definition of parental permission needs to be examined; their results do not necessarily implicate formal written consent processes for individual

research projects. Notably, that survey was designed only to assess parental attitudes; no consent forms or NBS were collected.

Telephone discussions with subjects also proved illuminating. Anecdotally, although some parents expressed surprise that their children's NBS may have been retained by state newborn screening programs, none voiced objections to the practice. On the contrary, many parents indicated interest in this potential avenue for research. Importantly, a few parents conveyed hesitation about releasing NBS in case their children need them in the future.

Epidemiologic research using NBS is shrouded in ethical debate. Overall, there is conflict between societal/public health benefits versus individual rights, and regarding ownership of the spots.[54, 116, 117] Specifically, opponents of NBS research are concerned about the preservation of data privacy, the potential for misuse of DNA, and the failure to obtain active consent for long-term storage and secondary uses.[54, 116, 117] If such concerns are not adequately addressed, public concern may lead to reduced availability of NBS. For example, genetic privacy advocacy groups sued the states of Texas and Minnesota in 2009, calling for the destruction of residual NBS stored without explicit parental consent.[118, 119] Although NBS were spared for this study, the settlement in Texas resulted in the destruction of 5 million NBS by April 2010.[120] At the time of publication, an appeal was expected in the dismissed Minnesota lawsuit.[121] Other states are reviewing their policies (see Table 3-3), presumably due to similar considerations. As with any

research involving humans, establishing transparency and trust with subjects and the greater public are critical.[j]

States storing NBS must also address logistical and budgetary issues.[116] Ideal conditions include individual storage with a desiccant and humidity indicator card at -20°C in sealed bags with low gas permeability; humidity should be maintained at <30%.[51] Maintaining these conditions can be costly,[71] especially in states with high birth rates. Some states report storage at ambient conditions, in which temperature and humidity can fluctuate with seasons, while others report refrigerator (2-8°C) or freezer (-20 to -30°C) storage, with or without a desiccant.[71, 122] Storage in ambient conditions could result in denatured DNA and/or degredation of analytes, limiting the utility of and contradicting the rationale for storing spots long-term.[116] Similarly, some states package NBS individually, while other states store them in bulk, with adjacent Guthrie cards touching; a concern of bulk storage is the potential for cross-contamination.[73]

In addition to state retention and release policies, other factors have been associated with NBS acquisition. In our study, spots were not retrieved for 2 of 310 consenting cases and there is no obvious explanation for this; identical data were provided to states for these cases as for those with retrieved specimens. In requesting NBS from the Maryland Department of Health and Mental Hygeine, Loffredo and Ewing observed significantly lower retrieval rates among 522 cases with congenital heart defects (65%) than among 1,645 population-based controls (84%).[68] Among controls, 10% had

[j] The Danish Newborn Screening Biobank can serve as an example of a successful NBS repository; it has been storing residual NBS from all newborns since 1982 and has provisions for the release of NBS to researchers conducting approved research. Importantly, there were no known abuses of data or samples from that repository as of 2007 (Norgaard-Pedersen B, Hougaard DM. Storage policies and use of the Danish Newborn Screening Biobank. J Inherit Metab Dis. Aug 2007;30(4):530-536).

no laboratory number to link the participant with his/her blood spot and 6% had a lab number, but the NBS was not located. Retrieval rates were significantly lower among low birthweight and preterm infants; there were no significant differences for other infant or maternal characteristics examined.

This study features unique strengths. To our knowledge, it is the first U.S. study to attempt NBS retrieval with subject consent on a national level. The study population was generated from a random sample of COG institutions and should therefore reasonably represent U.S. pediatric and adolescent hematologic cancer cases, since COG institutions treat the vast majority of leukemia and lymphoma cases ages 0-14 years in the U.S.[44, 123] Correspondingly, responding cases were born in 42 states and consent was received for 39 of these, providing an excellent survey of the feasibility of this methodology nationally. These results are also applicable to etiologic studies of other rare pediatric/adolescent conditions requiring retrospective designs.

This study was designed to obtain completed questionnaires and residual NBS. Questionnaire response rates were of interest for two reasons. First, this design simulates the likely design of future case-control investigations of childhood cancers involving NBS, since some pediatric malignancies are thought to be initiated *in utero* and since it is useful to have information regarding exposures, potential confounders, and/or stratification variables. Second, it serves as a test of the live CCRN (implemented in December 2007), since a primary purpose of this registry is to facilitate future contact regarding non-therapeutic studies.[47k] The 41% response rate observed in the current study is lower than can be expected in using the current iteration of the CCRN simply because

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[k] A previous CCRN pilot study included an interview component conducted between April and December, 2004; 75 of 107 participants sampled (70%) completed an interview shortly after registration onto CCRN.

more subjects will likely be reached if they are contacted closer to diagnosis and CCRN registration. Further, parents and states may be less likely to provide NBS for a feasibility study than for etiologic studies with specific analytic hypotheses. Importantly, this response rate may indicate selection bias, which should be accounted for in interpreting study results. For example, individuals with higher socio-economic status tend to participate in case-control studies of childhood cancer,[124] and parents of higher educational levels have expressed greater willingness to permit long-term NBS storage.[67]

The chief study limitation is that state NBS policies are continually evolving; this report represents a snapshot in time. Some states have recently passed legislation allowing long-term storage of NBS (Table 3-3). Conversely, 2 states have been sued within the past year due to data privacy concerns, resulting in destruction of stored NBS in 1, and others are reviewing their policies.

Etiologic studies of pediatric/adolescent cancers usually utilize retrospective case-control designs, however, we did not query families of controls, who are expected to be less motivated to participate. Parents of healthy children may be less likely to recognize the importance or utility of NBS research or have concerns about data privacy and therefore may be less likely to provide consent for NBS release and/or storage.[67] Consistent with this idea, Tarini *et al* found that parents with children with very good to excellent health were less likely to agree to NBS release or storage.[67]

Ideally, NBS from all U.S. infants would be cataloged and properly stored such that they could be retrieved and released for etiologic research with appropriate scientific justification, ethics review board oversight, and subject consent. In practice, state retention policies are evolving. Currently, retrospective population-based case-control

studies of cancers or other rare disorders in children/adolescents using NBS are limited to a few states. For example, NBS were retrieved with signed consent from 5 of 39 states queried in this study. Fortunately, many of these have large populations to provide reasonably sized pediatric case and control groups. Depending on subject ages and future policies, NBS may be retrievable from an additional 9 states that store NBS ≥5 years. Studies of conditions in young children may have the greatest success and the most relevance to prenatal exposures. It will be of interest to observe future state policies as they unfold.

Chapter 4: Review of the literature on infant leukemia, folic acid supplementation, and genes related to folic acid metabolism

Infant leukemia

Leukemias are diagnosed in children ages <12 months (heretofore denoted "infant leukemia") at a rate of 40 per 1,000,000 infants (19/1,000,000 for acute lymphoblastic leukemia, ALL; 15/1,000,000 for acute myeloid leukemia, AML; and 6/1,000,000 for other leukemias).[3] Infant leukemias are distinct from leukemias in older children and adolescents with respect to several notable characteristics. The proportions of ALL and AML cases are more similar among infants (47% vs. 37%, respectively), while ALL predominates among children and adolescents (78% vs. 17% AML).[3] Unlike leukemias diagnosed in older children and adolescents, infant leukemia is associated with a poor survival rate (ALL: 5-year survival: 57%, 10-year survival: 37%; AML: 5-year survival: 39%, 10-year survival: 34%); these survival rates have improved substantially compared with rates in earlier decades.[3]

Most cases of infant leukemia (~75% of ALLs and ~60% of AMLs) are preceded by rearrangements of the mixed lineage leukemia (*MLL*) gene on chromosome 11 band q23.[125, 126] Notably, *MLL* gene rearrangements (denoted "MLL+") are observed among just 6% of pediatric ALL patients overall, but the vast majority of these occur in infants (80%).[127] Similarly, 14% of childhood AML cases present with MLL rearrangements and 65% of these are found in infants.[127] These rearrangements also occur commonly in secondary AML patients (70-90%) as a result of treatment with DNA topoisomerase II inhibitors, such as epipodophyllotoxins and anthracyclines;[127] MLL+ secondary

leukemias are also associated with a markedly brief latency period (26 months on average).[8] The presence of an *MLL* gene rearrangement confers an appreciably lower survival in infant ALL cases compared with MLL- cases (5-year survival: 5-34% versus 42-92%, respectively),[128] but does not appear to affect the prognosis of infant AML.[129]

Twin studies have demonstrated a high concordance rate for infant leukemia between monozygotic twins (~100% versus ~10% in older children/adolescents); transmission of an affected clone via shared monochorionic placental circulation is thought to be responsible.[8] (Of note, the first case report of discordance was recently published.[130]) This observation, coupled with evidence from backtracking studies of neonatal blood spots demonstrating that *MLL* translocations are consistently present at birth in infant leukemia cases[5] provide compelling evidence that the leukemias are initiated *in utero*. Further, the extremely short latency period indicates that the disease may progress to subclinical preleukemia prior to birth.[8] Infant leukemia therefore provides a unique opportunity for etiologic research, since it occurs at the very beginning of the life cycle, thus limiting the time period under study and the possibility of recall bias in exposure measurement.

Folate metabolism

Vitamin B9, commonly called folic acid (in supplements) or folate (in food and *in vivo*),[131] plays a critical role in embryonic development due to its contributions to DNA synthesis, cell and tissue proliferation, and DNA methylation.[132] Sufficient maternal consumption of folic acid during pregnancy has been shown in randomized controlled trials to significantly reduce the incidence of neural tube defects (NTDs, including spina

bifida, anencephaly, and encephalocele) in offspring[133-135] and is also postulated to play a preventative role in the pathogenesis of childhood leukemia (as summarized below). Since 1992, the U.S. Public Health Service has recommended a minimum daily folate intake of 400 µg (up to a maximum of 1000 µg/day) for women of childbearing age to reduce the prevalence of NTDs.[136] To ensure this minimum level is achieved, vitamin supplements are recommended, but tend to be underutilized in the general population (25-41% of pregnant women across 19 U.S. states reported regular multivitamin supplementation in the month prior to pregnancy),[137] leading the governments of the U.S. and Canada to implement mandatory folic acid fortification programs in 1998.[138, 139] Specifically, the U.S. Food and Drug Administration mandated in March of 1996 that all enriched grain products be fortified with folic acid by January 1, 1998.[140] Similarly, Health Canada required the fortification of flour, as well as some corn and rice products, by November 1, 1998.[139]

A comparison of mean serum folate concentrations among women ages 15 to 44 years participating in NHANES pre- and post-fortification showed a significant increase between NHANES III (1988-1991) and NHANES 1999 (6.3 vs. 16.2 ng/mL, respectively); similar results were observed for red blood cell folate concentrations.[141] In addition, a 19% reduction in the incidence of neural tube defects was detected among children conceived after the mandatory fortification program was introduced.[142] Parallel results have been observed in Canada.[139, 143] These observations indicate that the fortification programs have been successful in increasing folic acid intake among women of childbearing age, notably decreasing the possibility of folate deficiency among participants in this study. Of note, an ecologic study of the effects of mandatory folic acid

fortification in Canada found evidence supporting a protective role for folate in neuroblastoma development (IRR = 0.55, 95% CI: 0.39-0.78), but no evidence with respect to infant ALL or hepatoblastoma.[144]

Another analysis of NHANES data post-fortification (2001-2004) by Yeung *et al* indicates that among non-pregnant U.S. adults, vitamin supplementation accounts for the greatest source of variation in dietary folic acid across the population and constitutes about 61% of total daily consumption; there was some variation in folic acid consumed via prepared breakfast cereals but little variation in folate consumed via enriched cereal-grain products.[145] These data indicate supplement use may be a useful measure of variation in exposure to folic acid, although supplement use is expected to be greater and the dietary composition different among pregnant women.

Folic acid is consumed in dietary supplements as 5-methyltetrahydrofolate monoglutamate, a bioavailable form that can be directly absorbed in the small intestine via a transport mechanism involving solute carrier family 19 member 1 (*SLC19A1*, also called reduced folate carrier, *RFC1*), prior to transport into the cell via the folate receptors *FOLR1* in adults and *FOLR2* in the fetus.[146, 147] Folate is a vital component of two overlapping cellular pathways, including (1) the *de novo* synthesis of purines and thymidylate, important building blocks of DNA, and (2) the conversion of homocysteine to methionine, ultimately resulting in the biosynthesis of S-adenosylmethionine (SAM), which contributes a methyl group to >100 biochemical reactions, including the methylation of cytosine in DNA.[148] These conjoined pathways are shown in Figure 4-1 below.

Some enzymes involved in the purine/thymidylate synthesis pathway include 5,10-methylenetetrahydrofolate reductase (*MTHFR*), 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*, also called methionine synthase), 5-methylenetetrahydrafolate-homocysteine methyltransferase reductase (*MTRR*, also called methionine synthase reductase), 5,10-methylenetetrahydrofolate dehydrogenase (*MTHFD1*), hydroxymethyltransferase (*SHMT1*), thymidylate synthase (*TYMS*), and dihydrofolate reductase (*DHFR*). Transcobalamin II (*TCN2*) is responsible for transporting Vitamin B12 (cobalamin), a *MTR* cofactor, into the cell. Additionally, enzymes catalyzing the synthesis and degradation of homocysteine include paraoxonase 1 (*PON1*), cystathionine-β-synthase (*CBS*), and betaine-homocysteine methyltransferase (*BHMT*), which is folate-independent.[147] Several non-synonymous single nucleotide polymorphisms (SNPs) in genes encoding folate metabolism enzymes (shown in black boxes in Figure 4-1) have been identified.

MTHFR, the most widely investigated of the folate metabolizing enzymes, catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methyl THF). Two non-synonymous SNPs in the coding exons of *MTHFR*, C677T (resulting in a valine substitution for alanine at codon 222) and A1298T (causing alanine to replace glutamic acid at codon 429), are in strong linkage disequilibrium and have been associated with reduced enzyme function.[149] Individuals that are heterozygous and homozygous carriers of the 677 T allele have 60% and 30% of the activity of the wild type enzyme, respectively, while individuals with the 1298 CC genotype display 60% of the enzymatic activity of the wild type; compound heterozygotes (CT/AC) exhibit 50-60% of the *MTHFR* wild-type activity.[149]

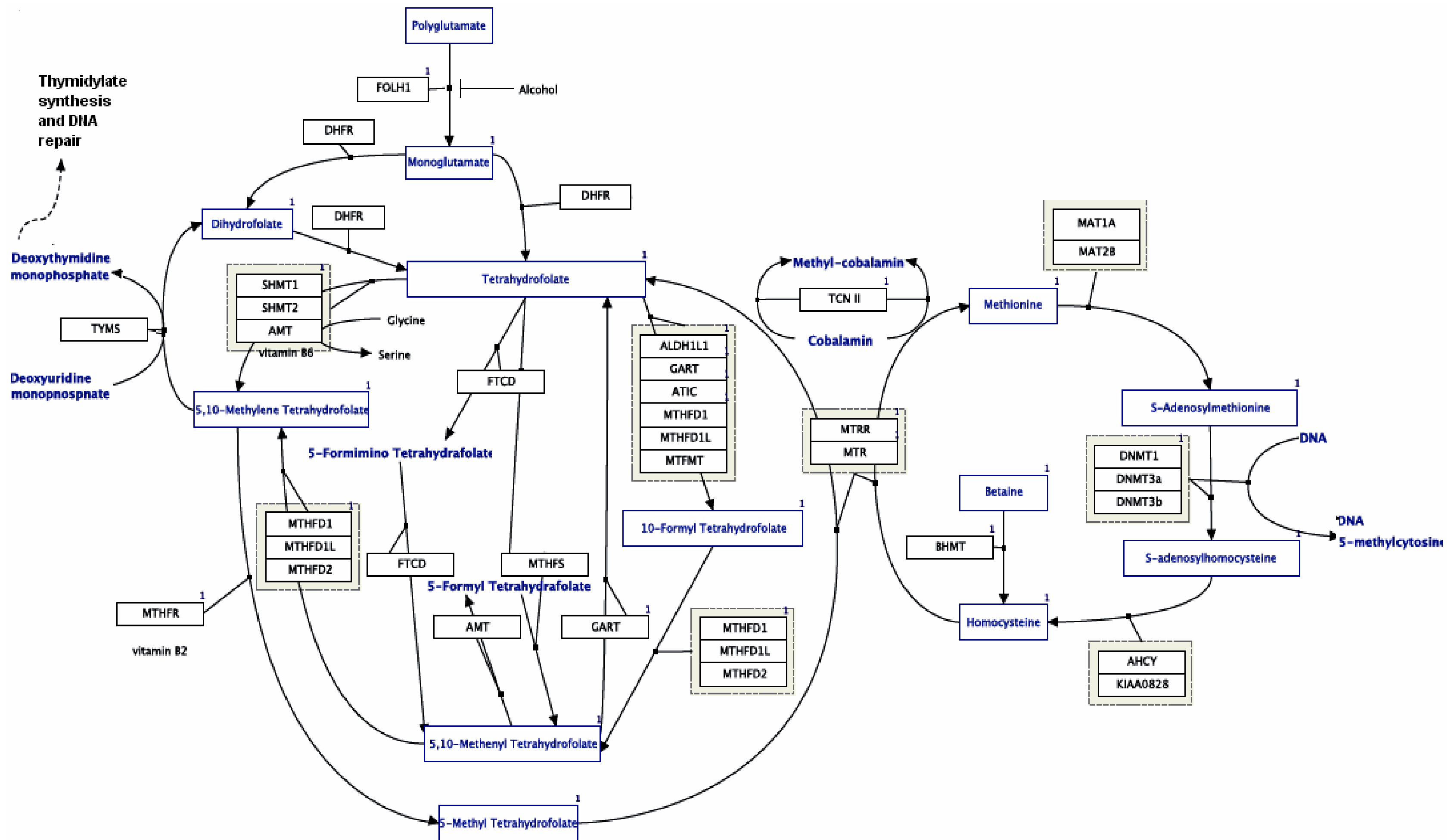

Figure 4-1. Diagram of the folate metabolism pathway. Source: Wikipathways One Carbon Metabolism (Homo sapiens) (http://www.wikipathways.org/index.php/Pathway:WP241).[150]

Review of literature on vitamins, folate, iron supplementation, and childhood leukemia

Case-control studies investigating the associations between maternal multivitamin/folic acid and/or iron supplementation during pregnancy and pediatric leukemia are summarized in Appendix H and are described below.

Infant leukemia. Two prior studies examining the associations between maternal prenatal supplementation and the development of infant acute leukemia in offspring were identified. Pombo-de-Oliveria *et al* reported no association between maternal consumption of vitamins or iron supplements during pregnancy and infant leukemia (including both ALL and AML) among those diagnosed at <21 months in Brazil (OR = 0.90, 95% CI: 0.63-1.28).[151] Similarly, in a report from the Children's Cancer Group (CCG), Wen *et al* observed no association with maternal vitamin use in the year before and during the index pregnancy (OR = 0.9, 99% CI: 0.1-7.1) or iron use during the index pregnancy (OR = 1.1, 99% CI: 0.3-3.9) in those diagnosed with ALL at <1 year.[41]

Childhood ALL. An additional 10 population-based case-control studies analyzed the associations with childhood leukemia (including infants). Of the 7 studies examining maternal vitamin supplementation and childhood ALL, 4 reported significant inverse effects in the range $0.40 \leq OR \leq 0.84$,[39-42] while another reported a nonsignificant inverse association[92] and 2 reported associations near the null.[93, 94]

The largest study to date, conducted by the CCG, reported a 30% reduction in risk of ALL associated with maternal vitamin consumption (99% CI: 0-50%).[41] The association was found to vary by time period of exposure, such that vitamin supplementation during the index pregnancy was associated with a reduction in risk of ALL (OR = 0.7, 99% CI: 0.5-1.0), while supplementation in the year before pregnancy was not. They also reported an inverse association for iron supplementation during pregnancy only (OR = 0.8, 99% CI: 0.7-1.0).

Ross *et al,* in a Children's Oncology Group (COG) study of leukemia among children with Down syndrome, observed an overall reduction in risk of ALL, although there was no evidence of a monotonic dose response with respect to the number of supplements consumed.[40] Importantly, they also noted differences in the effect based on the time period of observation. Vitamin supplementation in the periconceptional period (before pregnancy and early in pregnancy prior to knowledge of pregnancy) was associated with reduced risk (OR = 0.51, 95% CI: 0.30-0.89), while supplementation after knowledge of pregnancy was associated with increased risk (OR = 1.70, 95% CI: 0.98-2.92). This latter observation is consistent with results from recent studies of folic acid supplementation and colorectal cancer, where a dual role for folate has been postulated. Supplementation prior to polyp formation (the initiating event in colorectal cancer) is thought to be protective, while supplementation after polyp formation is thought to promote tumor growth.[152, 153] No association was observed between iron supplement use and ALL.[40] The questionnaire items regarding vitamin and iron supplementation were identical to those used in the current study.

Thompson *et al* also observed an inverse effect (OR = 0.40, 95% CI: 0.21-0.73), although they were unable to determine whether folic acid alone or in combination with iron was important etiologically.[42] (Iron supplementation without folic acid was not significantly associated with ALL.) Unlike the COG study, the initiation of vitamin supplementation across each of the three time periods examined, as well as each of the three durations of use, was associated with a significant reduction in risk; the strength of the association did not vary by either of these exposure variables.

Schüz *et al* observed an overall inverse association with vitamin, folate, and/or iron use; however, they note that the prevalence of maternal vitamin consumption was positively correlated with familial income levels in their study.[39] Since individuals with higher socioeconomic status (SES) tend to participate in case-control studies of childhood cancer,[124] they caution that the observed effect may be attributable to selection bias.[39]

Kwan *et al* reported an inverse association between iron supplement use from 3 months prior to pregnancy through the end of breastfeeding and childhood leukemia (OR: 0.70, 95% CI: 0.51-0.97),[154] while McKinney *et al* did not find evidence of an association between iron use during pregnancy and leukemia or ALL, respectively.[155]

Two meta-analyses were conducted on subsets of these studies with respect to childhood ALL. Upon pooling of 3 studies, the fixed effects summary odds ratio computed by Goh *et al* indicated a significant reduction associated with childhood ALL (OR = 0.61, 95% CI: 0.50-0.74).[31] Dockerty *et al* did not find evidence of an association with ALL upon pooling results from 3 different studies (OR = 0.9, 95% CI: 0.8-1.1).[93]

An analysis from the Northern California Childhood Leukemia Study examining total folate consumption and iron intake, respectively, in the pre-pregnancy diet (a surrogate for maternal prenatal diet) resulted in nonsignificant inverse associations.[156] The authors acknowledge the limitation that supplement use generally increases during pregnancy and therefore, pre-pregnancy diet may not be an adequate surrogate measure. They also noted the positive correlation between SES and vitamin use as a possible source of selection bias.

Finally, a case-case analysis did not find evidence of an association between maternal prenatal vitamin consumption and *ras* gene mutations among childhood ALL cases.[157]

Childhood AML. For AML (and acute non-lymphocytic leukemia, ANLL), each of the 3 identified studies failed to find evidence of an association with maternal prenatal vitamin and/or iron supplementation.[39, 40, 95]

It is important to note that there was heterogeneity in the specific exposures measured and the exposure windows considered across all of the identified studies.

Review of the literature regarding SNPs in the folate metabolism pathway and childhood leukemia

Infant Leukemia. A single study examining folate metabolism SNPs and infant leukemia has been reported in the literature, to our knowledge. Wiemels *et al* examined the distribution of two non-synonymous coding SNPs in *MTHFR* at nucleotide positions 677

(C>T) and 1298 (A>C) in 37 cases of MLL+ infant leukemia versus 200 cord blood controls.[158] Although the number of cases was modest, they reported a reduced risk for acute leukemia (ALL and AML) associated with the CT (OR = 0.29, 95% CI: 0.09-0.79) and TT (OR = 0.67, 95% CI: 0.16-2.53) genotypes at position 677, as well as for the CT and TT genotypes combined (OR_{CT+TT} = 0.36, 95% CI: 0.15-0.85). They did not find evidence of an association with the SNP at nucleotide 1298 (OR_{AC} = 1.35, 95% CI: 0.53-3.63; OR_{CC} = 1.33, 95% CI: 0.32-5.29) or among carriers of the C allele (OR_{AC+CC} = 1.14, 95% CI: 0.49-2.73). In contrast, there were no significant associations for SNPs at these loci among childhood ALL cases with TEL-AML1 translocations.

Although subject ages were not specified, Lightfoot *et al* observed an association between MLL+ leukemia (n = 34 cases) and the homozygous variant GG genotype of the *MTR* SNP at position 2756 (OR = 4.90, 95% CI: 1.30-18.45).[159] They did not observe associations for polymorphisms in *MTHFR*, *SHMT1*, or *TYMS*, however.

Childhood leukemia. Results of prior childhood leukemia studies of polymorphisms in the folate metabolizing genes *MTHFR, MTR, MTRR, MTHFD1, NNMT, RFC1, SHMT1,* and *TYMS*, as well as those examining gene-gene and gene-environment interactions, are described below. Of note, all but 2 of these investigations were restricted to childhood ALL (2 examined AML[159, 160]) and all but 2 of them[159, 161] evaluated the child's, but not the mother's, genotype. Only 1 childhood leukemia study has examined gene-environment interactions of 2 SNPs in the folate metabolism pathway[162] and 3 studies have assessed gene-gene interactions.[159, 163, 164]

MTHFR.

A number of studies have investigated the association between childhood ALL and SNPs in *MTHFR*, allowing for the conduct of two meta-analyses published in 2006. The first meta-analysis by Zintaras *et al* included 4 studies of the C677T SNP.[165] An analysis contrasting the frequency of the T versus C allele yielded a statistically significant inverse summary OR of 0.74 (95% CI: 0.57-0.96); the inverse association at position 677 held when different allelic contrasts were conducted (i.e., comparison of homozygotes, recessive model, dominant model). In addition, 3 studies investigating the A1298C SNP were pooled to compare the frequency of the C versus A allele and resulted in a nonsignificant inverse summary estimate (OR = 0.87, 95% CI: 0.68-1.11). The authors caution that there is moderate to high heterogeneity among the results of the included studies for the SNP at position 1298.

The second meta-analysis by Pereira *et al* included a greater number of studies (677: 10 studies and 1289: 8 studies) but failed to find evidence of an association at either locus under a recessive model (677: $OR_{TT\ vs.\ CT+CC}$ = 0.88, 95% CI: 0.73-1.06; 1289: $OR_{CC\ vs.\ AC+AA}$ = 0.80, 95% CI: 0.56-1.16).[166] The authors note that the results of the pooled analysis for the 677 locus are robust to model selection (fixed versus random effects and different genetic models) and various sensitivity analyses. There was modest heterogeneity in the studies of the 1298 SNP. In contrast to the pediatric results, they observed a statistically significant inverse association for the 677 SNP among adult ALL patients upon pooling data from three studies ($OR_{TT\ vs.\ CT+CC}$ = 0.45, 95% CI: 0.26-0.77). There was no association at nucleotide position 1298 in adult cases.

At least 5 additional studies of childhood ALL and *MTHFR* SNPs have been published subsequent to the two meta-analyses. In an analysis of UKCCS data, Lightfoot *et al* observed an inverse association with the T allele at position 677 among AML (n = 58; OR_{CT} = 0.51, 95% CI: 0.30-0.87; OR_{CT+TT} = 0.61, 95% CI: 0.38-0.98) but not ALL cases.[159] Conversely, an inverse association with the C allele at position 1298 was found among ALL (n = 685; OR_{AC} = 0.79, 95% CI: 0.64-0.97; OR_{AC+CC} = 0.79, 95% CI: 0.64-0.97) but not AML cases. The latter association was restricted to B-lineage ALL cases upon stratification by subtype. In a Dutch study of 245 pediatric ALL cases and 500 controls, de Jonge *et al* detected an inverse association with the 677 T allele (OR_{CT} = 0.7, 95% CI: 0.5-1.0; OR_{CT+TT} = 0.7, 95% CI: 0.5-1.0) and failed to detect an association for the 1298 C allele.[164] In a Slovenian study, Petra *et al* reported no significant difference in the distribution of the C677T/A1298C haplotypes between 68 ALL cases and 258 controls; the ORs were >1 for 5 of the 6 variant haplotypes observed (range: $0.81 \leq OR \leq 2.34$).[163]

A Korean study involving 66 cases and 100 controls did not find evidence of an association with one or more copies of the T allele at position 677, although a monotonic dose response was suggested (no p-value was provided for the trend).[167] They reported elevated odds of ALL associated with the AC genotype at position 1298 (OR_{AC} = 2.22, 95% CI: 1.09-4.51) but there were only 1 case and 2 control participants with the CC genotype, limiting their power to detect an association. Similarly, a Filipino study, which included 191 ALL cases and 394 controls, did not report a greater frequency of the CT and TT genotypes at nucleotide 677 among cases, but did observe a higher case

frequency of the AC and CC genotypes at position 1298 (OR_{AC} = 1.48, 95% CI: 1.00-2.19; OR_{CC} = 1.86, 95% CI: 1.11-3.10; OR_{AC+CC} = 1.57, 95% CI: 1.08-2.28).[168] Interestingly, the 677 T allele is much less prevalent in Filipino children then in Caucasian children (10% versus 30%, respectively) while the 1298 C allele is more common (37% versus 32%, respectively). The elevated ORs reported in the latter two studies for the 1298 SNP are in direct opposition to the inverse associations reported earlier.

A Brazilian study examined the distribution of the same *MTHFR* SNPs among 182 childhood and adolescent AML patients and 315 healthy controls.[160] They did not observe an association with respect to either SNP among white children/adolescents; however, among non-whites they found a 2.7-fold decreased risk associated with the heterozyote genotype at position 677 (OR_{CT} = 0.37, 95% CI: 0.14-0.92), as well as an inverse association for one or more copies of the variant (T) allele (OR_{CT+TT} = 0.45; 95% CI: 0.20-1.02), and a 2.9-fold increased risk associated with the 1298 heterozygote genotype (OR_{AC} = 2.90, 95% CI: 1.26-6.71), with an increased risk for one or more copies of the variant (C) allele (OR_{AC+CC} = 2.32; 95% CI: 1.06-5.11).

Two studies have examined maternal genotype in reference to leukemia in offspring. UKCCS investigators examined associations between maternal genotype for *MTHFR* SNPs C677T and A1298C and childhood ALL and AML, respectively; they did not find evidence for an association with either locus for either malignancy.[159] In a Canadian case-control study, Krajinovic *et al* also failed to observed an association between maternal genotype for these SNPs and childhood ALL, although the relatively

small number of mothers included in this analysis (n = 94) may have limited statistical power to detect an association.[161]

MTR. The A-to-G transition at nucleotide position 2756 of *MTR* has been investigated in 4 prior studies of childhood leukemia. Lightfoot *et al* reported significant positive associations for ALL (OR_{AG} = 1.24, 95% CI: 1.00-1.53; OR_{GG} = 1.88, 95% CI: 1.16-3.07; OR_{AG+GG} = 1.31, 95% CI: 1.07-1.60) and AML (OR_{GG} = 2.74, 95% CI: 1.07-7.01; OR_{AG+GG} = 1.62, 95% CI: 1.01-2.60) upon genotyping the index children, but no associations among their mothers.[159] Gast *et al* genotyped 460 childhood ALL patients and 552 controls and found a positive association among heterozygotes (OR_{AG} = 1.2, 95% CI: 1.0-1.8), but no association among homozygotes.[169] Petra *et al* reported no association for one or more copies of the variant allele, although their results may indicate an inverse association.[163] No association was observed in the Dutch study for this locus.[164]

MTRR. Associations between childhood ALL and 4 *MTRR* polymorphisms have been explored in the literature. For the A66G SNP, Gast *el al* reported an inverse association for both heterozygotes (OR_{AG} = 0.7, 95% CI: 0.5-1.0) and homozygotes (OR_{GG} = 0.6, 95% CI: 0.4-0.9),[169] while de Jonge *et al* and Petra *et al* observed nonsignificant inverse associations.[163, 164] Gast *et al* did not compute significant ORs for SNPs at positions 524 (C>T), 1049 (A>G), or 1783 (C>T), however.[169] Results of a haplotype analysis (A66G/C524T/A1049G/C1783T) indicate that the ACAC (OR = 2.3, 95% CI: 1.8-2.9)

and GTAC (OR = 1.8, 95% CI: 1.4-2.3) haplotypes are associated with increased risk of ALL, while the GCAC haplotype is associated with reduced risk (OR = 0.5, 95% CI: 0.4-0.6).[169]

MTHFD1. Gast *et al* evaluated two polymorphisms of *MTHFD1*, including SNPs at positions 401 (G>A) and 1958 (G>A), yielding no statistically significant associations with pediatric ALL.[169]

NNMT. de Jonge *et al* examined the IVS -151 (C>T) polymorphism and found a positive association for pediatric ALL among homozygous variants (OR_{TT} = 2.2, 95% CI: 1.1-4.6).[164]

RFC1. In examining the *RFC1* SNP at position 80 (G>A), Gast *et al* reported a positive association with ALL among heterozygotes (OR_{GA} = 1.4, 95% CI: 1.1-1.9) and a null association among homozygous variants. de Jonge *et al* also observed a positive association with ALL at this locus (OR_{AA} = 2.1, 95% CI: 1.3-3.2; OR_{GA+AA} = 1.5, 95% CI: 1.1-2.1).[164]

SHMT1. There were no observed associations for the C-to-T transition at position 1420 in relation to childhood ALL in the studies by Lightfoot *et al*, de Jonge *et al,* and Gast *et al.*[159, 164, 169] There was also no association observed for pediatric AML in the UKCCS study.[159]

TYMS. The *TYMS* polymorphisms of interest include a 28-base pair (bp) repeat in the 5' untranslated region (UTR), where 2 repeats (2R) is the more prevalent allele and 3 repeats (3R) can be considered the variant, and a 6-bp deletion in the 3'UTR. deJonge *et al* observed a reduced risk for pediatric ALL among heterozygotes for the 2R/3R polymorphism ($OR_{2R/3R}$ = 0.7, 95% CI: 0.5-1.0);[164] none of the other 3 studies examining this polymorphism indicated an effect with respect to childhood ALL[159, 163, 169] or AML,[159] however, although a monotonic dose response was suggested in the Slovenian study (no p-value was provided for the trend). Lightfoot *et al* observed a positive association between homozygous variants for the 6-bp deletion among both ALL ($OR_{6bp-/6bp-}$ = 1.46, 95% CI: 1.02-2.08) and AML cases ($OR_{6bp-/6bp-}$ = 2.04, 95% CI: 1.03-4.03).[159] There was no evidence of an association with the 6-bp deletion in the German study, however.[169]

Gene-gene interactions. Petra *et al* evaluated multiplicative gene-gene interactions between *MTHFR, MTR, MTRR* and *TYMS* via logistic regression.[163] They report an interaction of borderline significance between carriers and non-carriers of the *MTHFR* 677 T allele and the *MTR* 2756 G allele across cases and controls (OR = 0.45, 95% CI: 0.19-1.04), as well as a significant interaction between carriers and non-carriers of the *MTHFR* 677 T allele, the *MTR* 2756 G allele, and the *MTRR* 66 G allele (OR = 0.31, 95% CI: 0.11-0.91). De Jonge *et al* found 2 significant multiplicative interactions, such that those who are homozygous for the wild-type *NNMT* IVS-151 C allele and carriers of

the *MTHFR* 677 T allele have reduced risk of pediatric ALL (OR = 0.5, 95% CI: 0.4-0.8), and those who are homozygous for the *RFC1* 80 A allele and carriers of the *NNMT* IVS-151 T allele have increased risk (OR = 4.2, 95% CI: 1.8-9.7).[164] Lightfoot *et al* observed evidence of a multiplicative interaction between the *MTR* 2756 SNP and the *TYMS* 6bp deletion among ALL cases, such that heterozygotes for the *MTR* SNP were more likely to have ≥1 copies of the *TYMS* deletion (p = 0.05).[159] There were also 2 suggested interactions between the *MTHFR* 1298 SNP and the *SHMT1* 1420 SNP (p = 0.09) and the *TYMS* 3R polymorphism (p = 0.11), respectively.

Gene-environment interactions. In a case-only analysis involving 82 case-mother pairs, Milne *et al* observed no statistically significant evidence of an interaction between child's genotype and maternal prenatal vitamin supplementation on the multiplicative scale for either the 677 or 1298 *MTHFR* SNP, after adjustment for sex, age, and the genotype at the other locus.[162] For both loci, the ORs suggested that the odds of ALL were greater than the products of the independent effects when one or more copies of the variant allele were present and the mother reported no prenatal folate consumption (OR_{677} = 1.40, 95% CI: 0.73-2.67, OR_{1298} = 1.25, 95% CI: 0.65-2.40), but were lower than expected in the presence of folate (OR_{677} = 0.59, 95% CI: 0.26-1.31, OR_{1298} = 0.49, 95% CI: 0.20-1.19). Of note, a prior case-control analysis from this study indicated an inverse association with maternal prenatal vitamin supplementation;[42] the main effects of the SNPs could not be investigated via this study design.

Krajinovic *et al* investigated the potential for modification of the effect of the two *MTHFR* SNPs by national folic acid fortification in Canada beginning in 1996.[161] Upon stratification on date of birth (before January 1, 1996 versus January 1, 1996 or thereafter), they observed a significant inverse association for *MTHFR* haplotypes including one or more variant alleles only among children born prior to 1996, with ORs in the range 0.3-0.6. The ORs among children born in 1996 or later were nonsignificant, but may suggest an increase in risk overall ($1.3 \leq OR \leq 3.0$).

Possible mechanisms of leukemogenesis

Two potential mechanisms involving DNA instability have been suggested in the literature to explain the association between folate deficiency and cancer (reviewed in [170, 171]). The first mechanism concerns folate's involvement in the synthesis of nucleotide precursors. The theory suggests that in a state of folate deficiency, the methylation of deoxyuridine monophosphate (dUMP, a uracil precursor) by *TYMS* to produce thymidine monophosphate (TMP) is diminished, causing uracil to be inserted into DNA instead.[170, 172] Uracil DNA glycosylase excises the misplaced uracil, thereby creating a transient single strand break (SSB); when two of these SSBs arise simultaneously in close proximity (within ~14 bp), double strand breaks (DSBs) ensue.[172] Of note, in situations of folate deficiency, high levels of uracil are misincorporated in DNA (at a rate of 4 million per cell) and results of mathematical modeling suggest the probability of two proximal uracil moieties increases rapidly with increasing uracil insertion.[172] It has also been demonstrated that DNA extracted from blood and bone marrow of folate deficient individuals has increased numbers of DSBs.[172]

DSBs may be repaired by either homologous recombination (HR) or by non-homologous end-joining (NHEJ).[10, 173] NHEJ is responsible for the majority of DSB repairs in multicellular eukaryotes and although it is a very flexible mechanism, it is also less accurate than HR and can result in translocations or other chromosomal rearrangements.[10, 173] Chronic folate deficiency with repeated uracil misincorporation and excision is thought to lead to a "catastrophic" DNA repair cycle, chromosomal aberrations, and ultimately to malignant transformation.[170]

There is support for this potential mechanism with respect to MLL+ infant leukemia. In a study of t(4;11) translocations, Reichel *et al* observed that (1) a minimum of one DSB on one chromosome and a SSB on another must have occurred prior to the translocation event and (2) small segments of DNA adjacent to the break points had been deleted, inverted, or duplicated in the translocation.[174] A follow-up report from the same research group indicates that the observed t(4;11) translocations are consistent with NHEJ repair.[175]

The most frequently observed *MLL* rearrangements include translocations t(4;11) (MLL-AF4), t(11;19) (MLL-ENL), and t(9;11) (MLL-AF9) in infant ALL[128] and t(6;11) (MLL-AF6), t(9;11) (MLL-AF9), t(10;11) (MLL-AF10) and t(11;19) (MLL-ENL) in infant AML;[10, 129] these translocations are similar to one another in that they occur in-frame and result in functional chimeric fusion gene proteins that act as modified transcription factors.[10] The novel transcription factors have been shown in to act in a dominant gain-of-function manner to promote hematopoietic cell transformation in mouse models.[176] They do not appear to be sufficient for leukemogenesis, however, as a

latency period follows the *MLL* rearrangement, suggesting additional hits are needed.[176] Possible mechanisms of action by which they increase cell susceptibility to additional genetic aberrations include inducing rapid proliferation of the affected cell, increasing DNA instability, or blocking DNA repair mechanisms.[8] Notably, MLL+ cells show an accumulation of other chromosomal abnormalities at diagnosis, such as alterations in tumor suppressor gene *p53*, the *ras* oncogene, and *FLT3*, which is important to the proliferation and differentiation of primitive hematopoietic stem cells.[8] (Copy number variation was notably absent, however, among infant MLL+ cases with t(4;11) translocations.[177]) A modified version of the mechanism as it applies to infant leukemia is shown in Figure 4-2 below.

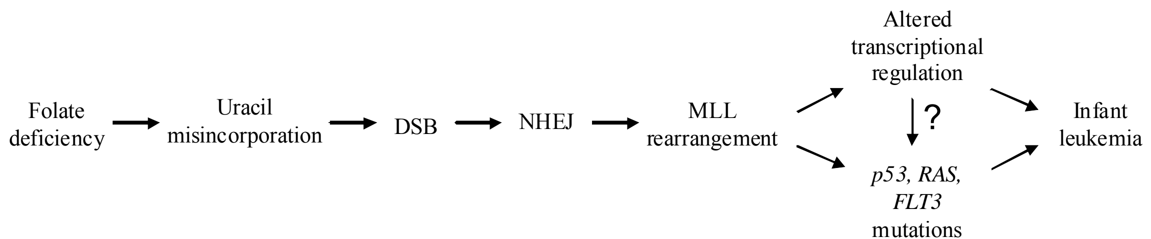

Figure 4-2. Proposed mechanism for the development of MLL+ infant leukemia

The second mechanism relates to potential epigenetic effects due to the participation of folate as a cofactor in the DNA methylation pathway. Here, folate deficiency reduces the available pool of SAM, the principle methyl donor for DNA, leading to DNA hypomethylation. Hypomethylation occurring within a proto-oncogene promoter region can result in the activation of the proto-oncogene, eventually resulting in malignant transformation.[170]

Global hypomethylation has been observed in several malignancies.[178] In a study of 307 childhood and adult ALL cases, Roman-Gomez *et al* observed global hypomethylation within leukemic cells; the functional significance of this observation is not yet known.[178] More specifically, prior studies of solid tumors have found hypomethylation within proto-oncogenes[170] and Watt *et al* observed that the promoter region of *HOX11* was demethylated in leukemic cells from ALL cases expressing the gene, suggesting that hypomethylation activates the proto-oncogene.[179] Importantly, results of murine feeding studies indicate that folate deficiency is sufficient to induce hypomethylation of proto-oncogenes.[170]

At this point it is not clear whether maternal or fetal genes, or a combination of the two, are the most important determinants of folate metabolism with respect to infant leukemia. Labuda *et al* reported that parental genes at some loci may be important predictors of ALL in offspring.[180] Most prior studies of childhood leukemia have been limited to evaluation of the child's genotype, however; the 2 prior studies examining maternal SNPs failed to show an association with ALL or AML in their children.[159, 161] Christensen *et al* observed that the risk of NTDs increased when mothers were carriers of the variant T allele for the *MTHFR* SNP at position 677; the observed risk was greater when both mother and child were carriers.[181] Further, research by Jirtle and colleagues has demonstrated that varying the levels of maternal gestational folic acid consumption can modulate offspring phenotype (i.e., size, coat color) via DNA methylation in the viable yellow agouti (A^{vy}/a) mouse.[182]

Iron supplementation and leukemogenesis

Prenatal iron supplementation may indicate low iron levels, anemia, or a desire to prevent anemia, and maternal anemia was associated with childhood leukemia in three prior case-control studies.[81, 82, 102] The inverse associations between maternal prenatal iron supplementation and childhood leukemia previously observed in some[41, 154], but not all, studies are consistent with an etiologic model in which iron deficiency is related to childhood leukemia. Notably, in a prior report from the first phase of the current study, there was no evidence for an association between maternal iron supplement use or maternal anemia during pregnancy, as recorded in the medical record, and infant leukemia, ALL, or AML, or among the *MLL* subgroups.[183]

On the contrary, high iron levels may be detrimental to developing fetuses[184] and have been associated with increased cancer risk in adults.[185] The biological mechanism is unknown, however, it has been hypothesized that excess iron may generate reactive oxygen species that exert a genotoxic effect or may act by increasing cell proliferation or altering immune function.[186] An association between prenatal iron supplementation and childhood leukemia may exist only in the presence of genetic variants. Associations between SNPs in two iron homeostasis genes (i.e., hereditary hemochromatosis gene *(HFE),* hepcidin antimicrobial peptide *(HAMP))* and childhood leukemia have been observed on the order of $2.00<OR<3.00$ among British populations.[187, 188]

Theoretical model of causation

The hypothesized association between maternal folic acid consumption and infant leukemia is depicted in the directed acyclic graph (DAG) in Figure 4-3 below, where

folic acid (F) causes underlying biological changes (B), leading ultimately to a reduced risk of infant leukemia (L).

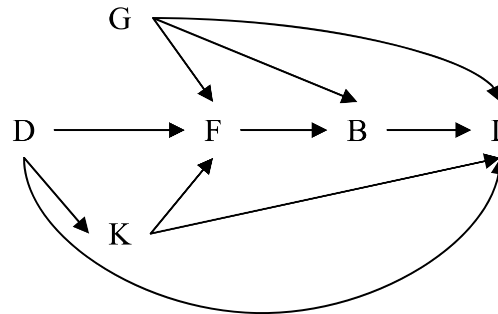

Figure 4-3. Directed acyclic graph depicting the hypothesized association between folic acid consumption and infant leukemia, where D = demographic factors (e.g., sex, race, maternal age, socioeconomic status), F = folic acid, B = underlying biological changes, L = infant leukemia, G = genetic susceptibility, and K = lifestyle factors (e.g., maternal prenatal consumption of alcohol or tobacco).

The commonly cited epidemiological definition of a confounder is a variable that is a risk factor for the outcome of interest, associated with the exposure of interest in the source population, and is not on the causal pathway.[189] This definition has been found to be insufficient, however. The structural definition of confounding is bias that is created by an unblocked backdoor path via a common cause of the exposure and disease of interest.[190] In the analysis, conditioning on one or more confounding variables in a path blocks the backdoor path.[190] Here, potential confounders include demographic factors (D), such as the sex and race of the child, maternal age, and familial socio-economic status (SES), and lifestyle factors (K), such as maternal consumption of alcohol or

tobacco products. A brief literature review of these factors is provided below. (Please note that it is difficult to represent gene-environment interactions in a DAG.)

Sex. The incidence rate of infant leukemia is 24% higher among female infants than among male infants ($RR_{Leukemia}$ = 1.24, 95% CI: 1.01-1.52; RR_{ALL} = 1.74, 95% CI: 1.29-2.37; RR_{AML} = 1.15, 95% CI: 0.80-1.66).[191] Although it seems improbable that mothers would vary their vitamin consumption based on the gender of their unborn child (if the gender was even known), the possibility remains that the vitamins may be processed differently by male versus female fetuses. Therefore, sex will be considered as a potential confounder.

Race/Ethnicity. Maternal vitamin consumption has been associated with race. A report from the CDC's Pregnancy Risk Assessment Monitoring System (PRAMS) 2000 data indicates that women reporting a race of white or other were significantly more likely than black women, and non-Hispanic women were more likely than Hispanic women, to report multi-vitamin consumption in the month before the index pregnancy (a surrogate for pregnancy vitamin use) in a majority of U.S. states surveyed.[137] These data do not support a protective role for folate with respect to leukemogenesis, however, as infant leukemia is diagnosed 30% and 15% more often among white children in the U.S. than among black or Asian/Pacific Islander children, respectively ($RR_{white/black}$ = 1.30 95% CI: 0.93-1.82; $RR_{white/API}$ = 1.15, 95% CI: 0.81-1.67).[191]

Maternal age. Increasing maternal age was positively correlated with multi-vitamin supplementation in the month prior to conception in the PRAMS report in all of the 19 states examined,[137] suggesting older mothers would be more likely to ingest prenatal vitamins. Similarly, a United Kingdom study of determinants of folic acid supplementation in the first trimester of pregnancy observed that women <21 years were less likely than women ≥21 years to report supplement use.[192] Maternal age at the time of the index child's birth has been inconsistently associated with infant leukemia, however. A recent report found the distribution of age among case mothers was significantly greater than that of control mothers,[151] while a second study indicated a lower risk for maternal age <20 years (OR = 0.53, 95% CI: 0.32-0.88) and no association with maternal ages ≥35 years,[193] and a third study did not report any statistically significant associations, but did observe a suggested protective effect for maternal age >20 years.[194] Two earlier studies did not find evidence of an association with maternal age.[195, 196] It is therefore difficult to predict the degree or direction of confounding that maternal age may have on the association of interest.

Maternal education level. In all 19 states queried in PRAMS, women reporting >12 years of education were significantly more likely to confirm preconceptional multivitamin use than women with ≤12 years of education.[137] The association between maternal educational attainment and infant leukemia has been inconsistently reported in three prior studies. A report from a series of three studies conducted by the CCG described a trend of reduced risk of infant leukemia with increasing maternal education compared with those

who had attended some high school or less ($OR_{HS\ graduate}$ = 0.49, 95% CI: 0.28-0.85; $OR_{>HS\ graduate}$ = 0.40, 95% CI: 0.24-0.69),[194] and a Brazilian study found a different distribution of education among case and control mothers (p = 0.001), but no obvious pattern was observed,[151] while a study in California reported null associations across all levels of maternal educational attainment.[193] Of note, each of these studies used slightly different definitions of "infants."

SES/Income. Medicare recipients were significantly less likely to indicate multivitamin use prior to conception than those not receiving Medicare across all 19 states participating in PRAMS.[137] Likewise, the UK study reported lower folic acid use in the first trimester of pregnancy among women in lower social classes.[192] An analysis of the 1987 National Health Interview Survey (NHIS) responses also indicated a similar finding; multivitamin use increased incrementally among U.S. adults with increasing income (<$5,000: 11.1% versus >$50,000: 20.9%).[197] In contrast, higher socioeconomic status is considered an established risk factor for childhood leukemia, although the underlying mechanism has not been confirmed.[4] Infant leukemia data presented by Pombo-de-Oliveira *et al* are consistent with this general finding; they observed a greater distribution of wealth among families of cases (p = 0.001).[151] Ross *et al* found the converse in their analysis of three CCG studies; compared to the lowest income group (<$10,000), they observed an inverse association with infant leukemia for all higher income groups examined (p_{trend} = 0.03).[194]

Maternal prenatal alcohol exposure. Alcohol consumption may block the absorption of folate from food.[149] An analysis of the 1987 NHIS data failed to find evidence of an association between frequency of alcohol use and multivitamin supplementation among U.S. adults, however.[197] Surprisingly, the 1999-2000 National Health and Nutrition Examination Survey (NHANES) data indicated that supplement use increased with increasing consumption of wine and distilled spirits, but found no differences by frequency of beer consumption.[198] The literature on the association between maternal prenatal alcohol use and childhood leukemias has also been inconsistent. In study of infant leukemia, Alexander *et al* reported no association with maternal alcohol consumption during pregnancy overall or for any of the diagnostic subgroups examined (MLL+, MLL-, ALL, AML).[97] Of the 11 studies evaluating maternal prenatal alcohol consumption and childhood leukemia or ALL, 5 observed no evidence of an association,[38, 85, 90, 96, 97] while 3 found significantly increased risk associated with any use during pregnancy, with ORs in the range of 1.4-2.0,[98-100] and a fourth study reported a nonsignificant positive association for any alcohol use during pregnancy.[101] Two studies observed inverse ORs of 0.57 (95% CI: 0.34-0.95)[102] and 0.7 (95% CI: 0.5-0.9),[103] respectively. Of the 7 studies investigating the association with AML, 3 failed to find evidence of an association,[97, 99, 100] while 3 found evidence of a sizable positive association with ORs in the range 1.9-2.6,[96, 98, 101] and a fourth reported a nonsignificant positive association.[104]

Maternal prenatal tobacco smoke exposure. Data from the 1987 NHIS indicate that current smokers (15.7%) are significantly less likely than never (23.6%) and former smokers (26.0%) to report multi-vitamin use.[197] This pattern was also observed in the 1999-2000 NHANES data (26.6% vs. 36.0% vs. 41.6%, respectively).[198] Similarly, the U.K. study indicated that smokers were less likely to consume folic acid supplements during the first trimester of pregnancy ($p<0.001$).[192] Although the data are consistent with respect to tobacco use and vitamin supplementation, they are less consistent with respect to maternal smoking during pregnancy and childhood leukemia. Alexander *et al* did not observe an association between maternal smoking during pregnancy and infant leukemia overall or for any of the subgroups examined (MLL+, MLL-, ALL, AML).[97] A meta-analysis did not provide evidence of increased risk of acute leukemias/ALL in children with maternal prenatal smoking (8 studies, OR = 1.05, 95% CI: 0.82-1.34). Several studies have been published since that meta-analysis was conducted. For acute leukemia, an additional 10 studies found no association,[85, 99-101, 107-112] while a large cohort study in Sweden computed an inverse association between maternal smoking in the first trimester and ALL (HR = 0.73, 95% CI: 0.58-0.91), but reported a nonsignificant positive association with AML (HR = 1.41, 95% CI: 0.74-2.67).[113]

Significance of the research

Leukemias diagnosed in infants have specific biological features compared to leukemias in older children, resulting in poor infant survival rates. These characteristics implicate a distinctive leukemic etiology among this unique patient population. Due to the early onset of disease, the prenatal time period and the maternal environment (as

determined by maternal genotype and exposure to other agents) are thought to play a particularly important role. The beneficial effects of prenatal folic acid consumption in preventing neural tube defects have been documented in randomized controlled trials. There is reasonable evidence to suspect a beneficial effect with respect to childhood leukemias as well, particularly among ALL cases. Based on one estimate, up to 900 cases of pediatric leukemia could be prevented in the U.S. annually by multivitamin supplementation;[31] it is unknown what proportion of these would be infant leukemias.

The Epidemiology of Infant Leukemia study is the largest study to date to investigate the unique etiology of acute leukemia among infants. The study enrolled 443 infant cases from participating Children's Oncology Group institutions across the U.S. and Canada, as well as 324 population-based controls. Mothers were asked to complete a one-hour telephone interview, provide access to their child's diagnostic information, and to provide biological specimens from themselves and/or their child for the purpose of genetic analysis.

In the current analysis, maternal prenatal vitamin and iron supplementation were assessed via maternal interview to estimate the main effects of these exposures with respect to the risk of infant leukemia.

Chapter 5: Maternal vitamin and iron supplementation and risk of infant leukemia: A report from the Children's Oncology Group

Sufficient maternal prenatal folic acid consumption reduces neural tube defects in offspring; epidemiologic evidence also suggests a preventative role in childhood leukemia. Infant leukemia originates *in utero* and may have a distinct etiology, as evidenced by the high prevalence of mixed lineage leukemia (*MLL*) gene translocations. This case-control analysis was performed to examine the associations between maternal prenatal vitamin and iron supplementation and infant leukemia. Mothers of 443 cases (n = 264 acute lymphoblastic leukemia (ALL), n = 172 acute myeloid leukemia (AML)) diagnosed at <12 months at COG institutions in 1996-2006 and mothers of 324 frequency-matched controls completed telephone interviews. The associations were evaluated via unconditional logistic regression, resulting in odds ratios (ORs) and 95% confidence intervals (CIs). After adjustment for race/ethnicity and income, there was little evidence supporting an association between periconceptional vitamin use (OR = 0.89, 95% CI: 0.64-1.24), use after knowledge of pregnancy (OR = 0.78, 95% CI: 0.48-1.28), or use in all periods (OR = 0.84, 95% CI: 0.62-1.14) and infant leukemia. Likewise, there was little evidence of an effect of periconceptional iron supplementation (OR = 1.23, 95% CI: 0.63-2.38), use after knowledge of pregnancy (OR = 1.06, 95% CI: 0.74-1.53), or use in all periods (OR = 2.54, 95% CI: 0.69-9.39). Similar results were observed among ALL and AML cases analyzed separately. Reduced risk was suggested in ALL MLL+ cases for vitamin use throughout the periconceptional/prenatal period (OR

= 0.66, 95% CI: 0.44-1.00). Overall, we found little evidence supporting these associations and speculate this may be attributable to high rates of supplementation and/or folic acid fortification programs implemented during the study period.

Introduction

Leukemias diagnosed in children ages <12 months ("infant leukemia") are distinct from leukemias in older children/adolescents. Most infant leukemias (~75% of acute lymphoblastic leukemias (ALLs) and ~60% of acute myeloid leukemias (AMLs)) are preceded by rearrangements of the mixed lineage leukemia (*MLL*) gene on chromosome 11 band q23.[125, 126] *MLL* rearrangements (denoted "MLL+") confer appreciably lower survival in infant ALL cases compared with MLL- cases (5-year survival: 5-34% versus 42-92%, respectively),[128] but may not affect the prognosis of infant AML.[129]

A high concordance rate of infant leukemia has been demonstrated for monozygotic twins (~100% versus ~10% in older children/adolescents); transmission of an affected clone via shared monochorionic placental circulation may be responsible.[8] This observation, coupled with results from backtracking studies of neonatal blood spots demonstrating that *MLL* translocations are consistently present at birth in infant leukemia cases[5], provides compelling evidence of *in utero* initiation. Further, the extremely short latency indicates the disease may progress to subclinical preleukemia prior to birth.[8]

There are few established risk factors for childhood leukemia, including predisposing genetic conditions, exposure to *in utero* or postnatal therapeutic irradiation, and exposure to chemotherapeutic agents.[4] Folic acid is a critical nutrient for developing fetuses; sufficient prenatal consumption of folic acid significantly reduces the incidence

of neural tube defects (NTDs) and congenital heart defects.[133, 135] Accordingly, the U.S. Public Health Service has recommended a minimum daily folate intake of 400 µg for women of childbearing age since 1992[136] and the U.S. and Canada implemented national folic acid fortification programs in 1998.[138, 139] Folic acid may be preventative for childhood leukemia as well, particularly among ALL cases.[39-42] Prenatal iron supplementation (30mg/day) is also recommended to meet high iron demands during pregnancy.[199] Maternal prenatal iron supplementation has been investigated as a risk factor for childhood leukemia with inconsistent results.[40-42, 93, 154, 155] The current analysis was conducted to investigate the effects of maternal prenatal vitamin and iron supplementation, respectively, on the risk of acute infant leukemia.

Methods

Details regarding this study have been previously published[200, 201] and are described below.

Participant eligibility/identification

<u>Cases.</u> Infants (<12 months) with confirmed diagnoses of ALL or AML (ICD-O codes[202]: 9801, 9803, 9805, 9821, 9823, 9835, 9836, 9837, 9861, 9863, 9867, 9871-9874, 9891, 9895-9897, 9910, 9913) during two time periods (Phase 1: January 1996 – October 2002 and Phase 2: January 2003 – December 2006) were eligible if they were diagnosed/treated at Children's Oncology Group (COG) institutions in the U.S. or Canada and had no prior Down syndrome diagnosis, physician approval for contact,

residential telephones, and biological mothers that spoke English (Phases 1 and 2) or Spanish (Phase 2) and consented to participate. Deceased cases were eligible.

Controls. Controls were frequency matched to cases on year of birth and location of residence. Controls were required to have a biological mother that spoke English or Spanish, consented to participate, and had a residential telephone.

In Phase 1, controls were identified via random digit dialing (RDD) via a revised version of the Waksberg method,[203] wherein telephone numbers were constructed from area codes and exchanges of cases, and remaining digits were randomly generated. Several contact attempts were made for each telephone number generated (≤9); if the number did not correspond to an eligible control, the family refused, or no contact was made, the next potential number was queried.

Due to secular trends in telephone usage,[204] RDD was not considered a viable option for Phase 2. Instead, rosters of potential controls, randomly selected based on the anticipated distribution of birth year of Phase 2 cases, were requested from state birth registries. Rosters were received from 15 states to represent the northeastern (NJ and PA), southeastern (FL, KY, NC, and TN), Midwestern (MI, MN, and MO), south central (LA and TX), northwestern (OR and WA), and southwestern (CA and UT) regions of the United States. Potential controls were randomly selected from these rosters. If mothers refused participation, replacement subjects were selected until willing participants were identified.

Data collection

Mothers were asked to complete 1-hour telephone interviews in which they were asked if they consumed vitamin supplements in four time periods (anytime in the year before or during the index pregnancy; in the year before pregnancy; early in but prior to knowledge of pregnancy; and after knowledge of pregnancy).[1] For each of these time periods, they were asked to specify the type of supplement(s) consumed and whether or not the supplement was prescribed by a health care professional. Indicator variables were then created to assess vitamin use in the periconceptional period (year before pregnancy and early in but prior to knowledge of pregnancy) and from 1 year before through the index pregnancy. An equivalent set of items concerned iron supplementation that exceeded the iron found in multivitamins.

Mothers of cases were also asked to release their child's diagnostic information, including results of any fluorescent in situ hybridization (FISH), Southern blot, or reverse transcription polymerase chain reaction (RT-PCR) analyses and/or other cytogenetics testing. Three independent reviewers (J.M.H., S.M.D., and N.A.H.) evaluated the submitted materials to determine if there was evidence of an *MLL* gene rearrangement (MLL+, n = 228), evidence of no translocation (MLL-, n = 146), or insufficient evidence to make a determination (n = 69).

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[1] Items from the interview instrument regarding maternal prenatal vitamin and iron supplementation are shown in Appendix I.

Statistical methods

Unconditional logistic regression (SAS 9.2, SAS Institute Inc., Cary, NC) was performed to quantify associations between maternal supplement consumption and acute leukemia among combined cases, and among ALL, AML, MLL+, MLL-, ALL MLL+, ALL MLL-, AML MLL+ and AML MLL- cases analyzed separately. Odds ratios (ORs) and 95% confidence intervals (CIs) were produced. Potential confounders selected *a priori*[m] are listed in Table 5-1. Variables were retained in the multivariable models if they substantially (≥10%) changed effect estimates for ALL and/or AML on the natural logarithm scale after adjustment for the other factors. Factors retained include maternal race/ethnicity (white, black, Hispanic, other) and household income in the year of the child's birth (≤\$30,000, \$30,001-\$75,000, >\$75,000). Adjustment for matching factors (year of birth, region of residence) did not materially alter point estimates, although confidence intervals were wider;[n] these factors were therefore not included in the final models, as they did not appear to act as confounders in these data.

To assess the robustness of the results, a number of stratifications were performed. The proportions of case mothers reporting vitamin use in the two phases and ORs corresponding to the two phases were compared to identify secular changes in vitamin use. To detect effects of folic acid fortification of the food supply, the ORs for vitamin use in three different time periods (before folic acid fortification, during implementation, after implementation) were compared. The time periods differed slightly

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[m] Potential confounders selected *a priori* included infant sex, birthweight, and gestational age; maternal age, race/ethnicity, and educational attainment; prior fetal loss; morning sickness; smoking during pregnancy; alcohol consumption during pregnancy; and household income in the year of the child's birth.
[n] Three cases born outside of North America had to be excluded for this analysis.

between the U.S. and Canada; these differences were accounted for in the analysis. Finally, ORs for vitamin use were compared across U.S. regions (defined above) or Canadian residence to detect geographic differences in supplementation.

Institutional Review Boards at the University of Minnesota and participating COG institutions approved the study. Ethics board approvals were also sought from states providing birth certificate data.

Results

A total of 443 case (n = 264 ALL and n = 172 AML) and 324 control interviews were completed across the two study phases. As previously reported, maternal interviews were completed for 240 of 348 eligible cases (69%) and for 255 of 430 eligible controls (59%) in Phase 1.[200] One control was subsequently excluded from analysis, as he/she was discovered in the interview to have Down syndrome. In Phase 2, 345 potential cases were identified through 133 participating COG institutions and 240 were enrolled. Of those eligible, 203 (59%) mothers completed interviews, while the remainder did not due to maternal refusal (22%), inability to locate the mother (11%), physician refusal (6%), and institutional failure to approach the mother during the study period (2%). Of 267 potential birth certificate controls, 70 completed interviews and 1 partially completed an interview (27%).[201] Results of our prior analysis indicate that controls from the two phases were similar enough on important demographic factors to warrant merging them.[201]

Cases and controls were similar with respect to infant gender, birthweight, and length of gestation, and maternal age at the index child's birth, prior fetal loss, and smoking during pregnancy (Table 5-1). Compared with control mothers, case mothers

were more likely to have less education (34% versus 28% with less than a high school diploma), be non-white (24% versus 15%), have a lower income (36% versus 30% making ≤$30,000, difference is nonsignificant), experience morning sickness (71% versus 63%), and report no alcohol consumption during the index pregnancy (86% versus 79%).

Notably, a large majority of case (91%) and control mothers (94%) reported any vitamin use in the year before and/or during the index pregnancy. After adjustment for race/ethnicity and income, there was little evidence supporting associations between any maternal vitamin use in the year before and/or during pregnancy (OR = 0.79, 95% CI: 0.44-1.42), in the periconceptional period (OR = 0.89, 95% CI: 0.64-1.24), after knowledge of pregnancy (OR = 0.78, 95% CI: 0.48-1.28), or use in all of these periods (OR = 0.84, 95% CI: 0.62-1.14) and infant leukemia (Table 5-2), although all ORs were <1.00. Restricting exposure to use only after knowledge of pregnancy generated comparable results to those described above (data not shown). Similar results were observed among ALL and AML cases analyzed separately (Table 5-2); ORs for ALL were consistently <1.00, while ORs for AML fluctuated around the null. Stratification on the presence of *MLL* translocations did not provide additional information, with the exception of ALL MLL+ cases, in whom a reduced risk was suggested for vitamin use throughout the periconceptional/prenatal periods (OR = 0.66, 95% CI: 0.44-1.00; Table 5-3).

The proportions of case mothers reporting vitamin use in the two phases were nearly identical (91% reported any vitamin use in both phases) and there was no evidence

of heterogeneity of the ORs for vitamin use across the phases (data not shown). There was also no evidence of heterogeneity upon stratification by folate fortification period or region of residence, although there was limited power to detect differences given the smaller cell counts after stratification (data not shown).

Fewer mothers reported any iron supplement use beyond multi- or prenatal vitamins (24% of cases versus 22% of controls). There was little evidence of an effect of additional iron supplement use any time in the year before and/or during pregnancy (OR = 1.07, 95% CI: 0.75-1.52), in the periconceptional period (OR = 1.23, 95% CI: 0.63-2.38), after knowledge of pregnancy (OR = 1.06, 95% CI: 0.74-1.53), or use in all periods (OR = 2.54, 95% CI: 0.69-9.39) after accounting for race/ethnicity and income (Table 5-2), although each of these ORs was >1.00. Among those who reported iron supplementation, there was no association with prescription by a health care professional (data not shown). Further analysis by leukemic subtype (ALL vs. AML, MLL+ vs. MLL-) did not yield additional insight (Tables 5-2 and 5-3).

Table 5-1. Selected characteristics of 443 infant leukemia cases and 324 controls and associations with leukemia.

	Controls	Combined Cases			ALL			AML		
	N (%)	N (%)	OR	95% CI	N (%)	OR	95% CI	N (%)	OR	95% CI
Infant characteristics										
Gender										
Male	156 (48.2)	218 (49.2)	1.00		133 (50.4)	1.00		84 (48.8)	1.00	
Female	168 (51.9)	225 (50.8)	0.96	0.72 – 1.28	131 (49.6)	0.91	0.66 – 1.27	88 (51.2)	0.97	0.67 – 1.41
Birthweight										
<2500 grams	17 (5.3)	23 (5.2)	0.99	0.52 – 1.90	9 (3.4)	0.65	0.29 – 1.50	14 (8.1)	1.57	0.75 – 3.29
2500-4000 grams	258 (79.6)	351 (79.2)	1.00		209 (79.2)	1.00		135 (78.5)	1.00	
>4000 grams	49 (15.1)	69 (15.6)	1.04	0.69 – 1.54	46 (17.4)	1.16	0.75 – 1.80	23 (13.4)	0.90	0.52 – 1.54
Length of gestation										
<38 weeks	35 (10.8)	55 (12.4)	1.17	0.75 – 1.84	31 (11.7)	1.10	0.66 – 1.84	23 (13.4)	1.27	0.72 – 2.23
38-42 weeks	288 (88.9)	387 (87.4)	1.00		232 (87.9)	1.00		149 (86.6)	1.00	
>42 weeks	1 (0.3)	1 (0.2)	0.74	0.05 – 11.95	1 (0.4)	1.24	0.08 – 19.98	0 (0.0)	--	
Maternal characteristics										
Age at index child's birth										
<35 years	265 (82.0)	372 (84.2)	1.00		228 (86.4)	1.00		139 (81.3)	1.00	
≥35 years	58 (18.0)	70 (15.8)	0.86	0.59 – 1.26	36 (13.6)	0.72	0.46 – 1.13	32 (18.7)	1.05	0.65 – 1.70
Previous fetal loss										
None	241 (74.4)	337 (76.1)	1.00		199 (75.4)	1.00		133 (77.3)	1.00	
1	64 (19.8)	76 (17.2)	0.85	0.59 – 1.23	44 (16.7)	0.83	0.54 – 1.28	31 (18.0)	0.88	0.54 – 1.42
≥2	19 (5.9)	30 (6.8)	1.13	0.62 – 2.05	21 (8.0)	1.34	0.70 – 2.56	8 (4.7)	0.76	0.33 – 1.79

Educational attainment										
< High school graduate	91 (28.2)	149 (33.7)	**1.47**	**1.02 – 2.11**	94 (35.7)	**1.52**	**1.01 – 2.29**	53 (30.8)	1.36	0.84 – 2.19
Some post-high school	112 (34.7)	125 (28.3)	1.00		76 (28.9)	1.00		48 (27.9)	1.00	
College graduate	120 (37.2)	168 (38.0)	1.25	0.89 – 1.77	93 (35.4)	1.14	0.77 – 1.70	71 (41.3)	1.38	0.88 – 2.16
Ethnicity										
White	273 (84.5)	334 (75.6)	1.00		199 (75.7)	1.00		130 (75.6)	1.00	
African-American	18 (5.6)	18 (4.1)	0.82	0.42 – 1.60	11 (4.2)	0.84	0.39 – 1.81	7 (4.1)	0.82	0.33 – 2.00
Hispanic	15 (4.6)	55 (12.4)	**3.00**	**1.66 – 5.42**	29 (11.0)	**2.65**	**1.39 – 5.08**	24 (14.0)	**3.36**	**1.71 – 6.62**
Other	17 (5.3)	35 (7.9)	1.68	0.92 – 3.07	24 (9.1)	**1.94**	**1.01 – 3.70**	11 (6.4)	1.36	0.62 – 2.98
Household income										
≤$30,000	95 (29.6)	157 (35.8)	1.27	0.91 – 1.77	100 (38.2)	1.45	1.00 – 2.12	54 (31.8)	1.01	0.65 – 1.55
$30,001 - $75,000	145 (45.2)	189 (43.1)	1.00		105 (40.1)	1.00		82 (48.2)	1.00	
>$75,000	81 (25.2)	93 (21.2)	0.88	0.61 – 1.27	57 (21.8)	0.97	0.64 – 1.48	34 (20.0)	0.74	0.46 – 1.20
Morning sickness										
No	120 (37.0)	128 (28.9)	1.00		76 (28.8)	1.00		51 (29.7)	1.00	
Yes	204 (63.0)	315 (71.1)	**1.45**	**1.07 – 1.96**	188 (71.2)	**1.46**	**1.03 – 2.06**	121 (70.4)	1.40	0.94 – 2.08
Smoking during pregnancy										
No	258 (79.9)	368 (83.3)	1.00		213 (81.0)	1.00		149 (86.6)	1.00	
Yes	65 (20.1)	74 (16.7)	0.80	0.55 – 1.15	50 (19.0)	0.93	0.62 – 1.41	23 (13.4)	0.61	0.37 – 1.03
Drinking during pregnancy										
No	254 (78.6)	377 (85.7)	1.00		220 (83.7)	1.00		151 (88.8)	1.00	
Yes	69 (21.4)	63 (14.3)	**0.62**	**0.42 – 0.90**	43 (16.4)	0.72	0.47 – 1.10	19 (11.2)	**0.46**	**0.27 – 0.80**

OR = odds ratio; 95% CI = 95% confidence interval; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia

Table 5-2. Association between vitamin use and infant leukemia

	Controls	Combined Cases			ALL			AML		
	N	N	OR*	95% CI	N	OR*	95% CI	N	OR*	95% CI
Prenatal vitamins										
Any prenatal vitamin consumption										
No	19	40	1.00		28	1.00		11	1.00	
Yes	303	402	0.79	0.44 – 1.42	235	0.63	0.34 – 1.18	161	1.20	0.53 – 2.75
Periconceptional consumption										
No	100	162	1.00		104	1.00		56	1.00	
Yes	222	280	0.89	0.64 – 1.24	159	0.77	0.54 – 1.11	116	1.05	0.68 – 1.61
Consumption during pregnancy, after confirmation of pregnancy										
No	29	58	1.00		39	1.00		18	1.00	
Yes	293	384	0.78	0.48 – 1.28	224	0.66	0.39 – 1.11	154	1.05	0.55 – 2.04
Consumption in year before and throughout pregnancy										
No	137	222	1.00		137	1.00		83	1.00	
Yes	185	220	0.84	0.62 – 1.14	126	0.77	0.55 – 1.09	89	0.88	0.60 – 1.31

	Controls	Combined Cases			ALL			AML		
	N	N	OR*	95% CI	N	OR*	95% CI	N	OR*	95% CI
Prenatal iron supplements										
Any prenatal iron consumption										
No	253	334	1.00		195	1.00		136	1.00	
Yes	70	108	1.07	0.75 – 1.52	68	1.22	0.82 – 1.80	36	0.82	0.51 – 1.33
Periconceptional consumption										
No	308	414	1.00		247	1.00		160	1.00	
Yes	15	28	1.23	0.63 – 2.38	16	1.30	0.62 – 2.72	12	1.26	0.55 – 2.88
Consumption during pregnancy, after confirmation of pregnancy										
No	260	345	1.00		201	1.00		141	1.00	
Yes	63	97	1.06	0.74 – 1.53	62	1.22	0.81 – 1.84	31	0.77	0.46 – 1.27
Consumption in year before and throughout pregnancy										
No	320	431	1.00		256	1.00		168	1.00	
Yes	3	11	2.54	0.69 – 9.39	7	3.04	0.77 – 12.03	4	1.72	0.33 – 9.06

OR = odds ratio; 95% CI = 95% confidence interval; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia

* ORs adjusted for maternal race (white, black, Hispanic, other) and household income (≤$30,000, $30,001 - $75,000, >$75,000).

Table 5-3. Association between vitamin use and infant leukemia by *MLL* gene status

	Controls	MLL+			MLL-		
	N	**N**	**OR***	**95% CI**	**N**	**OR***	**95% CI**
Prenatal vitamins							
Any prenatal vitamin consumption							
No	19	22	1.00		7	1.00	
Yes	303	205	0.72	0.37 – 1.41	139	1.34	0.54 – 3.32
Periconceptional consumption							
No	100	87	1.00		50	1.00	
Yes	222	140	0.83	0.57 – 1.22	96	0.94	0.61 – 1.46
Consumption during pregnancy, after confirmation of pregnancy							
No	29	30	1.00		14	1.00	
Yes	293	197	0.75	0.43 – 1.33	132	1.01	0.51 – 2.01
Consumption in year before and throughout pregnancy							
No	137	116	1.00		71	1.00	
Yes	185	111	0.81	0.56 – 1.17	75	0.85	0.56 – 1.28

	Controls	MLL+			MLL-		
	N	**N**	**OR***	**95% CI**	**N**	**OR***	**95% CI**
Prenatal iron supplements							
Any prenatal iron consumption							
No	253	174	1.00		105	1.00	
Yes	70	53	1.10	0.72 – 1.68	41	1.23	0.77 – 1.97
Periconceptional consumption							
No	308	218	1.00		132	1.00	
Yes	15	9	0.85	0.35 – 2.03	14	1.89	0.87 – 4.11
Consumption during pregnancy, after confirmation of pregnancy							
No	260	176	1.00		113	1.00	
Yes	63	51	1.18	0.76 – 1.82	33	1.04	0.64 – 1.71
Consumption in year before and throughout pregnancy							
No	320	222	1.00		143	1.00	
Yes	3	5	2.55	0.57 – 11.36	3	2.08	0.40 – 10.87

OR = odds ratio; 95% CI = 95% confidence interval; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; *MLL* = mixed lineage leukemia

* ORs adjusted for maternal race (white, black, Hispanic, other) and household income (≤$30,000, $30,001 - $75,000, >$75,000).

Table 5-3 (Continued). Association between vitamin use and infant leukemia by *MLL* gene status

	Controls	ALL MLL+			ALL MLL-			AML MLL+			AML MLL-		
	N	N	OR*	95% CI	N	OR*	95% CI	N	OR*	95% CI	N	OR*	95% CI
Prenatal vitamins													
Any prenatal vitamin consumption													
No	19	18	1.00		3	1.00		4	1.00		4	1.00	
Yes	303	138	0.60	0.29–1.21	74	1.54	0.44–5.45	64	1.13	0.35–3.68	62	1.14	0.37–3.55
Periconceptional consumption													
No	100	66	1.00		25	1.00		21	1.00		24	1.00	
Yes	222	90	0.69	0.45–1.05	52	1.00	0.57–1.73	47	1.11	0.60–2.04	42	0.89	0.50–1.59
Consumption during pregnancy, after confirmation of pregnancy													
No	29	24	1.00		8	1.00		6	1.00		6	1.00	
Yes	293	132	0.64	0.35–1.17	69	0.86	0.37–2.01	62	1.10	0.42–2.91	60	1.17	0.45–3.01
Consumption in year before and throughout pregnancy													
No	137	87	1.00		35	1.00		29	1.00		35	1.00	
Yes	185	69	0.66	0.44–1.00	42	0.95	0.56–1.59	39	1.13	0.64–2.01	31	0.73	0.42–1.28

	Controls	ALL MLL+		ALL MLL-		AML MLL+		AML MLL-	
	N	N	OR* 95% CI	N	OR* 95% CI		N	N	OR* 95% CI
Prenatal iron supplements									
Any prenatal iron consumption									
No	253	119	1.00	53	1.00	53	1.00	51	1.00
Yes	70	37	1.13 0.70–1.80	24	1.43 0.80–2.54	15	1.02 0.52–2.01	15	0.94 0.49–1.81
Periconceptional consumption									
No	308	151	1.00	68	1.00	64	1.00	61	1.00
Yes	15	5	0.73 0.25–2.09	9	2.31 0.95–5.63	4	1.19 0.35–3.99	5	1.49 0.51–4.37
Consumption during pregnancy, after confirmation of pregnancy									
No	260	120	1.00	58	1.00	54	1.00	54	1.00
Yes	63	36	1.22 0.76–1.98	19	1.16 0.63–2.15	14	1.04 0.52–2.10	12	0.79 0.39–1.61
Consumption in year before and throughout pregnancy									
No	320	152	1.00	75	1.00	67	1.00	65	1.00
Yes	3	4	2.89 0.60–13.84	2	2.39 0.37–15.29	1	1.32 0.11–16.04	1	1.27 0.12–13.35

OR = odds ratio; 95% CI = 95% confidence interval; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; *MLL* = mixed lineage leukemia

* ORs adjusted for maternal race (white, black, Hispanic, other) and household income (≤$30,000, $30,001 - $75,000, >$75,000).

Discussion

Overall, we found little evidence supporting an association between prenatal vitamin or iron supplementation and infant leukemia. These results are consistent with a prior report of no association between maternal consumption of vitamin or iron supplements during pregnancy and infant leukemia (ALL and AML) among those diagnosed at <21 months in Brazil,[151] and another report indicating no association with maternal vitamin or iron use during the index pregnancy in those diagnosed with ALL at <1 year.[41]

Of 7 studies examining maternal vitamin and/or iron supplementation and childhood ALL, 4 observed significant inverse effects in the range $0.40 \leq OR \leq 0.84$,[39-42] while another reported a nonsignificant inverse association[92] and 2 found associations near the null.[93, 94] Iron supplement use was associated with reduced odds of ALL in 2 studies [41, 154] and was not associated in 4 others.[40, 42, 93, 155] Notably, Thompson *et al* observed an inverse effect for folic acid supplementation (OR = 0.40, 95% CI: 0.21-0.73), although they were unable to determine whether folic acid alone or in combination with iron was important etiologically; the OR for iron supplementation without folic acid was not significant.[42] For AML (and acute non-lymphocytic leukemia, ANLL), each of 3 identified studies failed to find evidence of an association with maternal vitamin and/or iron supplementation.[39, 40, 95]

In examining different time periods, we found no evidence of an association with vitamin or iron supplementation, respectively, relevant to the year before pregnancy and prior to knowledge of pregnancy, or after knowledge of pregnancy, or use in all of these

periods. In contrast, Thompson *et al* reported that the initiation of vitamin supplementation across each of three periods examined, as well as each of three durations of use, was associated with a considerable reduction in risk; the strength of the association did not vary by either of these exposure variables. Wen *et al* observed that prenatal vitamin and iron supplementation, respectively, were associated with reduced risk of ALL ($OR_{vitamins}$ = 0.7, 99% CI: 0.5-1.0; OR_{iron} = 0.8, 99% CI: 0.7-1.0), while supplementation in the year before pregnancy was not.[41] Ross *et al* found that vitamin supplementation in the periconceptional period was associated with reduced risk of leukemia in children with Down syndrome (OR = 0.51, 95% CI: 0.30-0.89), while supplementation after knowledge of pregnancy was associated with increased risk (OR = 1.70, 95% CI: 0.98-2.92).[40]

The reduced odds observed in ALL MLL+ cases is of interest, given that maternal vitamin supplementation has been inversely associated with childhood ALL but not AML,[39-42, 95] folate deficiency has been associated with increased levels of DNA double strand breaks (which precede *MLL* translocations[174]) in blood and bone marrow,[172] and that mothers of ALL MLL+ cases are expected to recall vitamin supplementation similarly to mothers in other diagnostic subgroups.

Two mechanisms have been suggested to explain the association between folate deficiency and cancer (reviewed in [170, 171]). The first mechanism concerns folate's involvement in the synthesis of nucleotide precursors. In a state of folate deficiency, biosynthesis of thymine is diminished, causing uracil to be inserted into DNA instead.[170, 172] In the process of excising a misplaced uracil, a transient single strand break (SSB) is

created; when two such SSBs arise simultaneously in close proximity (within ~14 bp), double strand breaks (DSBs) ensue.[172] Errors in DSB repairs can result in translocations or other chromosomal rearrangements.[10, 173] Chronic folate deficiency with repeated uracil misincorporation and excision is thought to lead to a "catastrophic" cycle of DNA repair, resulting in chromosomal damage, and ultimately in malignancy.[170] The second mechanism relates to potential epigenetic effects due to the participation of folate as a cofactor in the DNA methylation pathway, wherein folate deficiency reduces the available pool of S-adenosylmethionine (SAM), the principle methyl donor for cytosine in DNA, leading to DNA hypomethylation.[170] Hypomethylation of a promoter region can result in activation of a proto-oncogene, and ultimately malignant transformation.[170]

Common variants in folate metabolizing genes may confer different risk for infant leukemia due to altered protein function. In the single published study examining folate metabolism single nucleotide polymorphisms (SNPs) and infant leukemia, Wiemels *et al* reported a reduced risk for acute infant leukemia associated with one or more copies of the variant allele (OR_{CT} = 0.29, 95% CI: 0.09-0.79; OR_{TT} = 0.67, 95% CI: 0.16-2.53; OR_{CT+TT} = 0.36, 95% CI: 0.15-0.85) at 5,10-methylenetetrahydrofolate reductase *(MTHFR)* position 677.[158] They did not find evidence of an association with the *MTHFR* SNP at nucleotide 1298.[158] Although subject ages were not specified, Lightfoot *et al* observed an association between MLL+ leukemia and the homozygous variant GG genotype of the *MTR* SNP at position 2756 (OR = 4.90, 95% CI: 1.30-18.45).[159] They did not observe associations for polymorphisms in *MTHFR*, *SHMT1*, or *TYMS*, however.

Several childhood leukemia studies have examined *MTHFR* SNPs[166] and a few have also assayed polymorphisms in 8 other folate metabolizing genes with varying results by locus.[159, 163, 164, 169]

It is also possible that maternal or fetal genotype may modify the effect of folic acid supplementation. Krajinovic *et al* investigated the potential for modification of the effect of the two *MTHFR* SNPs by national folic acid fortification in Canada beginning in 1996.[161] Upon stratification on birth year, they observed a significant inverse association for *MTHFR* haplotypes including ≥1 variant alleles among children born prior to 1996, with ORs in the range 0.3 to 0.6. The ORs among children born in 1996 or later were nonsignificant, but may suggest an increase in risk overall ($1.3 \leq OR \leq 3.0$). In a case-only analysis, Milne *et al* observed no statistically significant evidence of a multiplicative interaction between child's genotype and maternal prenatal vitamin use for either the 677 or 1298 *MTHFR* SNPs.[162]

Prenatal iron supplementation may indicate low iron levels, anemia, or a desire to prevent anemia, and maternal anemia was associated with childhood leukemia in three prior case-control studies.[81, 82, 102] The inverse associations between maternal prenatal iron supplementation and childhood leukemia previously observed in some[41, 154], but not all, studies are consistent with an etiologic model in which iron deficiency is related to childhood leukemia. Notably, in a prior report from the first phase of this study, there was no evidence for an association between maternal iron supplement use or maternal anemia during pregnancy, as recorded in the medical record, and infant leukemia, ALL, or AML, or among the *MLL* subgroups, respectively.[183]

On the contrary, high iron levels may be detrimental to developing fetuses[184] and have been associated with increased cancer risk in adults.[185] The biological mechanism is unknown, however, it has been hypothesized that excess iron may generate reactive oxygen species that exert a genotoxic effect or may act by increasing cell proliferation or altering immune function.[186] An association between prenatal iron supplementation and childhood leukemia may exist only in the presence of genetic variants. Associations between SNPs in two iron homeostasis genes (i.e., hereditary hemochromatosis gene *(HFE),* hepcidin antimicrobial peptide *(HAMP))* and childhood leukemia have been observed on the order of 2.00 < OR < 3.00 among British populations.[187, 188]

The current study features unique strengths. It comprises the largest study of infant leukemia conducted to date, and likely ever to be conducted; 2 prior infant leukemia studies included 136 and 202 cases, respectively.[97, 151] Use of the COG registry in case ascertainment results in a nearly population-based sample, since COG institutions see nearly 100% of childhood leukemia cases aged 0-4 years.[123]

Differential recall across cases and controls is a noteworthy concern, as case mothers may exert extra effort to accurately recall prior exposures to understand why their child developed cancer. The majority of women in a validation study accurately reported their folic acid supplement use during pregnancy, while ≤7% over-reported supplementation.[205] In another study of pregnancy outcomes, self-reported rates of vitamin use dropped significantly from early pregnancy to post-delivery among mothers of cases (7.3% versus 2.5%, respectively) and controls (6.0% versus 3.5%); the resulting ORs did not differ significantly across the two time points, however.[206] Case mothers in a

study of sudden infant death syndrome recalled prenatal iron use 3% (95% CI: -9%-13%) better than control mothers and were 7% (95% CI: -20%-8%) more likely to report iron use that was not recorded in the medical record.[207] These differences equated to a modestly higher interview OR than that calculated from medical records. Together these results suggest that although the maternal recall of supplementation may vary somewhat by case-control status and time period of assessment, the inferences to be drawn are similar. In the current study, infant leukemia provides a unique opportunity for etiologic research, since it limits the recall time period and therefore the possibility of recall bias.

There are additional potential sources of misclassification in these data. Most mothers reported taking multi- or prenatal vitamins, which contain many vitamins and minerals including folic acid and iron; it is therefore impossible to identify which is the etiologically relevant component. To partially address this concern, we restricted our analysis to vitamins containing folic acid and found that a large majority of mothers (98% of cases and controls, respectively) consumed vitamins with folic acid; results were nearly identical to those shown in Tables 5-2 and 5-3 (data not shown). Similarly, questions regarding iron supplementation requested information regarding supplementation in excess of multi- and prenatal vitamins, but all mothers taking multivitamins received some level of iron supplementation.

We were also unable to assess the contribution of maternal diet to total folate or iron intake. An analysis examining total folate and iron consumption, respectively, in the pre-pregnancy diet (a surrogate for prenatal diet) resulted in nonsignificant inverse associations with childhood ALL.[156] The authors acknowledged the limitation that pre-

pregnancy diet may not be an adequate surrogate measure because supplement use generally increases during pregnancy.[156] An analysis of National Health and Nutrition Examination Survey (NHANES) data post-fortification (2001-2004) indicated that vitamin supplementation accounted for the greatest source of variation in dietary folic acid among non-pregnant U.S. adults and constitutes ~61% of total daily consumption; there was some variation in folic acid consumed via prepared breakfast cereals but little variation in folic acid consumed via enriched cereal-grain products.[145] These data suggest supplement use may be a useful measure of variation in folic acid exposure, although supplement use is expected to be greater and the dietary composition different among pregnant women.

Another potential limitation is that we might only expect to see evidence of an effect in the presence of folate deficiency,[149] however, fortification programs have been successful in increasing folic acid intake among women of childbearing age,[139, 141-143o] thereby decreasing the prevalence of folate deficiency among participants in this study. Of note, an ecologic study of the effects of fortification in Canada found evidence supporting a protective role for folate in neuroblastoma development (IRR = 0.55, 95% CI: 0.39-0.78), but no association with infant ALL or hepatoblastoma.[144]

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[o] A comparison of mean serum folate concentrations pre- and post-fortification among U.S. women ages 15-44 years showed a significant increase between NHANES III (1988-1991) and NHANES 1999 (6.3 vs. 16.2 ng/mL, respectively); similar results were observed for red blood cell folate concentrations (Folate Status in Women of Childbearing Age - United States, 1999. MMWR Weekly 49: 962-965). A 19% reduction in the incidence of neural tube defects was also detected among children conceived after the mandatory fortification program was introduced (Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY (2001) Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. Jama 285: 2981-6). Parallel results have been observed in Canada (Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DE (2002a) Association of neural tube defects and folic acid food fortification in Canada. Lancet 360: 2047-8; and Ray JG, Vermeulen MJ, Boss SC, Cole DE (2002b) Increased red cell folate concentrations in women of reproductive age after Canadian folic acid food fortification. Epidemiology 13: 238-40).

Differential response rates across cases and controls are a potential limitation, as they may indicate selection bias. Individuals with higher SES tend to participate in case-control studies of childhood cancer,[124] and maternal prenatal vitamin use has been shown to vary by SES, race, and educational attainment.[137, 192] A comparison of baseline characteristics showed that RDD and birth certificate controls were very similar on important demographic factors, however, participants differed from the U.S. population in maternal age, race, education, and marital status and in the child's gestational age and birthweight.[201] Participating mothers identified by birth certificates were also older and had higher educational levels than non-participants.[201] We adjusted for maternal race/ethnicity and income in our analysis to minimize confounding by SES, but acknowledge that residual confounding may remain.

Overall, we found little evidence supporting an association between maternal prenatal vitamin use and infant leukemia, and speculate that this may be attributable to high rates of folic acid supplementation, including personal vitamin use and national folic acid fortification programs implemented in the U.S. and Canada early in the study period. Similarly, we did not observe an association with iron supplement use above that found in multi- or prenatal vitamins.

Chapter 6: Review of the literature regarding the association between childhood and adolescent leukemia and atopic disease

Childhood/adolescent leukemia

Leukemia is the most commonly diagnosed cancer among children and adolescents in the United States with an incidence rate of 42 cases per 1,000,000 persons; the incidence has been increasing in recent years (average annual percent change for 1992-2004: 0.7%, 95% confidence interval (CI): -0.1-1.5%).[3] The incidence varies by age, with 40 cases/1,000,000 diagnosed among infants, 85/1,000,000 among those ages 1-4 years at diagnosis, 38/1,000,000 among those 5-9 years, 28/1,000,000 in those ages 10-14 years and 27/1,000,000 among those aged 15-19 years.[3]

Acute leukemia is a malignancy of the bone marrow, resulting in the excess production of leukocytes. It is a heterogeneous disease, affecting the lymphocytes in acute lymphoblastic leukemia (ALL), myeloid cells in acute myeloid leukemia (AML), or both (biphenotypic or mixed lineage leukemia). ALL is further classified depending on the involvement of precursor B cells or T cells. The peak in ALL incidence observed in early childhood (≤5 years) is mainly attributable to a subtype of B-lineage cells expressing CD10 surface antigens and is therefore often called common ALL (cALL).[208] Immune-related hypotheses specific to the development of cALL have been proposed by Greaves[11, 12] and Schmiegelow,[209] and Kinlen has also described a more general hypothesis for leukemogenesis;[210, 211] these hypotheses are discussed briefly below.

As noted in the Overview above, there is a paucity of information regarding the etiology of childhood leukemia; established risk factors include predisposing genetic

conditions, such as Down syndrome,[13] neurofibromatosis type I,[14] and fanconi anemia,[15] exposure to *in utero*[16] or postnatal therapeutic irradiation,[17-19] and exposure to chemotherapeutic agents, such as alkylating agents[20] or epidopophyllotoxins.[21, 22] There is also reasonable evidence for an association with high birthweight,[75] although the underlying mechanism is unknown. Atopic disease has been studied as a risk factor for a variety of malignancies[212, 213] and is the subject of the current meta-analysis.

Atopy

The prevalence of atopic disease has been increasing over time worldwide, including the U.S.[214, 215] The estimated prevalence for any atopic condition among pediatric populations ranges from 32% to 52%,[37, 43, 216] while the estimated population prevalence for the individual atopic conditions ranges from 7% to 22%[37, 43, 215-218] (see Appendix J for reported prevalence rates). The reasons for the observed increase are not entirely known, however, the hygiene hypothesis (described below) presents one potential mechanism.[219]

Varying definitions of atopy are found in the literature;[220-223] we will use the definition presented by Bottema *et al*: "a hereditary predisposition to produce [immunoglobulin E] IgE antibodies against environmental allergens that is associated with one or more atopic diseases such as bronchial asthma, urticaria [hives], eczema, and allergic rhinitis [hay fever]".[223, 224] Atopy is the consequence of gene-environment interactions.[224] Atopic individuals generate an exaggerated response to environmental allergens initiated by CD4+ Type 2 helper T (Th2) cells. The allergen is processed and presented to Th2 cells by the antigen presenting cells.[225] This precipitates the release of

cytokines (interleukin-4, -5, -9 and -13) by Th2 cells, leading to IgE antibody production by B cells, as well as the growth and activation of mast cells, basophils, and eosinophils.[225, 226] The activation of eosinophils stimulates toxic enzyme release in the mucosa and dermis, ultimately causing chronic inflammation in affected tissues.[222]

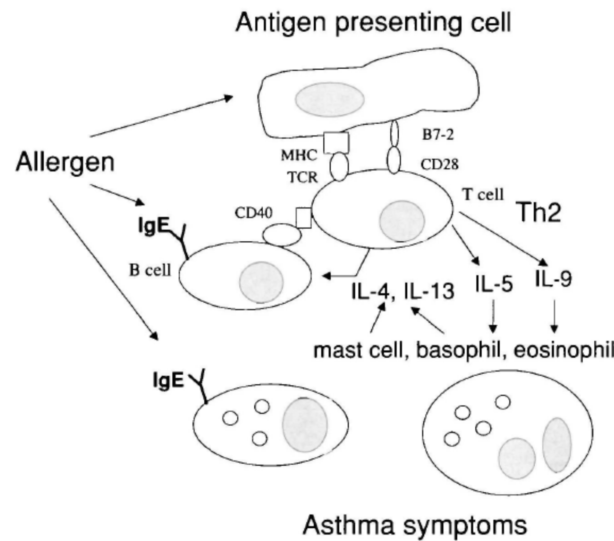

Figure 6-1. Diagram of the atopic response to environmental allergens. Source: Robinson, DS. *Br Med Bull* 2000; 56:956-968.[225]

Allergic conditions are detected via positive skin prick tests or elevated serum IgE levels, while asthma diagnosis involves evaluation of bronchial hyperresponsiveness,[224] although all forms of asthma involve elevated serum IgE levels.[227] The majority of published studies regarding atopy and cancer have measured self-reported atopic phenotype via interview or questionnaire.[212] Biomarkers for disease, including positive skin prick tests to specific allergens, and total and allergen-specific IgE levels, have been used less commonly; eosinophil levels have not been previously utilized.[212, 213]

Total IgE levels vary by age, sex, and race. Low levels have been observed among healthy newborns (mean = 0.20 IU/mL);[228] levels rise during childhood to reach a maximum between 8-12 years before declining.[223] IgE concentrations within an individual are correlated over time,[229] such that infants with high IgE serum concentrations tend to have higher levels in adulthood.[223] Sensitization to specific allergens, resulting in elevated levels of specific IgE antibodies, generally varies by age as well. In infants, transient food allergies are observed, while enduring sensitization to aeroallergens increases during childhood and adolescence, to reach a peak between ages 20-30 years.[223] There is little difference in IgE levels in neonates of both genders; however, the concentrations rise faster in males than females, such that males have greater levels beginning at 6 months and extending throughout the life cycle.[223] With respect to specific allergens, the evidence does not point to any clear distinctions between the sexes.[223] White individuals generally have lower IgE levels than those of other races.[230]

Brief descriptions of the specific atopic conditions are provided below.

Allergic rhinitis. Hay fever is an allergic response of the nasal passages to airborne allergens. Marked by nasal itching, sneezing, redness, and tenderness, and by postnasal drip,[231] hay fever is usually first observed in children aged 8-11 years.[214] Common allergens include any type of tree or grass, components of house dust, cigarette smoke, cosmetics, cleaning products, and industrial chemicals.[231]

Atopic Dermatitis. Often underdiagnosed, eczema is a reaction of the skin characterized by intense itching, scaling or lichenification, swelling, and very dry skin.[214, 231] An important symptom considered in the diagnosis is flexural involvement, meaning there is eczema present in skin folds (i.e., behind the knees, inside the elbows).[34] Atopic eczema may result from stress, or as an allergic response to plant resins, fruit or vegetable consumption, cleaning products, chlorine, perfumes, or topical medications.[231] The age of onset is typically 12-24 months, which is earlier than the peak age for cALL.[34]

Bronchial asthma. Asthma is a condition of airway constriction and inflammation; the most notable symptoms are wheezing, coughing, tightness in the chest, dyspnea, and nasal congestion.[214, 231] There are several environmental triggers for asthma, including animal dander, house dust, indoor mold, pollen, industrial chemicals, tobacco smoke, and cold air. A vast majority of asthmatics (80-90%) are diagnosed by the age of 6 years.[214]

Urticaria. Hives are a hypersensitivity reaction of the skin resulting in red, swollen, itchy patches lasting 1-7 days typically. Common causes include foods, such as nuts, seafood, eggs, wheat, milk, strawberries, and additives/preservatives, antibiotics including penicillin, tetanus vaccination, gamma globulin, and insect bites.[231]

Possible biological mechanisms linking atopic disease and childhood leukemia

Although no biological mechanism has been established with respect to atopic disease and childhood leukemia, the immune system is known to play a role in both atopy and malignancy. Two conflicting hypotheses have been proposed for a causal relationship between atopy and cancer. The first theory is the immune surveillance hypothesis, which asserts that the immune system recognizes antigens of malignant cells as foreign and mounts a response to them, thus preventing a majority of potential cancers from developing.[232] The presence of an atopic condition is thought to increase the vigilance of the immune system in monitoring for, identifying, and eliminating malignant cells.[212, 233] An important piece of evidence in support of this hypothesis is the observation that immunocompromised individuals have higher incidence rates of specific malignancies than those with intact immune systems (reviewed in [234]). Notably, immunosupressed persons have an increased risk of non-Hodgkin lymphoma,[26-28] a malignancy with an identical histology to ALL.[4] Further, increased levels of natural killer cells, eosinophils, and cytokines interleukin (IL)-4 and IL-10 in atopic individuals may contribute to a reduction in cancer risk (reviewed in [212]). Experiments conducted in laboratory animals indicate that tumor advancement can be slowed or prevented by IgE-mediated hypersensitivity responses (reviewed in [235]). Finally, the observation that some tumors have the capacity to evade the immune system[232] provides further support for this hypothesis.[212]

The second hypothesis is that chronic stimulation of the immune system by allergens could increase the risk of carcinogenesis.[233, 234] A greater number of

proliferating cells increases the probability of genetic errors, such as pro-oncogenic mutations, that may not be repaired prior to subsequent divisions.[233] This mechanism has been proposed as an explanation for the positive association observed between autoimmune disease and malignancy,[236] for example.

The principal factor linking atopic disease and childhood leukemia is thought to be the rate at which the immune system matures,[34] which is mediated by exposure to various factors. The concept of Th2 versus Th1 cell predominance is often presented with respect to atopy, wherein Th2 cells express cytokines IL-4, stimulating IgE production by B cells, and IL-5, activating eosinophils, and do not express interferon (IFN)-γ.[237] Conversely, Th1 cells are marked by expression of IFN-γ (inhibiting IgE production) and IL-2, but not IL-4 and IL-5.[237] Infants are born with a Th2-dominated immune profile; non-atopic infants gradually migrate to a Th1-dominant process by the age of 2 years, while infants with a family history of atopy fail to make the transition from Th2 to Th1.[238] The currently available evidence in immunology indicates a complex relationship between infections and atopy, with a significant body of research implicating IL-10 as an immune regulator that is upregulated in response to infection and that serves as a suppressor of allergic responses.[219] Two additional T-cell types, T-regulatory and Th17, may also play a role in the complex interplay between atopy, infections, and autoimmune diseases (reviewed in [239]). A complicating factor is the idea that not all cases of asthma, eczema or hay fever are considered atopic,[240, 241] and may be attributable to other factors, such as infections, cold air, exercise, stress, and anxiety.[231, 241]

The hygiene hypothesis, originally proposed by Strachan in a 1989 publication about the reduced incidence of hay fever among those with older siblings,[242] postulates that several factors in developed countries have contributed to the rise in atopic/allergic conditions, including smaller family size, good public sanitation, increased use of antibiotics, low incidence of helminth infestation and oralfecal transmission of infection, and a stable population of micro-flora in the intestines.[219] A study by Smith *et al* demonstrated that the hygiene hypothesis may also apply to childhood leukemia.[243] In that ecologic study, decreases in the prevalence of hepatitis A infection, a marker for hygiene due to the fecal-oral mode of transmission, was associated with subsequent increased risk of ALL in the United States and Japan.[243] Importantly, the incidence of childhood leukemia is generally greater in developed countries,[244] however, this observation may result partially or wholly from better ascertainment in developed countries. Further, individuals in developed countries with higher socioeconomic status (SES) have a (modestly) greater risk of leukemia than those with lower SES (reviewed in [245]), where higher SES may be considered a surrogate marker for better hygiene.

Three hypotheses have been proposed that suggest an etiologic role for the immune system in the development of childhood leukemia via exposure (or lack thereof) to early life infections. Greaves' Hypothesis provides a potential explanation for the peak in cALL incidence in early life (2-5 years).[12] He postulates that two spontaneous mutations are required for the leukemic transformation of B cell precursors, both of which are due to proliferative stress.[11] The first mutation is thought to arise *in utero*, as a result of the high rate of precursor B cell proliferation in the prenatal period. The affected

cell then replicates, creating many clones. The second spontaneous mutation is thought to occur after birth in a clonal cell, as a result of increased precursor B cell proliferation due to an abnormal response to delayed exposure to a common infectious agent.[11, 12] Factors thought to modulate this relationship include breastfeeding, exposure to infectious agents during infancy (via older siblings, crowding, daycare attendance, or lack of hygiene), vaccinations, and HLA type (or other genetic factors affecting susceptibility).[11, 12] Greaves cites a body of laboratory and epidemiologic research as consistent with or supportive of his hypothesis,[11, 12] including research by Kinlen.[12]

Kinlen's Hypothesis is not restricted to a particular subtype of childhood leukemia and is based on observations of population mixing.[210, 211] He argues that isolated, sparsely populated areas may lack herd immunity to common infectious agents. During periods of intense population growth, immigrants may introduce specific infectious agents to the isolated population, resulting in outbreaks/mini-epidemics. In rare cases, an abnormal response to the infective agent among susceptible individuals may result in childhood leukemia.[211] A notable difference between the two hypotheses is that Kinlen speculates that the common infectious agent acts directly upon the precursor cells to induce leukemogenesis,[210, 211] while Greaves favors an indirect effect.[11, 12]

The adrenal hypothesis, compatible with the hypotheses by Greaves and Kinlen, has been proposed more recently.[209] It postulates that early childhood infections are protective for childhood leukemia (and cALL in particular) because they alter the hypothalamus-pituitary-adrenal axis with the effect of raising plasma cortisol levels. The increased cortisol concentrations may in turn shift the Th1 versus Th2 balance toward the

production of Th2 cytokines and/or may eliminate circulating leukemic or preleukemic cells directly. Importantly, no virus or other infectious agent responsible for human leukemias has been identified to date.[246, 247]

It is not clear if any of the mechanisms described above are applicable to childhood cancers;[35] however, the leukemia studies identified to date provide evidence in support of the immune surveillance hypothesis.

Identified studies of the atopy-leukemia association

Ten prior studies examining the association between atopic disease and the development of childhood/adolescent leukemia were identified and included in the meta-analysis.[34-38, 43, 248-251] These studies are summarized below and in Table 7-1.

Leukemia overall. United Kingdom Childhood Cancer Study (UKCCS) results suggested an inverse association between allergies and leukemia overall (OR = 0.87, 95% CI: 0.73-1.04), which was attributable to ALL but not AML cases, as described below.[34] Results of the National Cooperative Leukemia Study, one of the first epidemiological investigations of the causes of childhood leukemia, were similar (OR = 0.82, 95% CI: 0.53-1.27),[251] however, the Tri State Survey reported a large, positive association between allergic diseases (such as asthma and hives) and pediatric leukemia (OR = 4.25, 95% CI: 2.54-7.12).[250]

ALL. Results of the UKCCS indicate an inverse association between ALL and a prior history of at least one allergy (OR = 0.77, 95% CI: 0.60-0.98), eczema (OR = 0.70, 95%

CI: 0.51-0.97), or hay fever (OR = 0.47, 95% CI: 0.26-0.85).[34] Rosenbaum *et al* observed that any parent-reported allergy in the index child (defined as asthma, eczema, pollen-dust, cat-dog dander, or food-drug-bee allergies) prior to ALL diagnosis was associated with a reduced risk of ALL (OR = 0.58, 95% CI: 0.38-0.88) in a population-based case-control study in New York.[35] Each of the individual exposures was associated with reduced risk; inhaled allergens and food-drug-bee allergies analyzed separately had statistically significant inverse associations. A population-based case-control study in Germany reported inverse associations between parent-reported atopic disease (including hay fever, asthma, and neurodermatitis, OR = 0.52, 95% CI: 0.40-0.68) and childhood ALL, as well as hay fever, neurodermatitis, and eczema examined separately.[36] The results of a Children's Cancer Group (CCG) case-control study by Wen *et al* indicated an inverse association between childhood ALL and any allergic disorder (i.e., asthma, hay fever, food or drug allergies, eczema or hives) (OR = 0.7, 95% CI: 0.6-0.8) and significant inverse associations with asthma, hay fever, food or drug allergies, and eczema examined independently.[37] Nishi and Miyake reported that a history of asthma or atopic dermatitis in the index child prior to a diagnosis of non-T-cell ALL was associated with significantly reduced risk (OR = 0.26, 95% CI: 0.11-0.59) in a Japanese case-control study.[38]

Two additional studies examined associations between asthma and pediatric/adolescent ALL. Investigators of a population-based case-control study in France observed a strong inverse association with maternally reported asthma (OR = 0.5, 95% CI: 0.3-0.9).[249] An analysis of the Swedish cancer registry linked to the Swedish

hospital registry reported very similar results for prior hospitalization for asthma (OR = 0.5, 95% CI: 0.2-1.1).[248] It should be noted that the latter measure of atopic disease was qualitatively different than the exposure classifications used in other studies, and likely involved substantial underascertainment of asthma; it is unlikely that false positive asthma diagnoses were included, however.

Conversely, no association was observed for any allergic condition diagnosed ≥1 year prior to a diagnosis of ALL in a case-control study of abstracted medical records in the western United States (OR = 1.24, 95% CI: 0.82-1.86). Further, a prior diagnosis of atopy or hives diagnosed ≥1 year prior to ALL diagnosis was associated with increased odds of ALL (OR = 2.20, 95% CI: 1.16-4.16).[43] Diagnoses of atopy or hives (OR = 3.78, 95% CI: 1.00-14.29) or asthma (OR = 3.10, 95% CI: 1.39-6.95) within the year prior to ALL diagnosis were also positively associated with ALL.

The fact that an inverse association was observed in a majority of studies involving different populations is intriguing.

AML. The UKCCS results did not show an association between AML and a prior history of at least one allergy (OR = 0.92, 95% CI: 0.55-1.53), nor was there an observed association with asthma, hay fever, or eczema examined separately.[34] Likewise, the German study failed to find an association between AML and a history of atopic disease overall (OR = 0.95, 95% CI: 0.55-1.63) or with hay fever, neurodermatitis, or contact eczema.[36] The French study reported a nonsignificant inverse association between asthma and AML (OR = 0.4, 95% CI: 0.1-1.7).[249] Conversely, the Swedish registry-based study

reported a nonsignificant increase in the risk of AML associated with prior hospitalization for asthma (OR = 1.7, 95% CI: 0.5-5.4).[248] Of note, there was considerably less power to observe an association in these studies, with 65 to 132 AML cases per study.

Family history of atopy. As indicated in the definition, atopy is a hereditary condition and therefore, investigators have also examined the association of cancer with a family history (in parents or siblings) of atopy. Two case-control studies observed a 20% reduction in the odds of childhood ALL associated with parental atopy[35, 36] and two studies also observed a 10-34% reduction in ALL if at least one sibling had an atopic disease.[36, 37] Conversely, eczema and dermatitis were reported more commonly in parents of children with leukemia and lymphoma by McKinney *et al*[78] and Buckley *et al* found a positive association between a family history of allergies and pre-B-cell ALL.[252] With respect to AML, the results of Schüz *et al* indicate inverse associations for maternal (OR = 0.58, 95% CI: 0.33-1.03) and sibling (OR = 0.48, 95% CI: 0.24-0.98) histories of atopic disease, but not with respect to paternal history (OR = 1.09, 95% CI: 0.64-1.86).[36]

Additional studies were identified but excluded from the meta-analysis due to hospital- or specialty clinic-based control groups, study populations overlapping included studies, and inclusion of subjects exceeding the age range of interest (Table 7-3).

Theoretical model of causation

The hypothesized association between atopy and childhood leukemia is depicted in the directed acyclic graph (DAG) in Figure 6-2 below, where atopy (A) causes underlying biological changes (B), leading ultimately to a reduced risk of childhood leukemia (L).

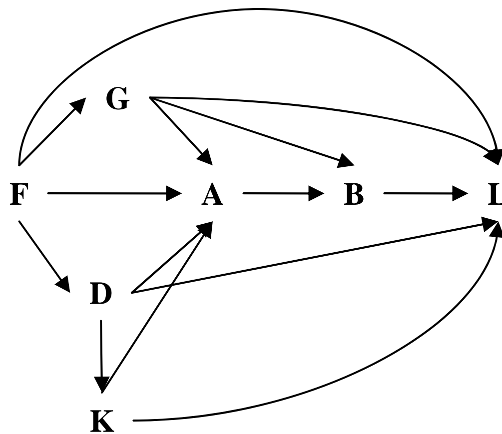

Figure 6-2. Directed acyclic graph depicting the hypothesized association between atopy and childhood leukemia, where F = family history of atopy, A = atopy, B = underlying biological changes, L = childhood leukemia, G = genetic susceptibility factors, D = demographic factors (e.g., sex, age, race, sibship, socio-economic status, location of residence), and K = method of infant feeding.

The commonly cited epidemiological definition of a confounder is a variable that is a risk factor for the outcome of interest, associated with the exposure of interest in the source population, and is not on the causal pathway.[189] This definition has been found to be insufficient, however. The structural definition of confounding is bias that is created

by an unblocked backdoor path via a common cause of the exposure and disease of interest.[190] In the analysis, conditioning on one or more confounding variables in a path blocks the backdoor path.[190] In this case, potential confounders include family history of atopy (F), which is likely causally related to genetic factors (G) modifying susceptibility to both atopy and leukemia and is also likely related to demographic factors (D), such as the sex, age, and race of the child, sibship, and the familial socio-economic status and location of residence. Method of infant feeding (K) is another potential confounding variable due to the observed associations with atopic disease and childhood leukemia. A brief literature review of these factors is provided below.

Age at diagnosis. The prevalence of atopic diseases and total serum IgE levels have been shown to increase with increasing age among children.[215, 223] For example, recent data collected by the National Health Interview Survey (NHIS) suggest rates of asthma increase between ages 0-4 (6.2%) to 5-9 years (10.6%) and then are stable throughout adolescence (10-17 years: 10.4%), while rates of hay fever peak later (4.7% in 0-4 year-olds versus 12.3% in 10-17 year-olds); conversely, rates of skin allergies decrease slightly over time (11.7% in 0-4 year-olds versus 8.9% in 10-17 year-olds).[253, 254] Our recent analysis of the Surveillance, Epidemiology, and End Results (SEER) Program data confirm that the age-standardized incidence of leukemia is greatest among those ages 1-4, with lower rates among infants (0 years) and children and adolescents ages 5-19 years.[3]

Sex. The prevalence of atopic diseases and IgE concentrations differ by gender.[215, 223, 255] The NHIS data indicate that males between the ages of 0-17 years have a greater prevalence of asthma (10.7% of males vs. 7.8% of females) and hay fever (10.5% of males versus 8.8% females), although the rates of skin allergies are more similar (9.7% in males versus 10.5% in females). [253, 254] These gender patterns are not consistent with a protective effect for leukemia, as an analysis of SEER Program data show that male children and adolescents (ages 0-19 years) are also 23% more likely to receive a diagnosis of childhood leukemia than females.[3]

Race/Ethnicity. Atopy and IgE levels have been associated with race/ethnicity.[215, 230, 255] In NHIS, 14.6% of black children had asthma compared with 8.2% of white, 8.3% of Hispanic and 5.4% of Asian children.[253] Hay fever was more prevalent among white children (10.3%) than among the other groups (7.5% in Asians, 7.5% in Hispanics, and 7.1% in blacks,), while skin allergies were more common in black children (12.7% versus 9.5% in whites, 8.9% in Asians, and 8.1% in Hispanics).[254] Childhood leukemia is diagnosed 88% and 41% more often among white children in the U.S. than among black or Asian/Pacific Islander children, respectively.[3]

Sibship. Having one or more older siblings has been consistently associated with decreased risk of atopy,[256, 257] atopic eczema,[258-260] and hay fever.[242, 257-259, 261] The association between birth order and asthma development has been less consistent.[258] Notably, results of a large cohort study in the UK indicate increased risk of asthma for

those diagnosed at ≤2 years and decreased risk thereafter.[259] Further, having one or more siblings has been positively associated with atopy, as measured by skin prick tests and specific IgE levels, but not with respect to airway hyperresponsiveness.[256, 258] The available evidence regarding a potential relationship between sibship and childhood leukemia is inconclusive, however. As reviewed by McNally, the association between having an older sibling and leukemia/ALL was examined in 14 studies from 1996-2004, with inconsistent findings.[262] A majority of studies failed to observe an association,[85, 102, 110, 155, 263-266] while 4 studies reported a positive association with one or more older siblings[193, 249, 267, 268] and 1 reported an inverse association.[269] Infante-Rivard *et al* reported a positive relationship among those diagnosed at <4 years and an inverse relationship among those diagnosed at ≥4 years.[270] Of the two subsequent reports on this topic, one failed to find an association between either ALL or AML and birth order,[271] while the other reported inverse associations between three or more older siblings and both ALL and acute monocytic leukemia, respectively.[272]

Socio-economic status (SES). Atopic disease has been related to SES.[212, 255] The NHIS-reported asthma rates are highest among the "poor" (11.7%) and lowest among the "non-poor" (8.2%), while rates of hay fever are lowest among the poor (6.6%) and highest among the non-poor (11.4%); rates of skin allergies are more similar among the socio-economic groups examined (10.3% in the poor and non-poor).[253, 254] Higher socioeconomic status is considered an established risk factor for childhood leukemia, although the underlying mechanism has not been confirmed.[4]

Location of residence. Children living in metropolitan areas were slightly more likely to have prevalent skin allergies (10.3%) than those living in non-metropolitan areas (8.8%), but were approximately equally likely to report asthma (9.5% and 8.4%, respectively) and hay fever (9.6% and 9.8%, respectively) in NHIS data.[253, 254] Higher population density has been associated with increased risk of childhood leukemia in 5 studies,[273-277] while the risk was greatest for intermediate population density in a sixth study.[278]

Method of infant feeding. After 17 years of follow-up, a prospective study found the highest rates of atopy among those not breastfed as infants; the authors concluded that breastfeeding is protective during childhood and adolescence.[279] Similarly, breastfeeding in the first three months after birth was associated with a 30% reduced risk of atopy in one study[280] and exclusive breastfeeding in infancy was associated with a 53% reduction of risk of atopic dermatitis in another.[281] With respect to childhood leukemia, two meta-analyses have been conducted. Kwan *et al* observed a significant inverse summary association between both short-term (≤6 months; OR = 0.88, 95% CI: 0.80-0.97)) and long-term (>6 months; OR = 0.76, 95% CI: 0.68-0.84) breastfeeding with childhood ALL upon pooling the results of 14 studies.[282] Similarly, they observed a nonsignificant 10% and 15% reduction in the odds of AML, respectively (8 studies).[282] Martin *et al* reported inverse associations for short-term and long-term breastfeeding and ALL (OR = 0.93, 95% CI: 0.86-1.00 and OR = 0.81, 95% CI: 0.72-0.91, respectively) in a meta-analysis of 12 studies and reported similar results for AML (8 studies).[283] Of note, both meta-

analyses indicate a dose response relationship, such that increased duration of breast-feeding results in lower odds of leukemia.

Significance of the research

Although there has been controversy surrounding the use of meta-analytic techniques on data from observational studies,[284] due to the vast potential for bias and the great heterogeneity of populations that can be included in epidemiologic studies, meta-analyses of observational studies are nevertheless growing in frequency in the medical literature.[285] Meta-analysis is a useful tool for childhood cancer epidemiologists and clinicians for at least two important reasons. First, it aids the researcher by condensing a vast quantity of literature and summarizes what is known about a given association in a systematic way. Childhood cancers are rare malignancies and as such, it is difficult to obtain sufficient power to detect modest associations in individual studies. Second, an important function of systematic reviews of observational studies is to carefully inspect existing heterogeneity in an effort to explain it.[286] Such heterogeneity may help in identifying areas warranting further research.

The current study aimed to examine the existing evidence regarding the role of atopy, including bronchial asthma, urticaria, atopic dermatitis, and allergic rhinitis, in the etiology of acute childhood leukemia among those diagnosed at ages 0-18 years. This research was of interest given that the incidence of leukemia and the prevalence of atopic conditions have both increased over time among children in the U.S. and other parts of the world. Further, experimental/clinical evidence has illustrated that the immune system plays an etiologic role in the development of both cancer and atopic disease. The

epidemiologic evidence gathered to date is not entirely consistent, however, with respect to childhood ALL and immune modulating factors.[262]

Ten studies examining associations between atopic disease and childhood/adolescent leukemia were selected for inclusion in the meta-analysis. For ALL, six of seven reported inverse associations,[34-38, 248] while one found an increased risk associated with atopy.[43] The four studies reporting associations with AML were less consistent.[34, 36, 248, 249] To achieve the study aims, a thorough search of the literature was conducted, in consultation with a biomedical librarian, followed by a survey of pediatric cancer epidemiologists, both in the U.S. and internationally, requesting any other relevant published or unpublished results. All retrieved data were summarized and random effects summary odds ratios were calculated for associations reported by 2 or more prior studies.

Chapter 7: The association between atopy and childhood/adolescent leukemia: A meta-analysis[p]

Atopic disease is hypothesized to be protective for several malignancies, including childhood/adolescent leukemia. To summarize the available epidemiologic evidence, meta-analysis of the associations between atopy/allergies, asthma, eczema, hay fever, and hives, and childhood/adolescent leukemia, acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML), respectively, was performed. To identify eligible studies, a MEDLINE literature search (1952–March 2009) and a query of international experts were conducted. Ten case-control studies were included; summary odds ratios (ORs) and 95% confidence intervals (CIs) were computed via random effects models. ORs for atopy/allergies were 1.42 (95% CI: 0.60-3.35) for three studies of leukemia overall, 0.69 (95% CI: 0.54-0.89) for six ALL studies, and 0.87 (95% CI: 0.62-1.22) for two AML studies, with high levels of heterogeneity detected for leukemia overall and ALL. Inverse associations were observed for ALL and asthma (OR = 0.79, 95% CI: 0.61-1.02), eczema (OR = 0.74, 95% CI: 0.58-0.96), and hay fever (OR = 0.55, 95% CI: 0.46-0.66) examined separately. ORs for ALL differed across strata of study design, exposure data source, and latency period, indicating these factors impact study results. Although these results should be interpreted cautiously given the modest number of studies, substantial

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[p] This work was published in the *American Journal of Epidemiology* (**Linabery AM**, Jurek AM, Duval S, Ross JA. The association between atopy and childhood/adolescent leukemia: A meta-analysis. *Am J Epidemiol.* doi:10.1093/aje/kwq004).

heterogeneity, and potential exposure misclassification, they are useful in designing future research.

Introduction

Leukemia is the most common malignancy among individuals ages 0-19 years; the incidence rate (42 per 1,000,000 person-years) increased at an average annual rate of 0.7% (95% CI: -0.1%-1.5%) in the U.S. in recent years.[3] Nonetheless, there are few established risk factors for pediatric/adolescent leukemia, including predisposing genetic conditions, and exposure to *in utero* or postnatal therapeutic irradiation, or chemotherapeutic agents.[4] Atopic disease (i.e., asthma, eczema, hay fever, hives) has been studied as a risk factor for several malignancies,[212] including childhood/adolescent leukemia, and is the subject of the current meta-analysis.

The prevalence of atopic disease increased worldwide over the latter part of the 20th century and continues to increase in some areas.[218] Estimated prevalences for asthma, skin allergies, and hay fever among those ages 0-17 years in the U.S. are 9.1%, 10.0%, and 9.7%, respectively.[287, 288] Reasons for the observed increase are not entirely known, however, the hygiene hypothesis presents one explanation.[242] This hypothesis postulates that several factors in developed countries contributed to the rise in atopic/allergic conditions, including smaller family size, public sanitation, increased antibiotic use, low incidences of helminth infestation and fecal-oral transmission of infection, and stable micro-flora population in the intestines.[219]

The current study aims to systematically summarize the etiologic evidence regarding the atopy-childhood/adolescent leukemia association, to identify sources of heterogeneity in the existing literature, and to identify gaps in the current state of knowledge. This research is of interest given secular trends described above in leukemia incidence and atopy prevalence, and the immune system's role in the development of both leukemia and atopic disease.

Methods

Study identification and selection

Eligible studies included individuals diagnosed with leukemia between ages 0 and 18 years; representative, healthy control groups; and reports of any atopic disease, allergies, asthma, atopic dermatitis/eczema, allergic rhinitis/hay fever, or urticaria/hives. Number of exposed and unexposed cases and controls and/or measures of association describing risk of childhood leukemia must also have been provided for inclusion; crude odds ratios (ORs) and standard errors (SEs) were computed from cell counts if not reported. Studies involving inappropriate control groups, such as siblings (who are not independent from cases in atopic status) and hospital- or specialty clinic-based controls (who may have been inadvertently selected on exposure status), and those with data overlapping other studies were excluded.

To identify all eligible studies published prior to April 1, 2009, the MEDLINE database was searched via PubMed, using a combination of MeSH subject headings and keywords ((("Allergy and Immunology"[Mesh] OR "Hypersensitivity"[Mesh]) OR "Asthma"[Mesh] OR ("Eczema"[Mesh] OR "Dermatitis, Atopic"[Mesh]) OR "Rhinitis,

Allergic, Seasonal"[Mesh] OR "Urticaria"[Mesh]))AND "Leukemia"[Mesh]) OR (((atopy OR atopic OR atop*) OR (allergy OR allergic OR allerg*)) AND leukemia) and limiting results to children ages 0-18 years and English language. The Cochrane Library Database of Systematic Reviews was also searched using relevant keywords. (The EMBASE database was not accessed, as it was deemed unlikely to yield additional references.) Abstracts from resulting articles were reviewed by two independent reviewers to determine eligibility (A.M.L. and A.M.J.). In the infrequent event of discrepancies, the two reviewers reached consensus through discussion. Reference lists from retrieved articles were manually examined to identify additional studies.

Additionally, 36 international experts in pediatric cancer etiology were surveyed to request any other relevant published or unpublished results, as direct contact with experts has been shown to be an effective method of study ascertainment.[289][q]

Data extraction

Data from eligible studies, including general study characteristics (study design, participant ages, diagnostic dates, number and source of cases and controls, source of exposure data, and matching variables), quality-related factors (listed below), and results (cell counts, ORs, and 95% confidence intervals (CIs)), were abstracted by the two reviewers onto a standardized form developed by the authors[r] and were compared to ensure accuracy. Dichotomous exposures abstracted included atopy, allergy, asthma, eczema, hay fever, or hives; dichotomous outcomes included childhood leukemia, acute lymphoblastic leukemia (ALL), or acute myeloid leukemia (AML). A dichotomous

[q] A list of the 36 international experts queried is provided in Appendix K.
[r] The data abstraction form developed for this study is shown in Appendix L.

variable indicating any report of atopy or allergies was created due to the variability in definitions of composite atopy/allergy variables across eligible studies. If >1 definition of an exposure was evaluated in a given study, results for the most general definition were abstracted to attain comparability across studies.[34, 249] If a definition included a latency period, the corresponding results were abstracted preferentially.[43] Adjusted estimates were generally selected over unadjusted estimates; however, in one study, estimates from the most parsimonious model were abstracted due to the wide spectrum of adjustment factors included in the full multivariate model and the similarity between the two estimates.[38] Two authors were contacted for study results specific to children/adolescents;[248, 290] additional information was received from one of these (Karin Söderberg, Karolinska Institutet, personal communication, 2009).

Statistical methods

Ten exposure-disease associations were examined, representing all identified associations with ≥2 eligible studies. Stata was used to conduct all analyses and produce forest plots.[291] Summary ORs and 95% CIs were computed from study-specific ORs and corresponding SEs using DerSimonian and Laird random effects (RE) models,[292] since RE models incorporate between-study heterogeneity. Fixed effect (FE) summary ORs were also calculated for comparison. To quantify the degree of heterogeneity across studies, Higgins' I^2 statistic and 95% CIs were generated, representing the proportion of the variance attributable to between-study variability;[293] CIs could not be calculated for statistics with degrees of freedom <2.

Analysis of the influence of individual studies was conducted, wherein each study was omitted in turn, to identify studies contributing disproportionately to the observed heterogeneity.[s] Summary ORs for ALL were calculated across strata of factors selected *a priori* as potentially related to study quality to examine potential sources of heterogeneity. (There were not enough studies of leukemia overall or AML to permit similar stratification.) These factors include study design (nested versus other case-control), source of controls (population-based versus other), source of exposure data (medical record versus parental report), latency period (yes versus no), response rates for cases and controls (≥80% versus <80%), and adjustment for potential confounders (yes versus no for age, sex, race, socioeconomic status (SES), method of infant feeding, and location of residence).

Notably, there were too few studies for a given association to warrant a formal assessment of publication bias; however, the thorough search strategy executed in combination with the query of experts should have eliminated most sources of publication bias (except possible English language bias).

Results

Of the 442 manuscripts identified via the MEDLINE search, 417 were excluded and 25 were retrieved for review; 10 were determined eligible upon further review

[s] Cumulative analysis was also conducted to assess whether the conduct of the most recent studies has advanced our understanding of these associations. Results of the cumulative analysis are shown in Appendix M. The summary OR for the ALL-atopy/allergy association has not changed materially since 2003, while the summary ORs for the associations between ALL and asthma and eczema, respectively, have remained fairly constant since the initial study in 2000. Results of the influence analysis are shown in Appendix N. With the possible exception of the study by Bross and Natarajan, there does not appear to be any other single study contributing too much weight to any of the summary ORs.

(Figure 7-1). [34-38, 43, 248-251] No additional eligible studies resulted from the Cochrane Database search, manual examination of references, or query of experts.

Characteristics of the 10 case-control studies meeting the inclusion criteria are provided in Table 7-1. A majority were published in 2000 or thereafter and half were conducted in the U.S.; the remainder originated in Europe and Japan. They collectively included 6,592 childhood/adolescent leukemia cases and 24,171 controls, although individual studies investigated different exposure-disease associations. Most examined children ages 0-14 years, with a few exceptions.

The distribution of selected quality-related factors is summarized in Table 7-2. A minority of studies (30%) were nested within a larger cohort, although most used population-based control groups (80%), which should better represent the cases had they not developed leukemia. Seven studies used parental report to determine atopic phenotype, while three used medical records; none used biomarkers for atopic disease classification. Four included a latency period between the onset of atopy and leukemia. Case response rates were ≥80% in half of the studies, while this high level of participation was only reached in 20% of control groups. Finally, all but one study adjusted for age, none adjusted for sibship, and an intermediate number adjusted for sex, race, SES, method of infant feeding, and location of residence, all of which are possible confounders.

Table 7-3 lists excluded case-control studies, reasons for exclusion, and key study results. Primary reasons for exclusion were hospital- or specialty clinic-based control

groups, study populations overlapping included studies, and inclusion of subjects exceeding the age range of interest.

Results of the meta-analysis are depicted in Figure 7-2. For leukemia overall, the summary OR for any reported atopic disease or allergies was 1.42 (95% CI: 0.60-3.35) with substantial heterogeneity present across the three studies reporting on this outcome (I^2 = 94%). The study by Bross and Natarajan[250] accounts for the observed variability; there was no detectable heterogeneity upon omission of this study (I^2 = 0%). Summary ORs were not calculated for specific atopic diseases, as these were not assessed in two of the three studies of leukemia overall.

A 0.69-fold reduced odds of ALL was observed for any atopy/allergies (95% CI: 0.54-0.89), although there was evidence of considerable heterogeneity across the six included studies (I^2 = 80%). The influence analysis did not reveal an obvious outlier among the six studies. For atopic diseases examined separately, inverse associations were observed for ALL and asthma (7 studies; OR = 0.79, 95% CI: 0.61-1.02), eczema (5 studies; OR = 0.74, 95% CI: 0.58-0.96), and hay fever (3 studies; OR = 0.55, 95% CI: 0.46-0.66), but not for hives (2 studies; OR = 0.93, 95% CI: 0.73-1.19).

For AML, a nonsignificant inverse association with any atopy/allergies was calculated (OR = 0.87, 95% CI: 0.62-1.22) among two studies; there was no appreciable heterogeneity detected (I^2 = 0%). Of note, there was less power to detect an association in these studies, with 164 and 101 AML cases, respectively. The summary ORs did not indicate evidence of an association for AML and asthma (3 studies; OR = 1.05, 95% CI:

0.56-1.95), eczema (2 studies; OR = 0.78, 95% CI: 0.53-1.15), or hay fever (2 studies; OR = 1.10, 95% CI: 0.65-1.86) assessed individually.

The FE models produced identical or very similar ORs to those generated by RE models, with identical or narrower CIs but equivalent interpretations. One possible exception is the FE OR for atopy/allergies and leukemia overall, which was attenuated compared with the RE OR (OR_{FE} = 1.01, 95% CI: 0.86-1.18). This is not surprising, considering that the high heterogeneity present in this set of studies is attributable to the smallest study with a large, positive association and this smallest study does not contribute substantially to the FE OR.

The stratified analyses indicate the inverse associations for ALL are nearly completely attenuated by a nested case-control study design, medical records as the source of exposure data, and inclusion of a latency period, collectively ($OR_{atopy/allergies}$ = 0.99, 95% CI: 0.72-1.37; OR_{asthma} = 0.97, 95% CI: 0.60-1.59; OR_{eczema} = 0.92, 95% CI: 0.77-1.11); the effect of each could not be evaluated, since the same studies mutually included these factors (Figure 7-3). Similarly, summary ORs for studies with case and control response rates ≥80% were closer to the null compared with rates <80%. Controlling for race, SES, or location of residence, respectively, did not produce meaningful differences in summary ORs across strata. Adjusting for sex or breastfeeding generated identical or very similar ORs across strata, although precision of the stratum-specific ORs differed.

Table 7-1. Characteristics of 10 Case-control Studies Included in the Meta-analysis of the Atopy-Leukemia Association

First author, Country (Year)	Study design	Age range (years)	Source of cases	Source of controls	N_{cases}	$N_{controls}$	Assessment of exposure	Exposure variables	Matching/ adjustment factors
Fraumeni, USA (1964)[251]*	Case-control study (National Cooperative Leukemia Study)	0-15	12 Medical centers participating in National Cooperative Leukemia Study Participation rate: NA	Neighborhood controls Participation rate: NA	498	498	Parental interview	Atopic allergy (i.e., asthma, eczema, hay fever)	Matching: Neighborhood, age, birth order, family size, race Adjustment: None
Bross, USA (1972)[250]†	Case-control study (Tri-State Survey)	1-14	Cancer registries in the sample areas (NY, MD, MN) Participation rate: NA	Multi-stage random sample of households in sample areas Participation rate: NA	141	357	Parental interview	Allergic diseases (i.e., asthma, hives)	Matching: NA Adjustment: Age group (1-4, 5-9, 10-14 years)
Nishi, Japan (1989)[38]	Case-control study	0-14	All children with immunologic-ally ascertained non-T-cell ALL at 9 hospitals in Hokkaido Prefecture, Japan Participation rate: 100%	Health centers and hospitals in case residential areas, recruited at routine health examinations Participation rate: NA	63 ALL	126	Maternal interview	Atopic diathesis (i.e., history of asthma or atopic dermatitis)	Matching: Age, sex, district of residence at diagnosis Adjustment: Age, sex, district of residence at diagnosis

First author, Country (Year)	Study design	Age range (years)	Source of cases	Source of controls	N_{cases}	$N_{controls}$	Assessment of exposure	Exposure variables	Matching/ adjustment factors
Wen, USA (2000)[37]	Case-control study (Children's Cancer Group (CCG))	0-14	CCG member institutions in the US Participation rate: 92%	RDD Participation rate: 76.5%	1842 ALL	1986	Maternal telephone interview	Allergic disorders (i.e., asthma, eczema, hay fever, hives, food or drug allergies), asthma, eczema, hay fever, hives	Matching: Age (within 25% of age of diagnosis, with a maximum of ±2 years), race (white, black, other), telephone area code and exchange Adjustment: Age, race, telephone area code and exchange, months of breastfeeding, maternal education, maternal race, family income
Schüz, Germany (2003)[36]	Pooled results from 3 case-control studies	0-14	Nationwide German Childhood Cancer Registry (>95% complete) Participation rate: 78%	Local resident registration offices Participation rate: 67%	1130 ALL, 164 AML	2957	Self-administered postal questionnaire completed by parents	Atopic disease (i.e., asthma, neurodermatitis, hay fever), asthma, neurodermatitis, hay fever, hives	Matching: Gender, date of birth (+/- 1 year), community Adjustment: Age, year of birth, gender, degree of urbanization (rural, urban, mixed), SES (average, high)

First author, Country (Year)	Study design	Age range (years)	Source of cases	Source of controls	N_{cases}	$N_{controls}$	Assessment of exposure	Exposure variables	Matching/ adjustment factors
Spector, USA (2004)[43]	Case-control study	0-6	Databases of 4 health maintenance organizations (HMOs) in Western USA Participation rate: 100%	Databases of 4 HMOs in Western USA Participation rate: 100%	180 ALL	718	Subjects' medical records (diagnosis by a physician)	Allergic conditions (i.e., asthma, eczema, atopy or hives, food-drug-bee allergy, pollen-dust-dander allergy), asthma, eczema	Matching: HMO, gender, date of birth (+/- 2 weeks) Adjustment: Age, gender, race, HMO. Individual allergic conditions mutually adjusted.
Jourdan-Da Silva, France (2004)[249]	Population-based case-control study	0-14	National Registry of Childhood Leukemia and Lymphoma in France (all cases <15 years) Participation rate: 73%	Randomly selected from general population via RDD based on expected distribution of cases Participation rate: 71%	408 ALL, 65 AML	567	Self-administered questionnaire completed by mothers	Asthma	Matching: Age, gender, region of residence at diagnosis Adjustment: Gender, age, region
Rosenbaum, USA (2005)[35]	Case-control study (31 counties of New York State)	0-14	Institutional tumor registries and departmental records at 4 major medical centers Participation rate: 71%	Randomly selected via Live Birth Certificate Registry (New York State Department of Health) Participation rate: 55%	255 ALL	760	Self-administered postal questionnaire completed by parents	Allergy history (i.e., asthma, eczema, food-drug-bee, pollen-dust, or cat-dog dander allergy), asthma, eczema	Matching: Sex, race (white/non-white), birth year Adjustment: Maternal smoking (yes/no), maternal education (years), breast fed (yes/no), race, birth year

First author, Country (Year)	Study design	Age range (years)	Source of cases	Source of controls	N_{cases}	$N_{controls}$	Assessment of exposure	Exposure variables	Matching/ adjustment factors
Söderberg, Sweden (2006)[248]‡	Population-based case-control study	0-18	All cases in Swedish Cancer Registry Participation rate: 100%	Randomly selected from Swedish nationwide population registry Participation rate: 100%	875 ALL, 132 AML	14865	Swedish Hospital Discharge Registry	Asthma	Matching: Sex, 5-year age group, year of diagnosis Adjustment: Sex, age, SES
Hughes, UK (2007)[34]	Population-based case-control study (United Kingdom Childhood Cancer Study)	0-14	Residents of Great Britain diagnosed with malignancy Participation rate: 92%	Randomly selected from primary care population registers Participation rate: 70%	839 (720 ALL, 101 AML)	1337	Primary care records	Allergies (i.e., asthma, eczema, hay fever), asthma, eczema, hay fever	Matching: Age, sex, region of residence at diagnosis Adjustment: Age (single years), sex, region of residence, deprivation index

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; HMO = health maintenance organization; NA = not available; RDD = random digit dialing; SES = socio-economic status

* Crude odds ratio (OR) calculated from data provided.

† Age-adjusted OR calculated from data provided in (55).

‡ Results for subjects ages 0-18 years obtained from first author via personal communication.

Table 7-2. Distribution of Factors Related to Quality in the 10 Case-control Studies Included in the Meta-analysis of the Atopy-Leukemia Association

	Total		Leukemia overall		ALL		AML	
Factor	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Study design								
Nested case-control study	3	30%	1	33%	3	38%	2	50%
Population-based controls	8	80%	2	67%	7	88%	4	100%
Exposure assessment								
Medical records	3	30%	1	33%	3	38%	2	50%
Latency period	4	40%	2	67%	3	38%	2	50%
Participation rates								
Cases (≥80%)	5*	50%	1*	33%	5	63%	2	50%
Controls (≥80%)	2*	20%	0*	0%	2*	25%	1	25%
Control for confounders								
Age at diagnosis	9	90%	2	67%	8	100%	4	100%
Sex	6	60%	1	33%	6	75%	4	100%
Race	3	30%	0	0%	3	38%	0	0%
Sibship	0	0%	0	0%	0	0%	0	0%
SES	5	50%	1	33%	5	63%	3	75%
Breastfeeding	2	20%	0	0%	2	25%	0	0%
Residence	5	50%	1	33%	5	63%	3	75%

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; SES = socio-economic status

* Participation rates not available for 2 studies of leukemia overall and 1 study of ALL.

Table 7-3. Description of Case-control Studies Excluded from the Meta-Analysis of the Atopy-Leukemia Association

First author, Country (Year)	Study design	Reason for exclusion	Results
Manning, USA (1957)[294]	Case-control	Specialty clinic-based control group	Suggestion of increased risk of childhood leukemia associated with allergies (i.e., asthma, eczema, hay fever, hives, other) (OR = 2.44, 95% CI: 0.97-6.14).*
Stewart, UK (1958)[295]	Case-control	Results presented for childhood cancer cases overall, not for leukemia cases	No evidence for an association between allergic conditions and childhood malignancy (OR = 1.12, 95% CI: 0.65-1.94).*
Ager, USA (1965)[296]	Case-control	Very low prevalence of atopic disease reported among cases and controls ages 0-4 years. This may be due to misclassification since exposure measured first via maternal interview, then followed up with medical record verification only for those with a positive maternal report.	No evidence for an association between death from childhood leukemia and asthma, eczema, or hives, respectively (OR_{asthma} = 0.66, 95% CI: 0.11-4.03; OR_{eczema} = 0.99, 95% CI: 0.14-7.16; OR_{hives} = 0.99, 95% CI: 0.14-7.16).*
Smith, USA (1973)[297]	Case-control	Study overlaps with Bross (1972).[250] Cell counts from this paper used to calculate overall age-adjusted OR.	Results included in meta-analysis
Natarajan, USA (1973)[298]	Case-control	Study overlaps with Bross (1972)[250]	The association between allergic disease (i.e., asthma, hives, eczema) and childhood leukemia is greater among those with maternal exposure to preconception radiation (OR = 4.6, P = 0.0001) than those with no preconception radiation (OR = 1.9, P = 0.03).
Viadana, USA (1974)[299]	Case-control	Results presented for leukemia cases ≥15 years of age. Unable to locate author to request data for 15-18 year olds.	Not applicable

First author, Country (Year)	Study design	Reason for exclusion	Results
Bross, USA (1974)[300]	Case-control	Study overlaps with Bross (1972)[250]	The association between allergic disease (i.e., asthma, hives, eczema) or bacterial disease (i.e., pneumonia, dysentery, rheumatic fever) and childhood leukemia is greater among those with exposure to maternal preconception, intrauterine, and postnatal radiation (OR = 4.1, $P = 0.0001$) than those with no prior radiation history (OR = 1.6, $P = 0.23$).
Gibson, USA (1976)[301]	Case-control	Results presented for leukemia cases ≥15 years of age. Unable to locate author to request data for 15-18 year olds.	Not applicable
Magnani, Italy (1990)[302]	Case-control	Hospital-based control group	Inverse association observed between allergic diseases and childhood ALL after adjusting for SES (OR = 0.4, 95% CI: 0.2-0.8).
Zheng, China (1993)[290]	Case-control	Results presented for leukemia cases ≥15 years of age. Per personal communication with first author, unable to obtain data for 15-18 year olds.	Not applicable
Buckley, USA (1994)[252]	Case-control	Results presented for family history of allergy, not for personal history of allergy	Family history of allergies (i.e., asthma, hay fever, hives, food or drug allergy) in siblings, parents and/or grandparents associated with a modest increased risk for childhood ALL after adjusting for birth year, race, income, geographical region, and family size (OR = 1.3, $P < 0.05$).
Petridou, Greece (1997)[102]	Case-control	Hospital-based control group and exposure of hospitalization for allergic disease	Nonsignificant inverse association observed between hospitalization for allergic disease and childhood leukemia after adjustment for gender, age, location of residence, and other biomedical variables of interest (OR = 0.36, 95% CI: 0.09-1.43).

First author, Country (Year)	Study design	Reason for exclusion	Results
Kaatsch, Germany (1998)[303]	2 case-control studies	Study overlaps with Schüz (2003)[36]	Nonsignificant inverse association observed between allergy and childhood leukemia after adjusting for age, sex, place of residence, and SES (OR = 0.90, 95% CI: 0.63-1.29).
Schüz, Germany (1999)[304]	2 case-control studies	Study overlaps with Schüz (2003)[36]	Inverse association observed between allergy and childhood leukemia after adjusting for gender, date of birth, district of residence, and SES (OR = 0.6, 95% CI: 0.5-0.8).

ALL = acute lymphoblastic leukemia; CI = confidence interval; OR = odds ratio; SES = socioeconomic status

* Crude OR calculated from data provided.

Figure 7-1. Flow diagram of the search strategy and study selection process.

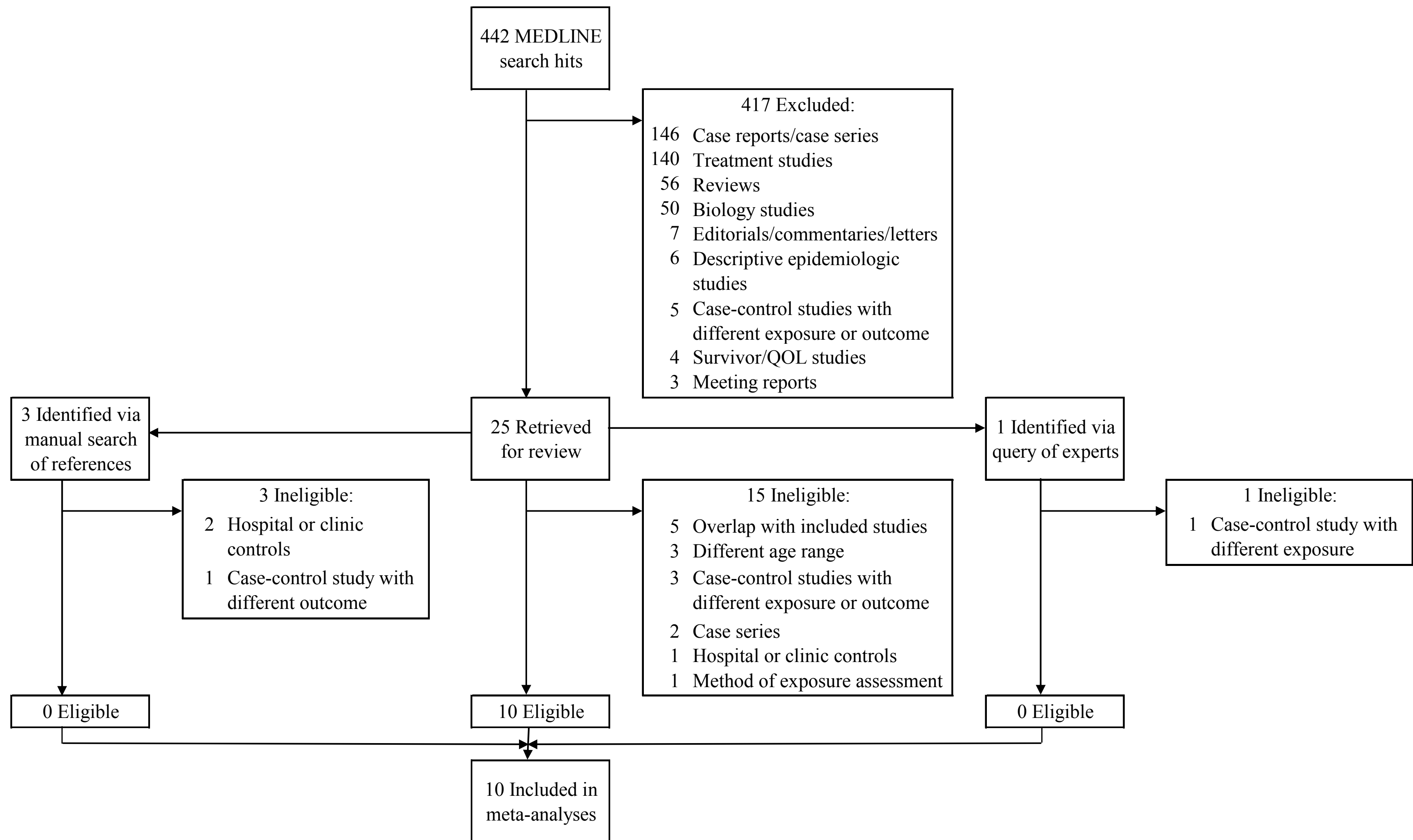
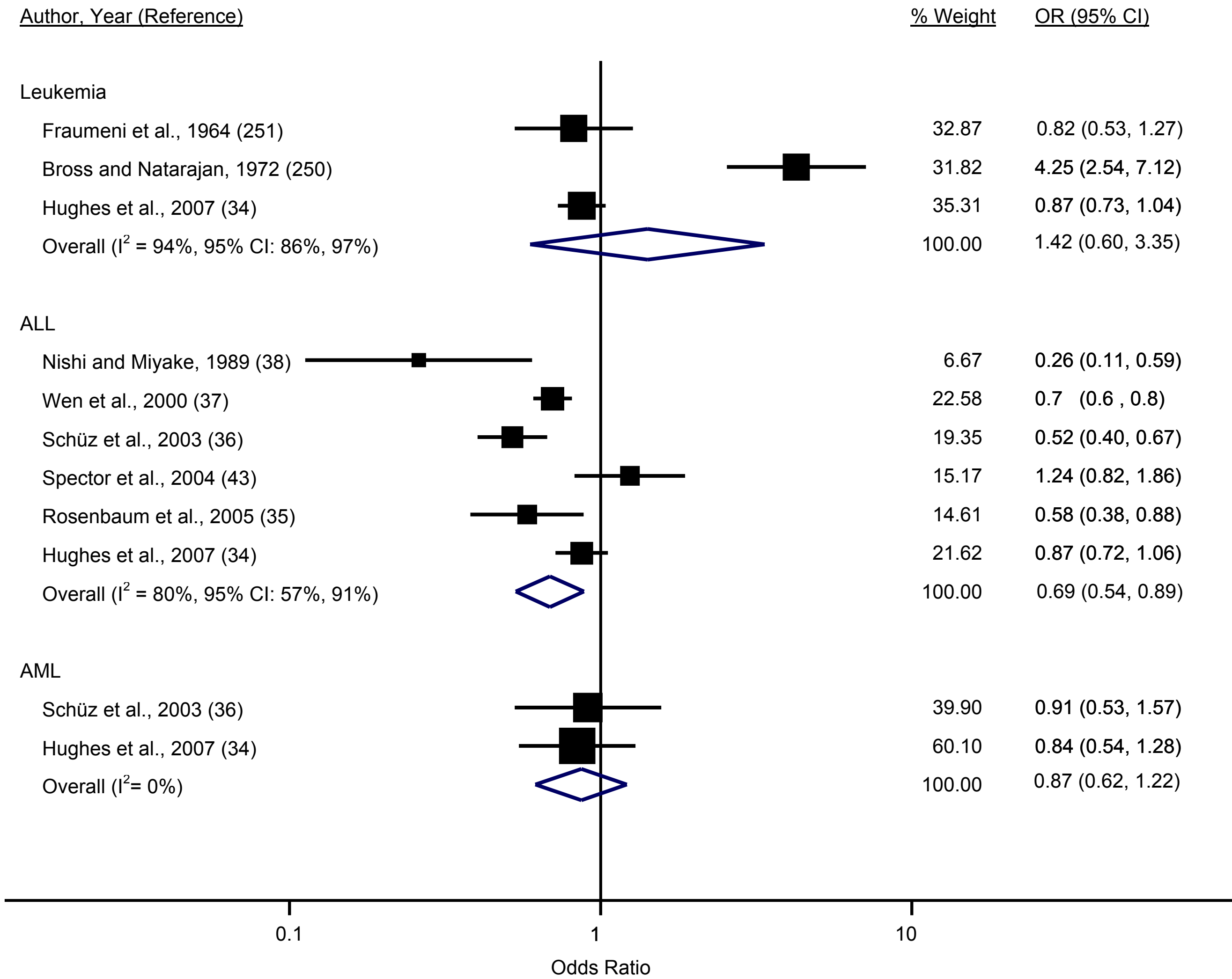
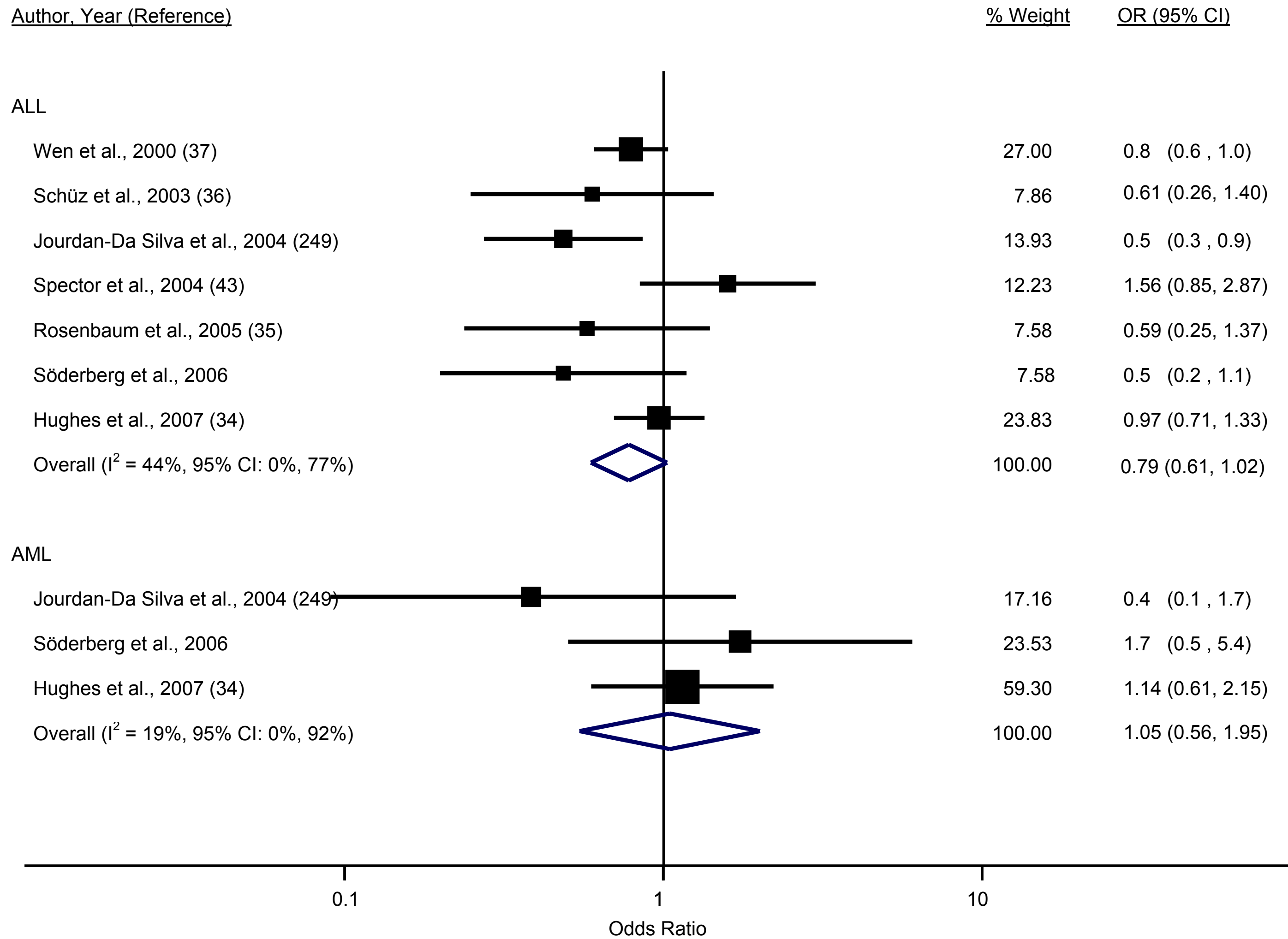


Figure 7-2. Study-specific and random effects summary odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between leukemia overall, acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML) and A) atopy or allergies, B) asthma, C) eczema, D) hay fever, and E) hives. The size of each box indicates the relative study weight; the bars indicate the 95% CI. Higgins' I^2 statistic and 95% CI, a measure of the degree of heterogeneity across studies, is also shown for each summary OR. Of note, the 95% CI could not be calculated for I^2 with fewer than 2 degrees of freedom. For Fraumeni *et al.*, 1964, the crude OR was calculated from data provided in [251]. For Bross and Natarajan, 1972, the age-adjusted OR was calculated from data provided in [297]. For Söderberg *et al.*, 2006, results for children/adolescents ages 0-18 years were obtained from first author (Karin Söderberg, Karolinska Institutet, personal communication, 2009).

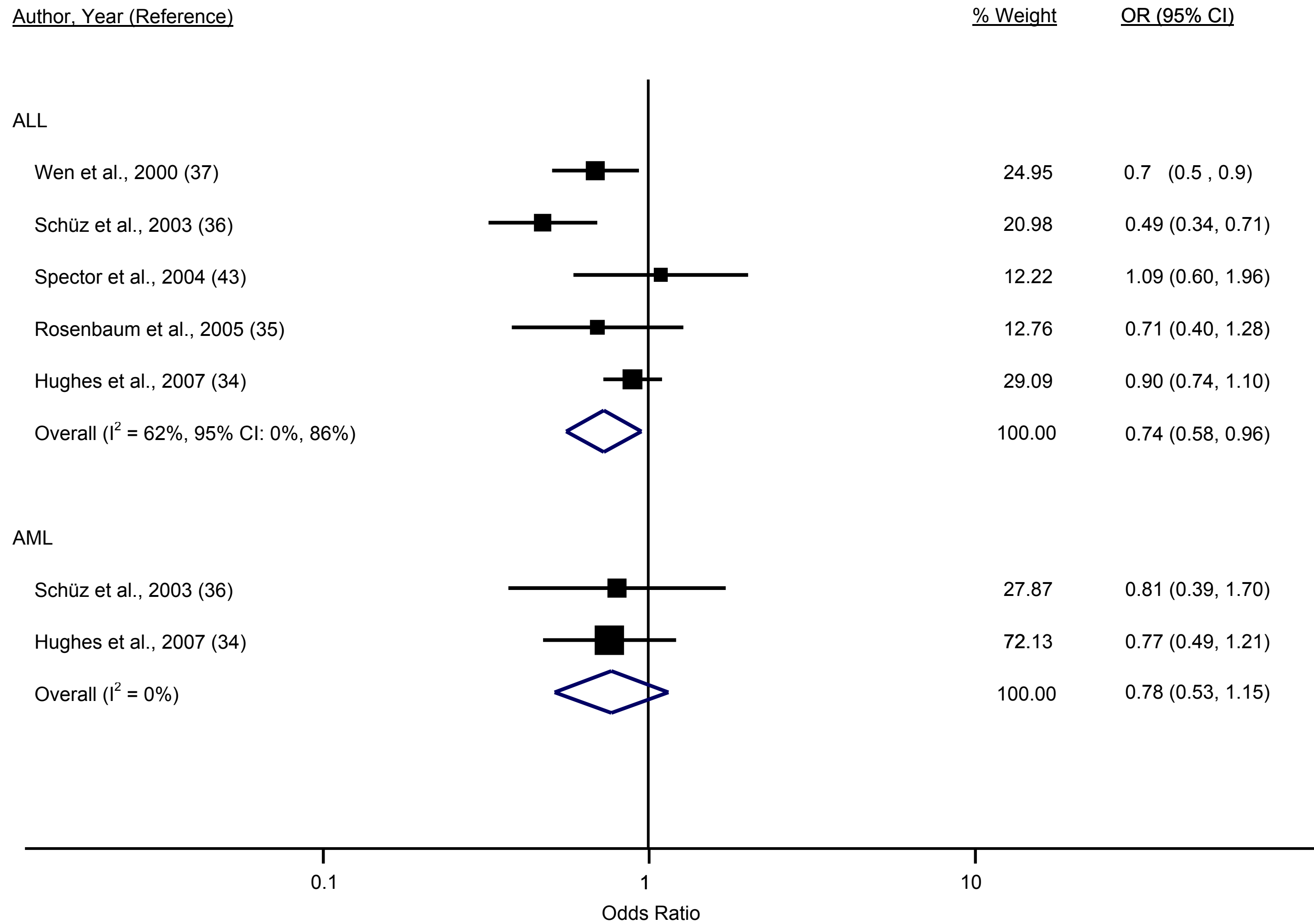
7-2A)



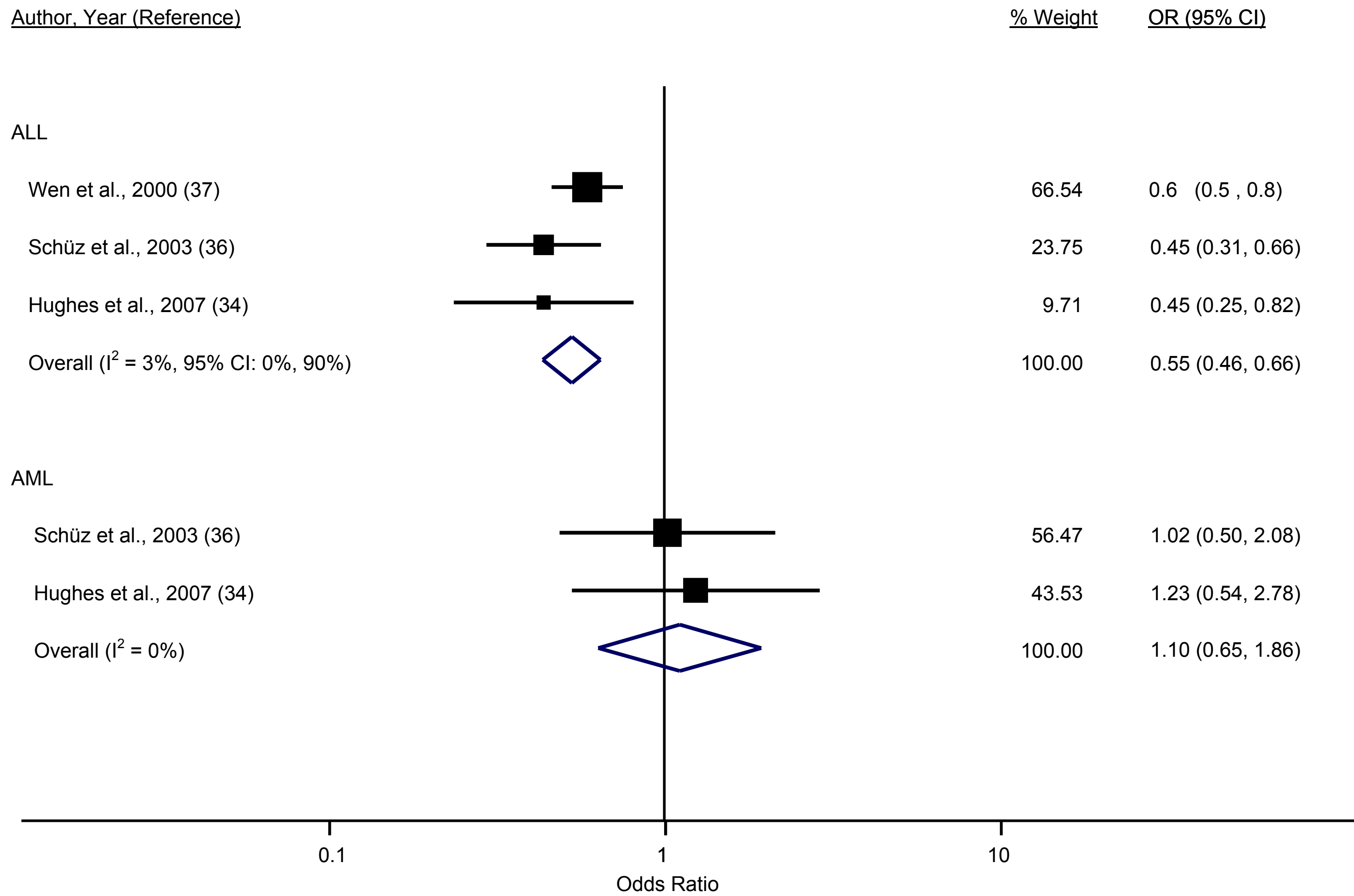
7-2B)



7-2C)



7-2D)



7-2E)

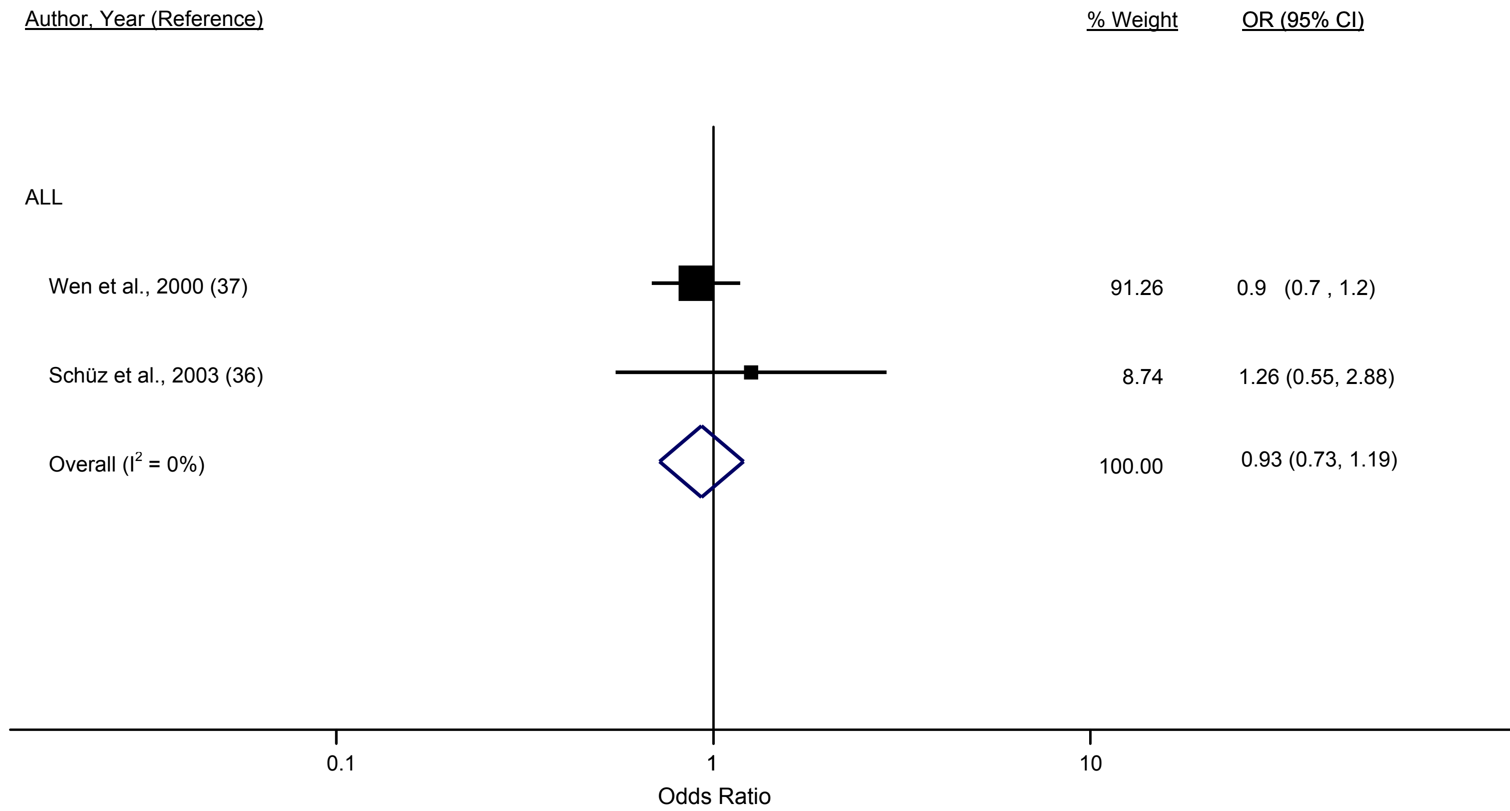
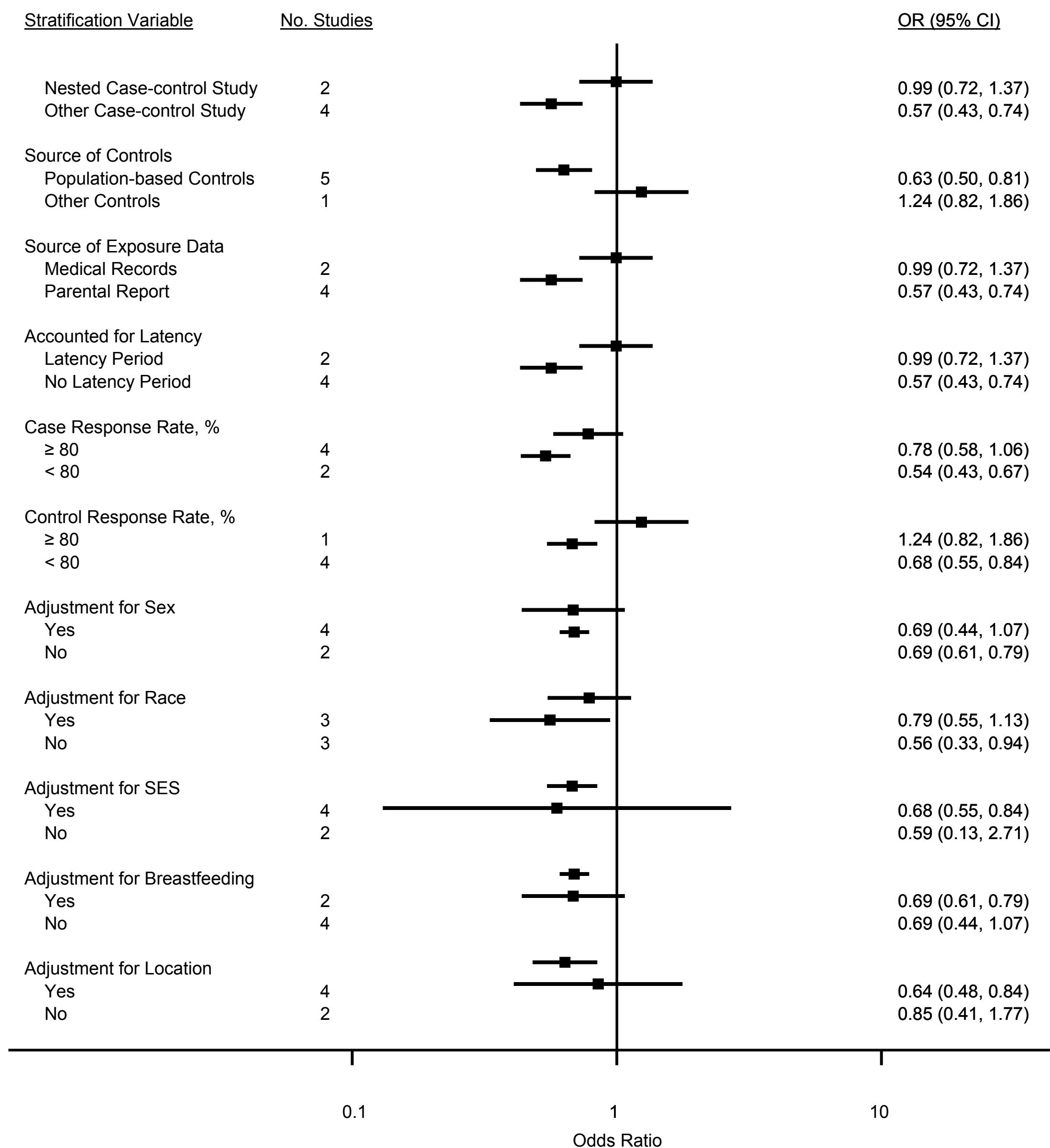
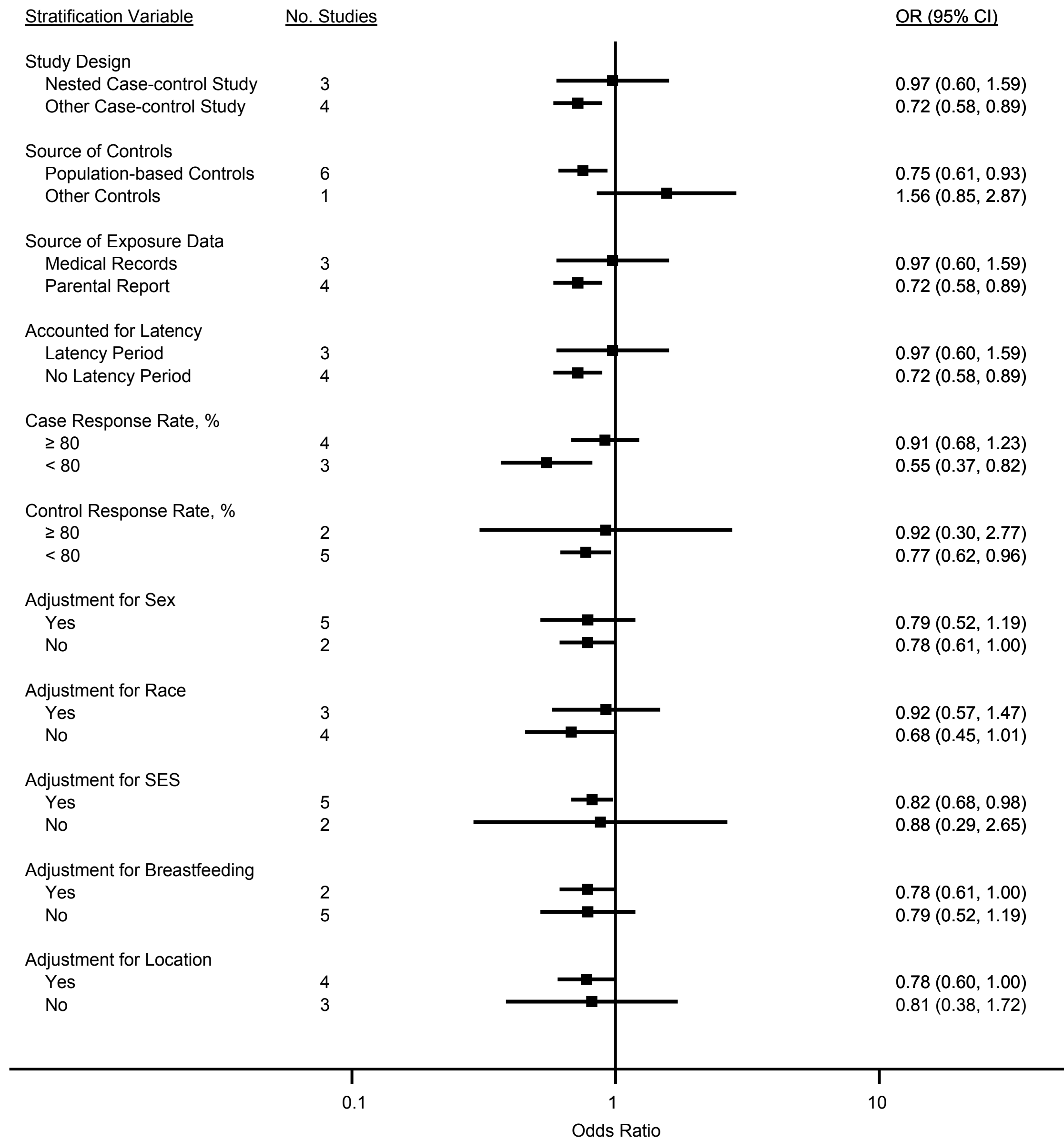


Figure 7-3. Random effects summary odds ratios (ORs) and 95% confidence intervals (CIs) upon stratification by quality-related factors for the association between acute lymphoblastic leukemia (ALL) and A) atopy or allergies, B) asthma, and C) eczema. Of note, the control response rate was not available for one study.[38]

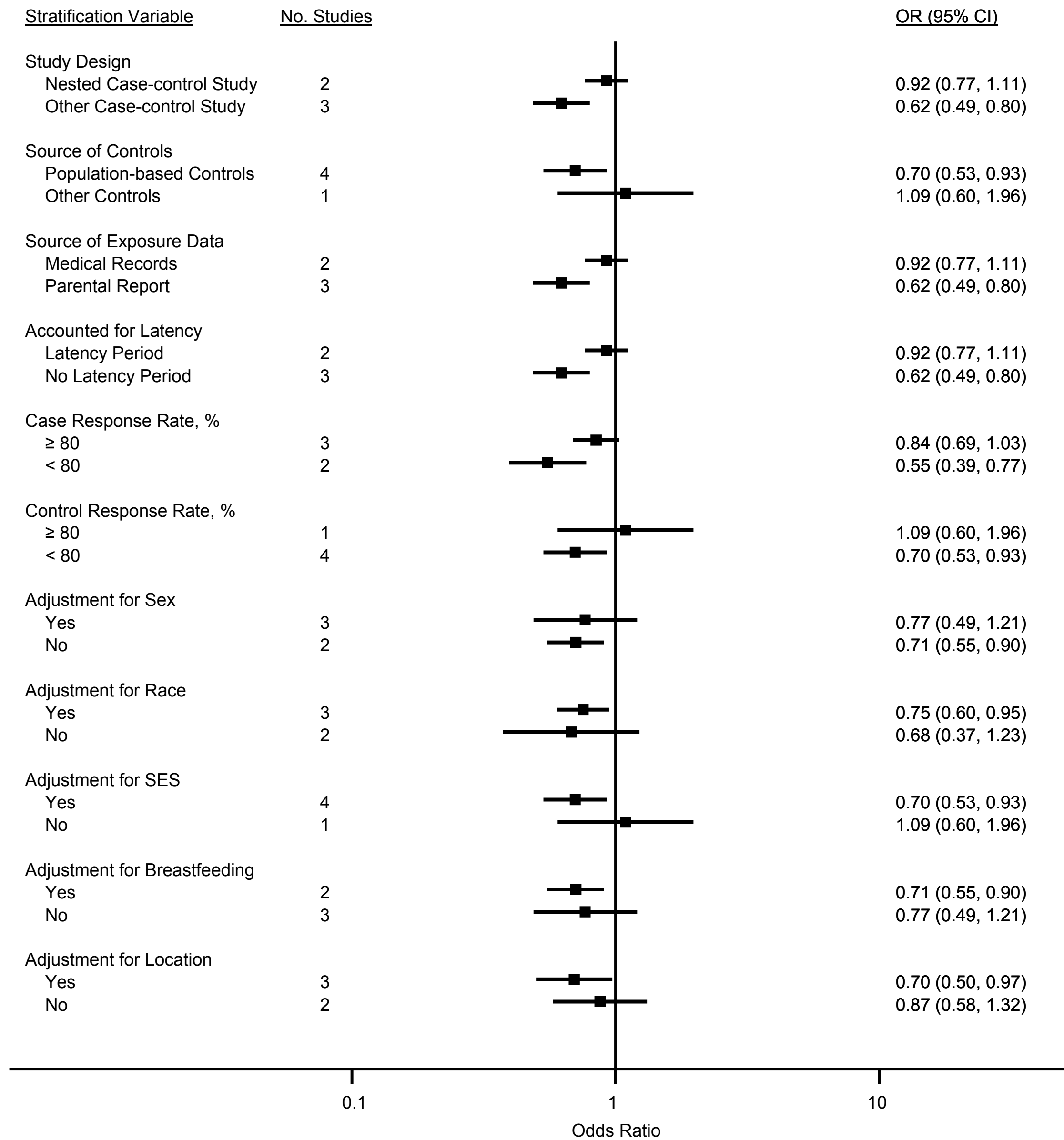
7-3A)



7-3B)



7-3C)



Discussion

Results of this meta-analysis indicate an inverse association between atopy/allergies and childhood ALL; the pooled odds of any reported atopy/allergy were 31% lower among cases than controls and was 21% lower for asthma, 26% lower for eczema, and 45% lower for hay fever, respectively. No association was observed for leukemia overall or AML, or for any of the specific atopic conditions and AML examined separately, although there were fewer studies of AML and fewer AML cases. The divergent results observed for ALL and AML are of interest, given that atopic status should have been captured similarly across the two case groups.

Two conflicting hypotheses have been proposed to explain a causal relationship between atopy and cancer. The immune surveillance hypothesis asserts that the immune system recognizes antigens of malignant cells as foreign and mounts a response to them, preventing a majority of potential cancers from developing.[232] The presence of an atopic condition is thought to increase the vigilance of the immune system in monitoring for, identifying, and eliminating malignant cells.[212, 233] In support of this hypothesis is the observation that immunocompromised individuals have higher incidence of specific malignancies than those with intact immune systems.[234] Notably, immunosupressed persons have increased risk of non-Hodgkin lymphoma,[26] a malignancy with identical histology to T-cell ALL.[4]

The second hypothesis is that chronic stimulation of the immune system by allergens increases the risk of carcinogenesis.[233, 234] A greater number of proliferating cells increases the probability of genetic errors, such as pro-oncogenic mutations, that

may not be repaired prior to subsequent divisions.[233] This mechanism has been proposed as an explanation for the positive association between autoimmune disease and malignancy,[236] for example.

It is not clear if either of these mechanisms is applicable to childhood/adolescent cancers; however, the majority of childhood/adolescent leukemia studies reported inverse associations, potentially supporting the immune surveillance hypothesis. Other possible explanations include reverse causality, where the leukemic process induces atopic manifestations, or a common etiology for both atopy and leukemia. There are case reports exemplifying the former explanation.[305-307] As an example of the latter, Smith *et al* demonstrated that the hygiene hypothesis may also apply to childhood leukemia.[243] In that ecologic study, decreases in the prevalence of hepatitis A infection, a marker for hygiene due to the fecal-oral mode of transmission, were associated with increased ALL risk in the U.S. and Japan.[243] Neither of these is consistent with the observed inverse association, however.

If an inverse relationship existed, then ALL incidence would be expected to decrease with increasing atopy prevalence. The observed increase in ALL incidence[3] is not inconsistent, however, as ALL is multifactorial, requiring ≥2 “hits,”[8] and atopy would constitute a single “hit” (i.e., it is neither sufficient nor necessary). Assuming $OR_{atopy/allergies} = 0.69$ and atopy $prevalence_{cases} = 30\%–40\%$, the attributable fraction for atopy is -14% to -18%.[308] It is possible that other putative risk factors with increasing secular trends (e.g., birthweight[75]) may contribute to the increase in ALL and offset any decrease attributable to atopy.

Although no biological mechanism has been established, the principal factor linking childhood/adolescent leukemia and atopic disease is the rate at which the immune system matures.[34] Hypotheses by Greaves and Kinlen suggest an etiologic role for the immune system in the development of childhood leukemia via delayed exposure and abnormal response to early life infections;[11, 210] no responsible virus or other infectious agent has been identified to date.[246, 247] T helper 2 cell (Th2) versus Th1 cell predominance is often described with respect to atopy,[237] where each cell type has a specific cytokine profile and associated sequelea. Infants are born with a Th2-dominated immune profile; non-atopic infants gradually migrate to a Th1-dominant process by 2 years, while infants with a family history of atopy fail to make the Th2-to-Th1 transition.[238] Early exposure to infectious agents is thought to stimulate this transition. Two additional T cell types, T-regulatory and Th17, may also play a role in the complex interplay between atopy, infections, and autoimmune diseases (reviewed in [239]).

There are several notable sources of heterogeneity across the 10 included studies. Results of the stratified analysis indicate the most important of these is case-control study design, source of exposure data, and/or inclusion of a latency period. Only three studies were nested within a larger cohort;[34, 43, 248] these have the advantage of temporality over other case-control designs, in that exposures were measured prior to leukemia diagnosis.

Two aspects of exposure measurement, which data were collected and how they were collected, likely contributed to the observed heterogeneity. As shown in Table 7-1, definitions for composite atopy/allergy variables differed across studies in that authors included different permutations of asthma, eczema, hay fever, and hives. One study

included neurodermatitis instead of eczema.[36] In two studies, composite variables incorporated food, drug, bee sting, pollen, dust, or pet dander allergies, accounting for a large proportion of exposed individuals and potentially driving the observed associations.[35, 37] Söderberg *et al* used the Swedish hospital discharge registry to identify those discharged for asthma, which is qualitatively different than exposure classifications used in other studies, and likely involves substantial underascertainment.[248] Of note, some authors presented definitions that included the presence of symptoms[34] or the use of medications.[249] Although not used in the current meta-analysis, these definitions, in concert with physician diagnosis, may increase sensitivity and could be considered in future studies.

Three studies used subject medical records for exposure assessment,[34, 43, 248] which also has the notable advantage of temporality. Medical records are typically considered the gold standard for medical history data; however, this is only applicable if medical records from all sources are obtainable and complete, which is not always the case.[309]

Atopic disease was measured via parental report in the remaining studies,[35-38, 249-251] introducing several potential sources of misclassification and/or recall bias. Subclinical atopy may not be diagnosed by physicians or recognized by parents. For example, it has been estimated that 30–40% of “healthy” individuals are atopic,[222] meaning they produce an immunoglobulin E response to an environmental stimulus. A related issue is that atopic individuals experience changes in symptoms over time.[223] Conversely, not all cases of asthma, eczema or hay fever are atopic.[240] Parents of cases

may be more motivated and more likely to recall exposures than parents of controls.[310] It is also possible that early symptoms of leukemia may be mistaken for atopic disease.[212] Or, parents of cases may be underreporting allergic diseases if immunosuppressive therapy administered for leukemia reduced allergic symptoms and parents report allergy status after initiation of treatment.[36] Parents of controls may report allergic disease arising after a specified reference date, since the reference date does not carry the personal significance of a diagnosis date.[36] A sensitivity analysis by Schüz *et al*, wherein reference dates for controls were reclassified to interview dates, revealed that the observed associations were attenuated somewhat, but they could not be wholly attributed to recall bias.[36]

Results of one validation study indicated that parental recall of an asthma diagnosis was high (87% agreement with medical records).[311] A second validation study by Hughes *et al* demonstrated that accuracy of maternal recall of asthma was high and approximately equal between cases and controls ($sensitivity_{cases}$ = 81% versus $sensitivity_{controls}$ = 83%), although the level of agreement between maternal recall and medical records was marginally higher among cases ($\kappa_{cases} = 0.69$ versus $\kappa_{controls} = 0.60$).[34] Recall of eczema was somewhat lower ($sensitivity_{cases}$ = 51% versus $sensitivity_{controls}$ = 57%), but agreement with medical records was equivalent between cases and controls ($\kappa = 0.46$). Parental recall of atopic diagnosis is therefore predicted to be similar across cases and controls, although degree of recall will likely vary by condition.

To minimize concerns about misclassification, a latency period should be incorporated between onset of atopic symptoms and leukemia diagnosis. Importantly,

only four included studies incorporated a latency period, and among those, the length of the latency period varied (3 months[34], 6 months[250], or 1 year[43, 248]).

The age range of included subjects is another potential source of heterogeneity. Most studies examined children/adolescents ages 0–14 years, however, Bross and Natarajan did not include infants,[250] Spector *et al* limited their analysis to children ≤6 years,[43] and Söderberg *et al* included children and adolescents ≤18 years (by request).[248] Age is an important consideration because the prevalence of atopy increases with increasing age.[223] Further, the age-standardized incidence of leukemia is greatest among those ages 1–4 years, with lower rates among infants and children/adolescents ages 5–19 years.[3] Importantly, three studies presented ORs by age group, however, categories differed across studies and could not be reasonably pooled.[37, 38, 250]

The principal limitation for any meta-analysis is the great potential for selection bias, encompassing publication, English language, citation, and multiple publication bias.[312] Failure to include all studies of a given association may produce a summary OR that overestimates the true effect.[313] To minimize publication bias, we contacted international experts of childhood cancer etiology to inquire about unpublished or unidentified studies in addition to the systematic electronic database search. Additional limitations include the small number of studies for each exposure-disease association and relatively high level of heterogeneity detected across studies, which may restrict the generalizability of the summary ORs produced.

There are also limitations within the individual case-control studies. Recall bias, as discussed above, is a primary concern with retrospective designs; selection bias is also

of concern. Selection bias was likely absent in the registry- or HMO-based studies[43, 248], but cannot be ruled out in other studies with lower participation rates, especially since ORs for ALL were closer to the null for both cases and controls for response rates ≥80% versus <80% in the stratified analyses. Also noteworthy, very sick or deceased cases were excluded from one study, potentially introducing survival bias.[249] Further, individuals with higher SES tend to participate in case-control studies of childhood cancer[124], and SES is associated with both atopy[314] and leukemia.[245] Adjustment for SES did not result in disparate stratum-specific ORs for ALL for atopy/allergies or asthma, but there was a possible effect for eczema. The potential effects of misclassification and other sources of bias are best evaluated by conducting a formal uncertainty analysis.[315]

Although the results of this meta-analysis indicate inverse associations between atopy/allergies and childhood/adolescent ALL, causes of the observed statistical associations must be investigated thoroughly to rule out explanations other than a direct causal relationship, such as reverse causality or selection or recall bias. Ideally, future studies would include a prospective design with analysis of biological specimens to avoid the pitfalls of temporality and misclassification plaguing existing studies.

Chapter 8: Conclusions

The ultimate aim of etiologic research is to identify underlying causes of disease. The current body of work has contributed to the general scientific knowledge about the causes of childhood and adolescent hematologic malignancies as described below.

NBS from all U.S. infants would ideally be cataloged and properly stored such that they could be retrieved and released for etiologic research with proper scientific justification, ethics review board oversight, and subject consent. From the FROGS Study, we can conclude that state newborn screening program policies regarding NBS retention and release for research are evolving rapidly; this report reflects a snapshot in time. Currently, retrospective population-based case-control studies of childhood/adolescent cancers using NBS are limited to a few states. For example, out of 39 states queried, residual NBS were retrieved with signed (parental or adult child) consent from California, Michigan, New York, Texas and Washington in this study. Fortunately, most of these states have large populations to provide reasonable pediatric case and control groups. Depending on the age range of participants and state policies in the future, NBS could potentially be retrieved from Iowa, Indiana, Maine, Maryland, Massachusetts, Minnesota, Missouri, North Carolina, and New Jersey as well. Studies of cancers in young children stand the greatest chance of success (versus studies in older children and adolescents) and may be the most relevant with respect to prenatal exposures. It will be of interest to observe state NBS retention and release policies moving forward.

In our analysis of the Epidemiology of Infant Leukemia Study dataset, we found little evidence supporting associations between maternal prenatal vitamin use in the year

before and/or during pregnancy, use in the periconceptional period (year before pregnancy and early in, but prior to knowledge of pregnancy), use after knowledge of pregnancy, or use in all of these periods and infant leukemia, ALL, or AML, although ORs for ALL were consistently <1.00. Similarly, we did not observe associations with iron supplement use above that found in multi- or prenatal vitamins, although the ORs for each of the etiologic time periods were >1.00. We speculate that the failure to observe associations with prenatal vitamin use in this study may be attributable to high rates of folic acid supplementation in the unique study population, including personal vitamin use and national folic acid fortification programs implemented in the U.S. and Canada early in the study period. The fortification programs have helped to ensure there is little chance for folate deficiency in these countries and therefore, the folic acid-leukemia association is no longer a relevant public health concern. It is still of interest from an etiologic perspective, however, and future research directions may include assessment of genetic susceptibility, such as an association study of SNPs in the conjoined pathways of folate and homocysteine metabolism, or experiments conducted in animal models.

In the meta-analysis of atopic disease and childhood leukemia, inverse associations were observed for ALL and atopic disease, and for asthma, eczema, and hay fever examined separately. High levels of heterogeneity were detected across studies. No association was observed for leukemia overall or AML, or for any of the specific atopic conditions and AML examined separately, although there were fewer studies of AML and fewer AML cases. The divergent results observed for ALL and AML are of interest, given that atopic status should have been captured similarly across the two case groups.

Upon further investigation, ORs for ALL differed across strata of study design, exposure data source, and latency period, indicating these factors impact study results. Although inverse associations between atopy/allergies and childhood/adolescent ALL were observed, causes of the observed statistical associations must be investigated thoroughly to rule out explanations other than a direct causal relationship, such as reverse causality or selection or recall bias. Ideally, future studies would include a prospective design with analysis of biological specimens to avoid the pitfalls of temporality and misclassification plaguing existing studies. Due to the difficulty in obtaining blood samples for immunoglobulin E (IgE) analysis prior to initiation of leukemia, genetic association studies may be a better avenue for future research.

The causes of most childhood hematologic malignancies remain elusive. Etiologic studies of pediatric malignancies have been generally restricted to registry-based or retrospective case-control study designs due to their rarity, and the interpretation of such studies must therefore be tempered by residual confounding, selection bias, and/or recall bias considerations. Novel exposure measurement methods will be needed to further the field. For example, analysis of biomarkers, such as substrates extracted from NBS, and genetic analyses, such as genome-wide association studies, allow investigators to circumvent some of these limitations.

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Appendix A. Children's Oncology Group Institutions participating in AADM01P1 Pilot study

†‡Albany Medical Center (NY)
Cancer Research Center of Hawaii (HI)
†Children's Hospitals and Clinics of Minnesota (MN)
Children's Medical Center Dayton (OH)
Connecticut Children's Medical Center (CT)
Dartmouth-Hitchcock Medical Center (NH)
†Driscoll Children's Hospital (TX)
East Tennessee Children's Hospital (TN)
†Hackensack University Medical Center (NJ)
Lutheran General Children's Medical Center (IL)
*McGill University Health Center - Montreal Children's Hospital (QC)
†Michigan State University (MI)
†Miller Children's Hospital/Harbor-UCLA (CA)
†Mission Hospitals (NC)
†New York University Medical Center (NY)
Primary Children's Medical Center (UT)
St. Christopher's Hospital for Children (PA)
*Stollery Children's Hospital (formerly Cross Cancer Institute) (AB)
The Children's Hospital of Southwest Florida, Lee Memorial Health System (FL)
‡Tod Childrens Hospital - Forum Health (OH)
University of Florida (FL)
†University of Texas Health Science Center at San Antonio (TX)
University of Vermont College of Medicine (VT)

* Canadian institution; not eligible for FROGS Study
† Institution located in state that stores NBS $\geq$5 years and state indicated it would release NBS for research
‡ Institution did not provide information for FROGS Study

Appendix B. NCCLS procedure for the collection of NBS

The National Committee on Clinical Laboratory Standards (NCCLS, now the Clinical and Laboratory Standards Institute, CLSI) standard procedure for the collection of NBS specifies that individuals responsible for the blood spot collection should:[51]

- Check the date printed on the card to ensure it has not expired.
- Fill out the form completely using a ballpoint pen, taking care not to touch the blood collection area at any time.
- Wash hands and wear powder-free gloves.
- Warm the child's heel and/or position the child's leg lower than heart level to ensure adequate blood flow.
- Disinfect the heel by wiping with 70% isopropanol/30% water solution
- Puncture the plantar surface of the heel (either the most medial or most lateral area) using a disposable, sterile lancet with a depth of 2.0 mm or less.
- Wipe away the first drop of blood using sterile gauze or a cotton ball (as it might contain tissue fluids, which would dilute the sample).
- Allow a large second drop to form and then gently apply the blood to the filter paper such that it completely fills a pre-printed circle via capillary action, taking care not to press the filter paper directly against the skin.
- Repeat for the remaining spots, filling from only one side of the paper. Spots should be examined to ensure the blood was uniformly and completely applied to the circles.
- Elevate the foot above the body to halt the bleeding.
- Lay the cards flat and allow them to dry on a non-absorbent surface at ambient temperatures (15-22 °C) out of direct sunlight for 3-4 hours.
- Send to the testing laboratory within 24 hours of collection, due to the urgent nature of the testing in reducing morbidity/mortality.

Appendix C. Examples of analytes that have been extracted from dried blood spots

TABLE 2

Analytes measured from human blood collected and dried on filter paper

- Acarboxyprothrombin
- Acylcarnitine
- Adenine phosphoribosyl transferase
- Adenosine deaminase
- Albumin
- α-Fetoprotein
- Amino Acids profiles
  - Arginine (Krebs cycle)
  - Histidine/urocanic acid
  - Homocysteine
  - Phenylalanine/tyrosine
  - Tryptophan
- Andrenostenedion
- Antipyrine
- Arabinitol enantiomers
- Arginase
- Benzoylecgonine (cocaine)
- Biotinidase
- Biopterin
- C-reactive protein
- Carnitine
- Carnosinase
- CD4
- Ceruloplasmin
- Chenodeoxycholic acid
- Chloroquine
- Cholesterol
- Cholinesterase chemokines
- Conjugated 1-β hydroxycholic acid
- Cortisol
- Creatine kinase
- Creatine kinase MM isoenzyme
- Cyclosporin A cytokines
- D-penicillamine
- De-ethylchloroquine
- Dehydroepiandrosterone sulfate
- DNA (polymerase chain reaction)
    - acetylator polymorphism
  - Alcohol dehydrogenase
  - α 1-Antitrypsin
  - Cystic fibrosis
  - Duchenne/Becker
  - Muscular dystrophy
  - Glucose-6-phosphate dehydrogenase
  - Hemoglobinopathies
    - A,S,C,E
    - D-Punjab
  - β-Thalassemia
  - Hepatitis B virus
  - HCMV
  - HIV-1
  - HTLV-1
  - Leber hereditory optic neuropathy
  - MCAD
  - mRNA
  - PKU
  - Plasmodium vivax
  - Sexual differentiation

- 21-deoxycortisol
- Desbutylhalofantrine
- Dihydropteridine reductase
- Diptheria/tetanus antitoxin
- Erythrocyte arginase
- Erythrocyte protoporphyrin
- Esterase D
- Fatty acids/acylglycines
- Free β-human chorionic gonadotropin
- Free erythrocyte porphyrin
- Free thyroxine (FT4)
- Free tri-iodothyronine (FT3)
- Fumarylacetoacetase
- Galactose/gal-1-phosphate
- Galactose-1-phosphate uridyl transferase
- Gentamicin
- Glucose
- Glucose-6-phosphate dehydrogenase
- Glutathione
- Glutathione perioxidase
- Glycocholic acid
- Glycosylated hemoglobin
- Halofantrine
- Hemoglobin variants
- Hexosaminidase A
- Human erythrocyte carbonic anhydrase I
- 17-α Hydroxyprogesterone
- Hypoxanthine phosphoribosyl transferase
- Immunoreactive trypsin
- Lactate
- Lead
- Lipoproteins
  - (a)
  - B/A-1
  - β
- Lysozyme
- Mefloquine
- Netilmicin
- Phenobarbitone
- Phenytoin
- Phytanic/pristanic acid
- Progesterone
- Prolactin
- Prolidase
- Purine nucleoside phosphorylase
- Quinine
- Reverse tri-iodothyronine (rT3)
- Selenium
- Serum pancreatic lipase
- Sissomicin
- Somatomedin C

- Specific antibodies
  - Adenovirus
  - Antinuclear antibody
  - Arbovirus
  - Aujeszky's disease virus
  - Dengue virus
  - *Dracunculus medinensis*
  - *Echinococcus granulosus*
  - *Entamoeba histolytica* enterovirus
  - *Giardia duodenalisa*
  - *Helicobacter pylori*
  - Hepatitis B virus
  - Herpes virus
  - HIV-1
  - IgE (atopic disease)
  - Influenza virus
  - Interlukins
  - *Leishmania donovani*
  - *Leptospira*
  - Measles/mumps/rubella
  - *Mycobacterium leprae*
  - *Mycoplasma pneumoniae*
  - *Onchocerca volvulus*
  - Parainfluenza virus
  - *Plasmodium falciparum*
  - Poliovirus
  - *Pseudomonas aeruginosa*
  - Respiratory syncytial virus
  - Rickettsia (scrub typhus)
  - *Schistosoma mansoni*
  - *Toxoplasma gondii*
  - *Trepenoma pallidium*
  - *Trypanosoma cruzi/rangeli*
  - Vesicular stomatis virus
  - *Wuchereria bancrofti*
  - Yellow fever virus
- Specific antigens
  - Hepatitis B virus
  - HIV-1
- Succinylacetone
- Sulfadoxine
- Theophylline
- Thyrotropin (TSH)
- Throxine (T4)
- Thyroxine-binding globulin
- Trace elements
- Transferrin
- Transferrin receptor
- Uridine diphosphate-galactose-4-epimerase
- Urea
- Uroporphyrinogen I synthase
- Vitamin A
- White blood cells
- Zinc protoporphyrin

Source: Mei, JV. *J Nutr.* 2001;131:1631S-1636S.[50]

Appendix D. FROGS Study self-administered paper questionnaire for mothers

UNIVERSITY OF MINNESOTA

FROGS Study Questionnaire

University of Minnesota
Department of Pediatrics
2009

How to fill out this survey

Please take about 20 minutes to fill out this survey. It asks for information about your pregnancy history, medication and vitamin use, personal habits, and allergies, as well as personal information about you and your child diagnosed with leukemia, lymphoma, or a similar illness. Please answer every question as best you can. If you do not know the answer to a question, please provide your best guess.

For some questions, you will **PUT AN X OR ✓ IN THE BOX** that goes with your answer, like this:

1. Are you? 1 ☒ Male 2 ☐ Female **OR** 1 ☑ Male 2 ☐ Female

You will sometimes be told to skip over some questions in this survey. When this happens, you will see an arrow with a note that tells you what questions to answer next, like this:

☐ Yes ⟶ **Go to Question 13**

For some questions, you will enter letters or numbers in boxes. Please enter one letter or number per box and stay within the box, like this:

A	B	2	8

Important Dates

The questions in this questionnaire are asking about your child that developed leukemia, lymphoma, or a similar illness ("your child").

1. What is today's date?

☐☐ / ☐☐ / ☐☐☐☐
month day year

2. What is your child's date of birth?

☐☐ / ☐☐ / ☐☐☐☐
month day year

3. What is your child's date of diagnosis?

☐☐ / ☐☐ / ☐☐☐☐
month day year

Pregnancy History

4. How many times have you been pregnant? *(Please include all live births, stillbirths, miscarriages, tubal/ectopic and molar pregnancies, and abortions.)*

☐☐ number of times pregnant

5. Before you became pregnant with your child, did you have any other babies who were born alive?

0 ☐ No ⟶ **Go to Question 8**
1 ☐ Yes

6. How many live children were born before your child?

☐☐ number of children

7. How many <u>total</u> live births have you had?

☐☐ number of births

8. Have you ever been diagnosed with diabetes (high blood sugar) by a physician or other health professional when you were <u>not</u> pregnant?

0 ☐ No
1 ☐ Yes

9. Have you ever been diagnosed with gestational diabetes (high blood sugar) by a physician or other health professional when you were pregnant?

0 ☐ No
1 ☐ Yes

10. How many pounds did you weigh just before you became pregnant with your child?

☐☐☐ pounds

11. Thinking about the first 3 months of your pregnancy, did your weight change?

1 ☐ Yes, I lost weight → ☐☐ pounds lost
2 ☐ Yes, I gained weight → ☐☐ pounds gained
3 ☐ No, my weight didn't change

12. Thinking about the entire pregnancy, did your weight change?

1 ☐ Yes, I lost weight → ☐☐ pounds lost
2 ☐ Yes, I gained weight → ☐☐ pounds gained
3 ☐ No, my weight didn't change

13. How tall are you without shoes?

☐ feet ☐☐ inches

14. Did you receive treatment from a doctor, nurse, or other health care worker to help you get pregnant with your child? (*This may include infertility treatments such as fertility-enhancing drugs or assisted reproductive technology.*)

0 ☐ No ⟶ **Go to Question 16**
1 ☐ Yes

15. Did you use any of the following treatments during the month you got pregnant with your child? *(Mark all that apply)*

☐ Fertility-enhancing drugs prescribed by a doctor *(fertility drugs include Clomid®, Serophene®, Pergonal®, or other drugs that stimulate ovulation)*

☐ Artificial insemination or intrauterine insemination *(treatments in which sperm, but NOT eggs, were collected and medically placed into a woman's body)*

☐ Assisted reproductive technology *(treatments in which BOTH a woman's eggs and a man's sperm were handled in the laboratory, such as in vitro fertilization [IVF], gamete intrafallopian transfer [GIFT], zygote intrafallopian transfer [ZIFT], intracytoplasmic sperm injection [ICSI], frozen embryo transfer, or donor embryo transfer)*

☐ Other medical treatment *(please tell us:)*________________________

☐ None of these

16. What was your child's due date?

☐☐ / ☐☐ / ☐☐☐☐
month / day / year

17. According to the due date you were given by a doctor or other health professional, was your child born more than 6 days early, more than 6 days late, or on time?

1 ☐ Early *(more than 6 days early)* ⟶ ☐☐ days early

2 ☐ Late *(more than 6 days late)* ⟶ ☐☐ days late

3 ☐ On time *(6 days or less before or after the due date)*

18. Was your child a twin or multiple birth?

1 ☐ Single birth ⟶ **Go to Question 20**
2 ☐ Twin
3 ☐ Triplet or more

19. In what order was your child delivered within that set of twins or multiples? (*for example*: *1st, 2nd, 3rd*)

____

20. How much did your child weigh at birth?

☐☐ pounds ☐☐ ounces

21. How long was your child at birth?

☐☐.☐☐ inches

22. Up until 12 months of age, was your child breastfed, formula fed, or both?

1 ☐ Breastfed
2 ☐ Formula fed
3 ☐ Both

23. For how many weeks or months was he/she breastfed?

☐☐ weeks **OR** ☐☐ months

1 ☐ Less than 1 week
2 ☐ I did not feed my baby breast milk

24. How old was he/she when he/she began formula feeding (in weeks or months)?

☐☐ weeks **OR** ☐☐ months

1 ☐ My baby was less than 1 week old
2 ☐ I did not feed my baby formula ⟶ **Go to Question 26**

25. For how many weeks or months did he/she drink formula?

☐☐ weeks **OR** ☐☐ months

1 ☐ Less than 1 week

Medication and Vitamin Use

26. Before you became pregnant with your child, did you ever use oral contraceptives (birth control pills) for any reason?

0 ☐ No ⟶ **Go to Question 30**
1 ☐ Yes

27. Before you became pregnant with your child, how many months or years total did you take birth control pills?

☐☐ months **OR** ☐☐ years

1 ☐ I took birth control pills for less than one month

28. What type of pill did you use for the longest period of time?

1 ☐ Low dose
2 ☐ Progesterone only
3 ☐ Standard
4 ☐ Don't know

29. What was the name of the pill you used for the longest period of time?

☐ Alesse-28
☐ Apri
☐ Aviane
☐ Brevicon
☐ Cyclessa
☐ Demulen
☐ Desogen
☐ Enpresse
☐ Estrostep
☐ Genora
☐ Jenest
☐ Kariva
☐ Lessina
☐ Levlite
☐ Levlen
☐ Levora
☐ Loestrin
☐ Lo-Ovral
☐ Low-Ogestrel
☐ Mircette
☐ Microgestin
☐ Micronor
☐ Modicon
☐ Necon
☐ Nelova
☐ Norlestrin
☐ Nordette
☐ Norinyl
☐ Nor-QD
☐ Nortrel
☐ Ogestrel
☐ Ortho-Cept
☐ Ortho-Novum
☐ Ortho-Cyclen
☐ Ortho-Tri-Cyclen
☐ Ovcon
☐ Ovral
☐ Ovrette
☐ Portia
☐ Tri-Norinyl
☐ Tri-Levlen
☐ Triphasil
☐ Trivora
☐ Yasmin
☐ Zovia

☐ Other (*please tell us*): ______________________

30. Before you became pregnant with your child, did you ever use any of the following for any reason? *(Mark all that apply, and indicate how long you used each of those you marked)*

☐ Contraceptive implants *(for example: Norplant®)*

Length of use: ☐☐ months **OR** ☐☐ years

1 ☐ I used implants for less than one month

☐ Contraceptive injections *(for example: Depo-Provera®, Lunelle®)*

Length of use: ☐☐ months **OR** ☐☐ years

1 ☐ I used injections for less than one month

☐ Cervical ring *(for example: NuvaRing®)*

Length of use: ☐☐ months **OR** ☐☐ years

1 ☐ I used rings for less than one month

☐ Contraceptive patches *(for example: OrthoEvra®)*

Length of use: ☐☐ months **OR** ☐☐ years

1 ☐ I used patches for less than one month

☐ None of these

31. Did you take vitamins in the year before you were pregnant?

0 ☐ No ⟶ **Go to Question 35**
1 ☐ Yes

32. What type of vitamin? *(Mark all that apply)*

- ☐ Multivitamin
- ☐ Pre-natal vitamin
- ☐ Folic acid supplement
- ☐ Iron supplement
- ☐ Other *(please tell us):* ______________________________

33. Was that vitamin(s) prescribed by a doctor?

0 ☐ No
1 ☐ Yes

34. During the <u>month before</u> you got pregnant, how many times a week did you take a multivitamin or a prenatal vitamin? (*These are pills that contain many different vitamins and minerals.)*

1 ☐ I didn't take a multivitamin or a prenatal vitamin at all
2 ☐ 1 to 3 times a week
3 ☐ 4 to 6 times a week
4 ☐ Every day of the week

35. Did you take vitamins in early pregnancy, <u>before you knew</u> you were pregnant?

0 ☐ No ⟶ **Go to Question 38**
1 ☐ Yes
↓

36. What type of vitamin? *(Mark all that apply)*

- ☐ Multivitamin
- ☐ Pre-natal vitamin
- ☐ Folic acid supplement
- ☐ Iron supplement
- ☐ Other *(please tell us):* ______________________________

37. Was that vitamin(s) prescribed by a doctor?

0 ☐ No
1 ☐ Yes

38. Did you take vitamins from the time you found out you were pregnant until your child was born?

0 ☐ No ⟶ **Go to Question 42**
1 ☐ Yes

39. What type of vitamin? *(Mark all that apply)*

☐ Multivitamin
☐ Pre-natal vitamin
☐ Folic acid supplement
☐ Iron supplement
☐ Other (*please tell us*):________________________________

40. Was that vitamin(s) prescribed by a doctor?

0 ☐ No
1 ☐ Yes

41. During the last 3 months of your pregnancy, how many times a week did you take a multivitamin or a prenatal vitamin? (*These are pills that contain many different vitamins and minerals.)*

1 ☐ I did not take a multivitamin or a prenatal vitamin at all
2 ☐ 1 to 3 times a week
3 ☐ 4 to 6 times a week
4 ☐ Every day of the week

Smoking

42. Before your child was born, did you ever smoke cigarettes on a regular basis, that is, more than a pack of cigarettes in your lifetime? *(A pack has 20 cigarettes.)*

0 ☐ No ⟶ **Go to Question 49**
1 ☐ Yes

43. In the <u>3 months before</u> you became pregnant, did you smoke any cigarettes?

0 ☐ No ⟶ **Go to Question 45**
1 ☐ Yes

44. On average, how many cigarettes per day did you smoke during this time? *(A pack has 20 cigarettes.)*

☐☐☐ cigarettes

45. Did you smoke in early pregnancy, <u>before you knew</u> you were pregnant?

0 ☐ No ⟶ **Go to Question 47**
1 ☐ Yes

46. On average, how many cigarettes per day did you smoke during this time? *(A pack has 20 cigarettes.)*

☐☐☐ cigarettes

47. Did you smoke from the time you found out you were pregnant until your child was born?

0 ☐ No ⟶ **Go to Question 49**
1 ☐ Yes

48. On average, how many cigarettes per day did you smoke during this time? *(A pack has 20 cigarettes.)*

☐☐☐ cigarettes

49. During the time you were pregnant, were you exposed to cigarette smoke from anyone at home?

0 ☐ No ⟶ **Go to Question 52**
1 ☐ Yes

50. On an average day, about how many hours a day were you in the same room at home with another person who was smoking?

☐☐ hours

1 ☐ Less than 1 hour a day
2 ☐ I was never in the same room with someone who was smoking

51. How many cigarette smokers, not including yourself, lived in your home during your pregnancy?

☐☐ number of smokers

52. During the time you were pregnant, were you exposed to cigarette smoke from anyone at work?

0 ☐ No ⟶ **Go to Question 54**
1 ☐ Yes

53. On an average day, about how many hours a day were you in the same room at work with another person who was smoking?

☐☐ hours

1 ☐ Less than 1 hour a day
2 ☐ I was never in the same room with someone who was smoking

54. Did your child's biological father smoke in the year before you became pregnant with him/her?

0 ☐ No
1 ☐ Yes

Alcohol Use

55. Did you ever drink at least two drinks per month of wine, beer, liquor or any alcoholic beverages for a period of one year or longer?

0 ☐ No
1 ☐ Yes

56. During the 3 months before you got pregnant, how many alcoholic drinks did you have in an average week? *(A drink is 1 glass of wine, wine cooler, can or bottle of beer, shot of liquor, or mixed drink.)*

1 ☐ 14 drinks or more a week
2 ☐ 7 to 13 drinks a week
3 ☐ 4 to 6 drinks a week
4 ☐ 1 to 3 drinks a week
5 ☐ Less than 1 drink a week
6 ☐ I didn't drink then

57. During the 3 months before you got pregnant, how many times did you drink 5 alcoholic drinks or more in one sitting?

1 ☐ 6 or more times
2 ☐ 4 to 5 times
3 ☐ 2 to 3 times
4 ☐ 1 time
5 ☐ I didn't have 5 drinks or more in 1 sitting
6 ☐ I didn't drink then

58. Early in your pregnancy, before you knew you were pregnant, how many alcoholic drinks did you have in an average week?

1 ☐ 14 drinks or more a week
2 ☐ 7 to 13 drinks a week
3 ☐ 4 to 6 drinks a week
4 ☐ 1 to 3 drinks a week
5 ☐ Less than 1 drink a week
6 ☐ I didn't drink then

59. Early in your pregnancy, before you knew you were pregnant, how many times did you drink 5 alcoholic drinks or more in one sitting?

1 ☐ 6 or more times
2 ☐ 4 to 5 times
3 ☐ 2 to 3 times
4 ☐ 1 time
5 ☐ I didn't have 5 drinks or more in 1 sitting
6 ☐ I didn't drink then

60. During the last 3 months of your pregnancy, how many alcoholic drinks did you have in an average week?

1 ☐ 14 drinks or more a week
2 ☐ 7 to 13 drinks a week
3 ☐ 4 to 6 drinks a week
4 ☐ 1 to 3 drinks a week
5 ☐ Less than 1 drink a week
6 ☐ I didn't drink then

61. During the last 3 months of your pregnancy, how many times did you drink 5 alcoholic drinks or more in one sitting?

1 ☐ 6 or more times
2 ☐ 4 to 5 times
3 ☐ 2 to 3 times
4 ☐ 1 time
5 ☐ I didn't have 5 drinks or more in 1 sitting
6 ☐ I didn't drink then

Allergic Diseases

Asthma

62. Has your child <u>ever</u> had wheezing or whistling in the chest at any time in the past?

0 ☐ No
1 ☐ Yes

63. Has your child <u>ever</u> had asthma?

0 ☐ No ⟶ **Go to Question 68**
1 ☐ Yes
↓

64. At what age did the asthma start?

☐☐ months **OR** ☐☐ years

65. Has your child ever taken medications for his/her asthma?

0 ☐ No ⟶ **Go to Question 67**
1 ☐ Yes

66. What is the name of the medicine used for his/her asthma? *(Mark all that apply)*

- ☐ Accolate
- ☐ Aerobid
- ☐ Albuterol
- ☐ Atrovent
- ☐ Azmacort
- ☐ Beclovent
- ☐ Cromolyn
- ☐ Flovent
- ☐ Foradil Aerolizer
- ☐ Formoterol
- ☐ Intal
- ☐ Iprotropium
- ☐ Nedocromil
- ☐ Omalizumab
- ☐ Pediapred
- ☐ Predisone
- ☐ Prelone
- ☐ Proventil
- ☐ Pulmicort
- ☐ Qvar
- ☐ Salmeterol
- ☐ Serevent Diskus
- ☐ Singular
- ☐ Slobid
- ☐ Theodur
- ☐ Theophylline
- ☐ Tilade
- ☐ Uniphyl
- ☐ Vanceril
- ☐ Ventolin
- ☐ Xolair
- ☐ Other (*please tell us*): ______________________________

67. Has your child ever been diagnosed with asthma by a physician or other health professional?

0 ☐ No
1 ☐ Yes

68. Were any of your child's family members ever diagnosed with asthma by a physician or other health professional?

0 ☐ No ⟶ **Go to Question 70**
1 ☐ Yes
↓

69. Who in your child's family was diagnosed with asthma? *(Mark all that apply)*

- ☐ You
- ☐ Your child or children (do not include the child that developed leukemia, lymphoma, or a similar illness)
- ☐ Your child's father
- ☐ Other family member (*please tell us*): ______________________

Hives

70. Has your child ever had hives? (Red, swollen, and itchy areas on the surface of the skin (wheals); red areas that change quickly in size, shape, and location; and possibly with swelling of the face, lips, and tongue, or wheezing)

0 ☐ No ⟶ **Go to Question 75**
1 ☐ Yes

71. At what age did the hives start?

☐☐ months **OR** ☐☐ years

72. Has your child ever taken medications for his/her hives?

0 ☐ No ⟶ **Go to Question 74**
1 ☐ Yes

73. What is the name of the medicine used for his/her hives? *(Mark all that apply)*

☐ Allegra	☐ Cyproheptadine	☐ Ranitidine
☐ Atarax	☐ Diphenhydramine	☐ Sinequan
☐ Benadryl	☐ Doxepin	☐ Singulair
☐ Cimetidine	☐ Hydroxyzine	☐ Tagamet
☐ Clarinex	☐ Montelukast	☐ Zantac
☐ Claritin	☐ Periactin	☐ Zyrtec

☐ Other *(please tell us):* ______________________

74. Has your child ever been diagnosed with hives by a physician or other health professional?

0 ☐ No
1 ☐ Yes

75. Were any of your child's family members ever diagnosed with hives by a physician or other health professional?

0 ☐ No ⟶ **Go to Question 77**
1 ☐ Yes
↓

76. Who in your child's family was diagnosed with hives? *(Mark all that apply)*

☐ You
☐ Your child or children (do not include the child that developed leukemia, lymphoma, or a similar illness)
☐ Your child's father
☐ Other family member (*please tell us*): ______________________

Eczema

77. Has your child ever had an itchy rash that was coming and going for at least six months?

0 ☐ No ⟶ **Go to Question 79**
1 ☐ Yes

78. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?

0 ☐ No
1 ☐ Yes

79. Has your child ever had eczema? (Recurring skin rashes often with one or more of these symptoms: redness, swelling, itching, dryness, crusting, flaking, blistering, cracking, oozing, or bleeding)

0 ☐ No ⟶ **Go to Question 84**
1 ☐ Yes

80. At what age did the eczema start?

☐☐ months **OR** ☐☐ years

81. Has your child ever taken medications for his/her eczema?

0 ☐ No ⟶ **Go to Question 83**
1 ☐ Yes

82. What is the name of the medicine used for his/her eczema? *(Mark all that apply)*

☐ Aclovate	☐ Elidel	☐ Psorcon
☐ Atarax	☐ Elocon	☐ Synalar
☐ Benadryl	☐ Hydrocortisone	☐ Temovate
☐ Cutivate	☐ Locoid	☐ Triamcinolone
☐ Cyclosporine	☐ Methoxsalen	☐ Ultravate
☐ Cyproheptadine	☐ Prednisone	☐ Vistaril
☐ Dermatop	☐ Protopic	☐ Zyrtec
☐ Diprolene		

☐ Other *(please tell us):* ___________________________________

83. Has your child ever been diagnosed with eczema by a physician or other health professional?

0 ☐ No
1 ☐ Yes

84. Were any of your child's family members ever diagnosed with eczema by a physician or other health professional?

0 ☐ No ⟶ **Go to Question 86**
1 ☐ Yes
↓

85. Who in your child's family was diagnosed with eczema? *(Mark all that apply)*

☐ You
☐ Your child or children (do not include the child that developed leukemia, lymphoma, or a similar illness)
☐ Your child's father
☐ Other family member (*please tell us*): ______________________

Hay Fever / Seasonal Allergies

The questions below are about problems that occurred when your child DID NOT have a cold or the flu.

86. Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu?

0 ☐ No
1 ☐ Yes

87. Has your child ever had hay fever/seasonal allergies?

0 ☐ No ⟶ **Go to Question 92**
1 ☐ Yes
↓

88. At what age did the hay fever/seasonal allergies start?

☐☐ months **OR** ☐☐ years

89. Has your child ever taken medications for his/her hay fever/seasonal allergies?

0 ☐ No ⟶ **Go to Question 91**
1 ☐ Yes

90. What is the name of the medicine used for his/her hay fever/ seasonal allergies? *(Mark all that apply)*

☐ Accolate
☐ Allegra
☐ Astelin
☐ Beconase
☐ Benadryl
☐ Chlor-trimeton
☐ Claritin
☐ Dimetane
☐ Flonase or Flixonase
☐ Hismanal
☐ Nasacort AQ
☐ Nasalcrom
☐ Nasonex
☐ Rhinocort
☐ Singulair
☐ Syntaris
☐ Tavist
☐ Zyrtec
☐ Other *(please tell us)*: ________________________________

91. Has your child ever been diagnosed with hay fever/seasonal allergies by a physician or other health professional?

0 ☐ No
1 ☐ Yes

92. Were any of your child's family members ever diagnosed with hay fever/ seasonal allergies by a physician or other health professional?

0 ☐ No ⟶ **Go to Question 94**
1 ☐ Yes
↓

93. Who in your child's family was diagnosed with hay fever/seasonal allergies? *(Mark all that apply)*

☐ You
☐ Your child or children (do not include the child that developed leukemia, lymphoma, or a similar illness)
☐ Your child's father
☐ Other family member (*please tell us*): ______________________

Personal Information

94. What is your child's full name?

____
first middle last

95. Is your child 18 years of age or older?

1 ☐ Yes
2 ☐ No
3 ☐ He/she is deceased → **Go to Question 97**

96. Is his/her home address and telephone number the same as yours?

1 ☐ Yes, he/she has the same contact information as me
2 ☐ No, he/she has the following contact information:

____
Street Address

____
City State/Province Zip Code

Phone number: ☐☐☐ - ☐☐☐ - ☐☐☐☐

97. What is your child's gender?

1 ☐ Female
2 ☐ Male

98. Where was your child born?

____
Hospital name | City | State/Province | Country

99. Please choose the answer that best describes your relationship with your child:

1 ☐ Birth mother
2 ☐ Adoptive mother
3 ☐ Stepmother
4 ☐ Grandmother
5 ☐ Other relationship (*please tell us*): ____________________________

100. What is your full name?

____
first | middle | last

101. What is your date of birth?

☐☐ / ☐☐ / 19☐☐
month day year

102. What is your telephone number?

☐☐☐ - ☐☐☐-☐☐☐☐

103. Where were you born?

____
City State/Province Country

104. What is the highest level of school that you completed?

1 ☐ Less than grade 9
2 ☐ Grade 9-12 (no diploma)
3 ☐ High school graduate or GED
4 ☐ Technical or vocational school
5 ☐ Some college credit but no degree
6 ☐ Associate degree *(example: AA, AS)*
7 ☐ Bachelor's degree *(example: BA, AB, BS)*
8 ☐ Master's degree *(example: MA, MS, MEng, MPH, MEd, MSW, MBA)*
9 ☐ Doctorate or Professional degree *(example: PhD, EdD, MD, DDS, DVM, LLB, JD)*

105. Which of the following best describes you?

1 ☐ White
2 ☐ African-American or Black
3 ☐ Asian, Asian-American, or Pacific Islander
4 ☐ Native American or Alaskan Native
5 ☐ Other *(please tell us):* ____________________

106. Are you Hispanic, Latina, or of Spanish origin?

0 ☐ No
1 ☐ Yes

107. What is your marital status?

1 ☐ Married
2 ☐ Living with someone in a marriage-like relationship
3 ☐ Separated
4 ☐ Divorced
5 ☐ Widowed
6 ☐ Single, never married
7 ☐ Other *(please tell us):* ____________________

108. During the year that your child was born, what was your total household income before taxes?

1 ☐ Less than $10,000
2 ☐ $10,000 - $19,999
3 ☐ $20,000 - $39,999
4 ☐ $40,000 - $59,999
5 ☐ $60,000 - $79,999
6 ☐ $80,000 - $99,999
7 ☐ $100,000 or more

109. What is the date of birth of your child's father?

☐☐ / ☐☐ / 19☐☐
month day year

110. What is the highest grade or level of school that your child's biological father completed?

1 ☐ Less than grade 9
2 ☐ Grade 9-12 (no diploma)
3 ☐ High school graduate or GED
4 ☐ Technical or vocational school
5 ☐ Some college credit but no degree
6 ☐ Associate degree *(example: AA, AS)*
7 ☐ Bachelor's degree *(example: BA, AB, BS)*
8 ☐ Master's degree *(example: MA, MS, MEng, MPH, MEd, MSW, MBA)*
9 ☐ Doctorate or Professional degree *(example: PhD, EdD, MD, DDS, DVM, LLB, JD)*

111. Which of the following best describes your child's biological father?

1 ☐ White
2 ☐ African-American or Black
3 ☐ Asian, Asian-American, or Pacific Islander
4 ☐ Native American or Alaskan Native
5 ☐ Other (*please tell us*): ______________________________

112. Is your child's biological father Hispanic, Latino, or of Spanish origin?

0 ☐ No
1 ☐ Yes

This page was intentionally left blank.

If there is anything else you would like to tell us about exposures you or your child may have encountered, please do so in the space provided below.

____

____

____

____

____

____

Thank you for taking the time to complete this questionnaire!

If you have any questions, or to request this questionnaire in Spanish, please call us toll-free at 888-663-2707.

Please return this questionnaire in the return envelope provided to:

FROGS Study
University of Minnesota
Division of Pediatric Epidemiology
420 Delaware Street SE, MMC 422
Minneapolis, MN 55455

Appendix E. FROGS Study forms and ancillary materials

Samples of the FROGS Study ancillary materials have been included herein. Please note that several versions of each of these materials may have been created due to the different groups contacted (i.e., mothers of children <8 years, mothers of children 8-17 years, mothers of children ≥18 years, guardians of children <8 years, guardians of children 8-17 years, guardians of children ≥18 years, children ≥18 at time of consent to COG AADM01P1, children ≥18 years at time of contact). Further, the materials were translated into Spanish as needed to facilitate participation of native Spanish-speakers. Sample materials include:

- Initial contact letter
- 1st questionnaire packet cover letter
- Instruction sheet
- Frequently asked questions
- Consent form
- Assent form
- HIPAA form
- Thank you/reminder postcard
- 2nd questionnaire packet cover letter
- Adult child contact information form (for guardians)
- Telephone script for telephone follow-up

<Date>

<First Name> <Last name>
<Street Address>
<City>, <State> <Zip>

Dear <First Name> <Last name>,

In the next two weeks, you will be sent a packet of materials and will be asked to participate in an important study of children's health. The study is being conducted by the Department of Pediatrics at the University of Minnesota in partnership with the Children's Oncology Group.

In this study, we will gather information about things that mothers or their children may have come into contact with during pregnancy or afterwards.

We wanted to let you know that you will receive these materials because many people like to think about their participation ahead of time. This study is important because little is known about the causes of leukemia, lymphoma, and similar illnesses in children and adolescents. It is only by asking questions about different experiences mothers and their children may have had that we can learn more about how these illnesses develop in young people.

Thank you in advance for considering taking part in our study.

Sincerely,

Amy M. Linabery and Julie A. Ross
Principal Investigators

<Date>

<First Name> <Last name>
<Street Address>
<City>, <State> <Zip>

Dear <First Name> <Last name>,

On behalf of the Department of Pediatrics at the University of Minnesota, and in partnership with the Children's Oncology Group, we invite you to take part in a study of children's health called the FROGS Study. The purpose of the study is to learn more about factors that may be related to the development of leukemia, lymphoma, and similar illnesses in children and adolescents. You were selected for this study because you participated in the Children's Oncology Group Childhood Cancer Research Network Pilot Study and agreed to receive information about future studies.

We are asking you to participate by completing and returning the questionnaire included in this packet. Questions in the survey booklet will ask for information related to pregnancy history, events surrounding your child's birth, personal habits such as smoking, medical history, and family health history. Please note that some of the questions are personal in nature.

We are also asking you to provide your written permission to obtain dried blood spots collected from your child at birth. These blood spots were created when a few drops of blood were taken at birth, placed on filter paper (also called "Guthrie Cards"), and dried. These blood spots were originally collected to check for some serious diseases (metabolic conditions) in newborns. Some states have stored the dried spots in case they could be used in the future. If you agree, we will ask the state where your child was born for the blood spots. The blood spots are useful because they provide a snapshot of your child's blood biology at the time of birth.

Please see the yellow sheet included in this packet for step-by-step instructions on what to do to take part in the study.

We want to stress that <u>we do not know the reasons</u> why most leukemias, lymphomas, and similar illnesses develop in children and adolescents. For that reason, we are studying a lot of different factors that mothers or their children may have encountered during pregnancy or afterwards. A good way to gather this type of information is to ask mothers to tell us. Another good way is to measure different components of the blood.

Your participation in this research study is entirely voluntary. All of the information we collect will be kept strictly confidential and your name will not be used in any publication of our findings.

If you have questions about the study, please feel free call us, toll-free, at 888-663-2707. If you do not want to be contacted further or if you decide not to participate, you can call us (888-663-2707), or write to us at the address on the letterhead. If you have any questions or concerns regarding this study and would like to talk to someone other than the researchers, you are encouraged to contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St. Southeast, Minneapolis, Minnesota 55455; 612-625-1650.

Thank you for your time and effort spent completing the questionnaire and other forms. We are grateful to you and to all mothers of children diagnosed with leukemia, lymphoma, and similar illnesses for providing information and biological materials for important research like this.

Sincerely,

Amy M. Linabery and Julie A. Ross
Principal Investigators

Feasibility of Retrospectively Obtaining Guthrie Spots

Study Instructions

The following have been included in this study packet:

- ☑ Cover letter
- ☑ Frequently asked questions and answers (see back of this sheet)
- ☑ Consent form (2 copies: 1 to keep and 1 to sign and return)
- ☑ Privacy (HIPAA) form (2 copies: 1 to keep and 1 to sign and return)
- ☑ Assent form (2 copies: 1 to keep and 1 for your child to sign and return)
- ☑ Questionnaire booklet
- ☑ Postage-paid return envelope

To participate in this study:

Step 1. **Read the pink consent form and decide if you would like to release your child's dried blood spots.** This form describes the blood spots, why they are of interest to researchers, and what will be done with them. If you agree to release the spots, please sign the back page of the consent form. There are three consent questions asked; please be sure to read each of them.

Step 2. **Read and sign the green privacy (HIPAA) form.** This form describes how we will keep the blood spots private. HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

Step 3. **Ask your child to read the blue assent form or read it to your child and ask him/her to decide if he/she would like to release the dried blood spots.** This form briefly describes the blood spots, why they are of interest to researchers, and what will be done with them. If he/she agrees to release the spots, please ask him/her to sign the bottom of the assent form.

Step 4. **Complete the questionnaire.** The questionnaire may take up to 30 minutes to complete. If you do not know the exact answers to some of the questions, please provide your best guess.

Step 5. **Mail the materials back to us.** Place the signed consent, assent, and privacy forms, along with the completed questionnaire, in the postage-paid return envelope and drop the envelope in the mail.

Questions? Please call the University of Minnesota study staff, toll-free, at 888-663-2707.

Thank you for your help!

Frequently asked Questions and Answers

Q: Why did I receive a letter about this study?
A: You are being contacted because you participated in the Children's Oncology Group (COG) Childhood Cancer Research Network (CCRN) Pilot Study. In that study, you agreed to receive information about future COG-approved non-therapeutic studies such as this one.

Q: What is this study about?
A: This is a national study that is being conducted in the United States by researchers at the University of Minnesota Department of Pediatrics in partnership with the Children's Oncology Group. It is funded by the Children's Cancer Research Fund. The study concerns factors during pregnancy or childhood that may be related to the development of leukemia, lymphoma, and similar illnesses in children and adolescents. We are contacting 1,000 mothers and children diagnosed with leukemia, lymphoma, and similar illnesses at ages 0-21 years that participated in the COG CCRN Pilot Study.

Q: What am I being asked to do?
A: We are asking mothers to complete a brief questionnaire. The questions ask about reproductive history, events surrounding your child's birth, personal habits such as smoking, medical history, and family health history. We are also asking mothers for permission to obtain dried blood spots collected at birth.

Q: What are dried blood spots?
A: Dried blood spots were created when a few drops of blood were taken at birth, placed on filter paper (also called a "Guthrie Card"), and dried. These blood spots were originally collected to check for some serious diseases (metabolic conditions) in newborns. Some states have stored the dried spots in case they could be used in the future. The blood spots are interesting to researchers because they provide a snapshot of a child's blood biology at birth. If you and your child agree (by providing your written consent and assent), we will contact the state where your child was born and ask for the blood spots.

Q: How much time will it take?
A: The questionnaire takes about 20 minutes to complete. Providing consent and assent for the release of dried blood spots takes about 10 minutes or less to complete.

Q: Can I receive the study results?
A: So little is known about the factors we are studying that the results will not provide useful information to you or your family. This information is for research purposes only and you will not be informed of the results. However, you can receive the overall findings from the study once the study has been completed. To request these results, please call the study staff, toll-free, at 888-663-2707.

Q: How can I get more information about this study?
A: If you have any questions or concerns about the study, now or later, you are encouraged to contact the study staff toll-free at 888-663-2707. If you have any questions or concerns regarding this study and would like to talk to someone other than the researchers, you are encouraged to contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St. Southeast, Minneapolis, Minnesota 55455; 612-625-1650.

CONSENT FORM

Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study Consent Form for Mothers

The Children's Oncology Group, a cooperative group of hospitals in the United States and elsewhere, along with the Department of Pediatrics at the University of Minnesota, are conducting a study of environmental and biologic factors affecting the health of children and their families. Amy Linabery, M.S., M.P.H., and Julie Ross, Ph.D., from the University of Minnesota Department of Pediatrics are responsible for this study. You were selected as a possible participant in this study because your child was diagnosed with leukemia, lymphoma, or a similar illness and because you participated in the Children's Oncology Group Childhood Cancer Research Network Pilot Study and agreed to receive information about future non-therapeutic studies. This study is funded by grants from the National Cancer Institute and the University of Minnesota Children's Cancer Research Fund.

We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Study Purpose

Not a lot is known about the causes of leukemia, lymphoma, and similar illnesses in children and adolescents. The purpose of the study is to learn about factors that may or may not be related to childhood illnesses. We are asking mothers to provide permission to release their child's dried blood spots. These blood spots were created when a few drops of blood were taken at birth, placed on filter paper (also called "Guthrie Cards"), and dried. These blood spots were originally collected to check for some serious diseases (metabolic conditions) in newborns. Some states have stored the dried spots in case they could be used in the future. If mothers agree, the researchers will ask the states where their children were born for the blood spots.

The blood spots are interesting to researchers because they provide a snapshot of a child's blood biology at birth. We will use the blood spots to see if we can measure different compounds in your child's blood in the laboratory. For example, we would like to look at the levels of different growth hormones, vitamins, and tobacco smoke markers that may have been present at the time of birth.

The researchers want to stress that we do not know the reasons why most leukemias, lymphomas, and similar illnesses develop in children and adolescents. For that reason, we are studying a lot of different factors that mothers or their children may have encountered during pregnancy or afterwards.

Study Procedures

If you agree to participate in this study, we would ask you to do the following:

- Agree to release your child's blood spots that were collected at birth and may have been stored by the state where your child was born. If you provide your written permission by signing this form, we will ask the state where your child was born to release one or two blood spots to us.

- We may also send you a separate release form and ask you to sign it. This form has been approved by the state where your child was born and allows the state to send the blood spots to the researchers.

So little is known about the factors we are studying that the results will not provide useful information to you or your family. This information is for research purposes only and you will not be informed of the results. However, if you wish, you can be informed of the overall findings from the study once the study has been completed.

Your participation in this study will last until your child's dried blood spots have been obtained from the state of birth, you request to withdraw from the study, or the study has ended.

Research that uses your child's blood spots may be done a long time after they are obtained by the researchers. These samples will be used for research by Amy M. Linabery, M.S., M.P.H. and Julie A. Ross, Ph.D. and their associates for the purposes of learning more about leukemia, lymphoma, and similar illnesses. In some cases, the spots could be sent to other research groups or laboratories for analysis. You would not be notified if this material was sent to another researcher, but any samples we send will be identified only with a number and will not be able to be traced back to you. If we ever want to study the blood spots for a reason other than for things related to this research study, we will get in touch with you and ask for your permission. You will not be identified in any publication or reports of this data.

You can request that your sample be destroyed at any time by contacting the researchers at the phone number provided below.

There are laws that require that research records that have your name on them be shown to people who make sure the research is being done the way it should be. These are the only people, besides Amy M. Linabery, M.S., M.P.H. and Julie A. Ross, Ph.D. and their associates, who would be able to trace these spots back to you.

Risks of Study Participation

The risks associated with allowing researchers to obtain dried blood spots from state health departments are few. There is a slight risk that the personal information collected in this study about you or your child could accidentally be released to someone other than study staff. We would keep all personal information in locked file cabinets or in computer databases protected by passwords. Only the study staff would have access to these documents and files.

Benefits of Study Participation

There are no direct benefits to you for participating. The results of this study may help us to better understand the biologic and environmental causes of childhood leukemias, lymphomas, and similar illnesses.

Alternatives to Study Participation

The alternative to participating in this study is to decide not to take part.

Study Costs/Compensation

There is no cost to you for participating in this study. You will not receive any payment for taking part in this study.

Confidentiality

If you agree to let us keep the blood spots in the storage bank, they will be labeled with a number, not your name.

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by the Children's Oncology Group and by departments at the University of Minnesota with appropriate regulatory oversight. To these extents, confidentiality is not absolute. Your participation in this study will not be noted in your medical record.

> The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act. The Certificate of Confidentiality will not protect against the required reporting by hospital staff of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.

Protected Health Information (PHI)

Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota or any of the Children's Oncology Group institutions. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions

The researchers conducting this study are Amy M. Linabery, MS, MPH and Julie A. Ross, PhD and their associates at the University of Minnesota Department of Pediatrics. If you have any

questions or concerns regarding the study, now or later, **you are encouraged to** contact them at 612-626-2707 or toll-free at 888-663-2707.

If you have any questions or concerns regarding this study and would like to talk to someone other than the researchers, **you are encouraged to** contact the Research Subjects' Advocate Line, D528 Mayo, 420 Delaware St. Southeast, Minneapolis, Minnesota 55455; 612-625-1650.

You will be given a copy of this form to keep for your records.

Statement of Consent – Dried blood spot release

I have read the above information. I have asked questions and have received answers. I consent to allow these researchers to obtain my child's dried blood spots from the department of health (or other agency) from the state where my child was born.

Signature of
Subject____Date__________________

Signature of
Investigator____Date__________________

Statement of Consent – Dried blood spot storage and future use

I have read the above information. I have asked questions and have received answers. I consent to allow these researchers to store my child's dried blood spots indefinitely for future use.

Signature of
Subject____Date__________________

Signature of
Investigator____Date__________________

Statement of Consent – Future contact regarding this study

I have read the above information. I have asked questions and have received answers. I consent to being contacted again in the future regarding this study.

Signature of
Subject____Date__________________

Signature of
Investigator____Date__________________

ASSENT FORM

Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study Assent Form for Children/Adolescents Ages 8-17 years

About the blood spots

We want to know if you want to take part in a research study. This study is being done by Amy Linabery and Julie Ross at the University of Minnesota. If you agree, we will ask the state that you were born in to give us one or two blood spots that were collected from you when you were born. All babies give a few drops of blood when they are born so that the blood can be checked. The drops of blood are placed on a special piece of paper and are then dried to make spots. Sometimes the state has extra spots left over. We want to ask if the state where you were born has any of your spots left over. If they do, we would like to do some tests on the blood spots in our laboratory to learn about vitamins and other things that might be in the spot. The research that is done with your blood spots will not be able to help you, but we hope we will learn things that may help us with the care that we can give to other kids with leukemia, lymphoma, and similar illnesses.

We will keep the spots for a long time in case there are things in the future that we want to look at that we haven't thought of yet. If we ever want to study the blood spots for a different reason, we will get in touch with you and ask if that's okay first.

Making your choice

You get to choose if you want to let us get your blood spots, do some tests on them, and store them for an unknown amount of time. No one will be mad at you if you don't want to do it.

You can ask any questions you have about letting us get your blood spots. If you have questions, you can have one of your parents call us. Our toll-free phone number is 888-663-2707.

If you sign here, it means you have read this paper or had it read to you and you are willing to be in the study. **If you don't want to give us permission to get, use, and store your blood spots, don't sign this paper.** Remember, being in this study is up to you and no one will be mad at you if you don't sign this or even if you change your mind later.

Signature of
Subject____Date_________________

Signature of
Investigator____Date_________________

HIPAA[1] AUTHORIZATION TO USE AND DISCLOSE INDIVIDUAL HEALTH INFORMATION FOR RESEARCH PURPOSES

1. Purpose. As a research participant, I authorize Amy M. Linabery and Julie A. Ross and the researchers' staff to use and disclose my individual health information for the purpose of conducting the research project entitled, "Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study" [HS # 0803M28241].

2. Individual Health Information to be Used or Disclosed. My individual health information that may be used or disclosed to conduct this research includes: neonatal blood spots collected at birth from ____ , as well as name and date of birth.
patient's name

3. Parties Who May Disclose My Individual Health Information. The researcher and the researcher's staff may obtain my individual health information from:

Hospitals: ____

Clinics: ____

Other Providers: ____

Health Plan: ____,

and from hospitals, clinics, health care providers and health plans that provide my health care during the study.

4. Parties Who May Receive or Use My Individual Health Information. The individual health information disclosed by parties listed in item 3 and information disclosed by me during the course of the research may be received and used by Amy M. Linabery and Julie A. Ross and the researchers' staff.

5. Right to Refuse to Sign this Authorization. I do not have to sign this Authorization. If I decide not to sign the Authorization, I may not be allowed to participate in this study or receive any research related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

6. Right to Revoke. I can change my mind and withdraw this authorization at any time by sending a written notice to Amy M. Linabery or Julie A. Ross, 420 Delaware Street SE, MMC 422, Minneapolis, MN 55455 to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

7. Potential for Re-disclosure. Once my health information is disclosed under this authorization, there is a potential that it will be re-disclosed outside this study and no longer covered by this

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

authorization. However, the research team and the University's Institutional Review Board (the committee that reviews studies to be sure that the rights and safety of study participants are protected) are very careful to protect your privacy and limit the disclosure of identifying information about you.

7A. Also, there are other laws that may require my individual health information to be disclosed for public purposes. Examples include potential disclosures if required for mandated reporting of abuse or neglect, judicial proceedings, health oversight activities and public health measures.

This authorization does not have an expiration date.

I am the research participant or personal representative authorized to act on behalf of the participant.

I have read this information, and I will receive a copy of this authorization form after it is signed.

______________________________	______________________________
signature of research participant or research participant's personal representative	date
______________________________	______________________________
printed name of research participant or research participant's personal representative	description of personal representative's authority to act on behalf of the research participant

FROGS Study
University of Minnesota
420 Delaware Street SE, MMC 422
Minneapolis, MN 55455

<First Name> <Last name>
<Address 1>
<Address 2>
<City>, <State> <Zip>

UNIVERSITY OF MINNESOTA

<Date>

We recently sent materials to you for the Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study. This national study is being conducted at the University of Minnesota in partnership with the Children's Oncology Group. The goal of the study is to learn more about environmental and biologic factors affecting children's health.

If you have already mailed a questionnaire and signed forms back to us, **Thank You!** If you have not yet completed the questionnaire, we would still like to hear from you.

If you have questions or do not want us to contact you further about the study, please call us toll-free at 888-663-2707. If you did not receive the study materials or if you need another questionnaire, please call us and we will send one out immediately.

Sincerely,

Amy Linabery, M.S., M.P.H., and Julie Ross, Ph.D.
Principal Investigators

<Date>

<First Name> <Last name>
<Street Address>
<City>, <State> <Zip>

Dear <First Name> <Last name>,

A number of weeks ago, we invited you to participate in the FROGS Study, a study of factors during pregnancy and childhood that may be related to the development of leukemia, lymphoma, and similar illnesses in children and adolescents. We sent you a questionnaire and other study forms, but to our knowledge, we have not yet received your completed materials.

We are contacting you again to ask for your help because of the importance of gathering information from as many mothers as possible. It is only by obtaining questionnaire answers and permission to obtain dried blood spots from nearly all mothers and children invited to participate that we can be sure the results are representative, accurate and useful.

Our end goal is to learn what causes leukemia, lymphoma, and similar illnesses to develop in young people. We think the results of this study will be very useful in deciding what environmental and biological factors should be researched further and whether or not we can use neonatal blood spots to do so.

The information you provide in this study will be kept strictly confidential and will only be seen by the research staff. Any information that can directly identify you or your child, such as your names, address, and telephone number will be stored separately from your other answers to the questionnaire and will be linked only through the questionnaire identification number. If you agree to let us use and store the blood spots, they will be labeled with a number, not your child's name. Your names will not be used in any publication or presentation of our findings.

We hope you will decide to fill out and return your questionnaire and other forms soon. The yellow page explains the materials included in this packet and provides step-by-step directions on what to do to participate. If you decide not to participate for any reason, please let us know by returning a blank questionnaire or a brief note in the envelope provided.

Sincerely,

Amy M. Linabery and Julie A. Ross
Principal Investigators

P.S. Please feel free to call us, toll-free, at 888-663-2707 with any questions about the study. Or, if you would like to speak to someone else about the research, please call the Research Subjects' Advocate Line at 612-625-1650.

Feasibility of Retrospectively Obtaining Guthrie Spots

Your Child's Birth and Contact Information

1. What is your child's full name?

____
First Middle Last

2. What is your child's date of birth?

___ ___ / ___ ___ / ___ ___ ___ ___
Month Day Year

3. Where was your child born?

____
Hospital name City State/Province Country

4. Is your child 18 years of age or older?

☐ Yes → **Go to question 5**

☐ No

☐ He/she is deceased

5. Is his/her home address and telephone number the same as yours?

☐ Yes, he/she has the same contact information as me

☐ No, he/she has the following contact information:

↓

____
Street Address

____
City State/Province Zip/Postal Code

Phone number: ___ ___ ___ - ___ ___ ___ - ___ ___ ___ ___

Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study
Telephone Script

Initial phone contact

Hello, my name is ___________ and I'm calling from the University of Minnesota Department of Pediatrics. We are working with the Children's Oncology Group on a childhood cancer study.

First of all, I want to check to see whether or not you received the questionnaire and other materials that we recently mailed to you. [Refer to prior mailings if needed.]

> IF NOT RECEIVED, VERIFY MAILING ADDRESS -- INCLUDING SPELLINGS

Do you have any questions about the materials you received?

I would like to ask whether or not you are interested in receiving more information about the study. Do you have a few minutes now to go over the material or would another time be better?

And I just need to double check a few things to make sure the information we have from COG is correct.

> Your child is a __boy/girl__ born on ___date of birth___. Is that correct? I have the __leukemia/lymphoma__ diagnosis date as __diagnosis date__. Is that right? (Within a few days is fine.)
>
> And what is your child's first name? (If we don't have it.)

As you saw in the materials, we are contacting you because you participated in the COG Childhood Cancer Research Network (CCRN) pilot study awhile back. We are conducting a study of factors that may be related to the development of leukemia and lymphoma in children and adolescents. We would like to do a questionnaire with you either in writing or over the telephone. The questions we ask are about medical and family health history, personal habits such as smoking, pregnancy history, and events surrounding your child's birth. Answering the questions usually takes about 20-30 minutes. [IF COMPLETING OVER THE TELEPHONE THEN THE INTERVIEW CAN BE SPLIT INTO DIFFERENT SESSIONS IF NECESSARY]

SILENT PROBE -- ALLOW FOR QUESTIONS

If it's okay, we can schedule a time now for sometime next week to run through the questions with you over the telephone.

IF NOT READY TO DECIDE → We will call back <<*next week OR in the amount of time specified by participant*>> to answer any questions you might have about the materials and to set up a time for the interview.

Also, there is a second, optional portion of the study where we are asking for your child's permission to get your child's dried blood spots from the state health department in the state where your child was born. Since your child is now aged 18 years or older, we would need to get consent from __him/her__ directly for this part of the study. What is the best way to get in touch with __him/her__? [TALK TO CHILD IF AVAILABLE OR FOLLOW INSTRUCTIONS PROVIDED BY MOTHER FOR GETTING IN TOUCH WITH THE CHILD. SEE SCRIPT BELOW.]

Our toll-free number is 1-8XX-XXX-XXXX and it is included it the packet of materials. Please call us with any questions regarding the study.

Thank you for your time. We appreciate your participation in this important study and look forward to talking to you/hearing from you soon.

If completing questionnaire over the telephone
We've gone over a lot of information (*or There's a lot of information in the study materials)* and I want to be sure we've gone over everything.

1. Can you describe for me the purpose of the study?

2. What more would you like to know about the study?

3. Do you have the toll-free number in case you have questions in the future?

4. And I would like to confirm whether or not you would like to participate.

At time of interview
Everything you tell us will be kept confidential and seen only by the researchers involved with the study. If there is any question you do not wish to answer, please let me know. If you have any questions as we go along please stop me. If you want to take a break let me know that too.

Discussion with the adult child

Hello, my name is ___________ and I'm calling from the University of Minnesota Department of Pediatrics. We are working with the Children's Oncology Group on a childhood cancer study. Your mom told us that this would be the best way to get in touch with you.

First of all, I want to check to see whether or not you received the materials that we recently mailed to you. [Refer to prior mailings if needed.]

Do you have any questions about the materials you received?

I would like to ask whether or not you are interested in receiving more information about the study. Do you have a few minutes now to go over the material or would another time be better?

As you saw in the materials, we are contacting you because you and your mom participated in the COG Childhood Cancer Research Network (CCRN) pilot study awhile back. We are conducting a study of factors that may be related to the development of leukemia and lymphoma in children and adolescents. In addition to the questionnaire we have asked your mom to fill out, there is a second, optional portion of the study where we are asking for your written permission to get your dried blood spots from the state health department in the state where you were born.

These blood spots are created when a few drops of blood are collected from babies at birth, placed on filter paper (also called "Guthrie Cards"), and dried. The blood spots are collected to check for some serious diseases (metabolic conditions) in newborns. Some states have stored the dried spots in case they could be used in the future. If you agree (by signing the forms we sent), we will ask the state where you were born for the blood spots. These blood spots are useful because they provide a snapshot of your blood biology at the time of your birth.

SILENT PROBE -- ALLOW FOR QUESTIONS

Do you have any questions about the study that I can answer?

Our toll-free number is 1-8XX-XXX-XXXX and it is included it the packet of materials. Please call us with any questions regarding the study or filling out the forms.

Thank you for your time. We appreciate your participation in this important study and look forward to hearing from you soon.

Appendix F. FROGS Study letters of approval

Includes letters from the COG Scientific Council, the University of Minnesota Cancer Protocol Review Committee (CPRC) and Institutional Review Board (IRB), and response letters from state IRBs, including: Minnesota Department of Health, State of New York Department of Health, Washington State Department of Health, Michigan Department of Community Health, and Texas Department of State Health Services. We currently await a response from the New Jersey Department of Health and Senior Services.

Children's Oncology Group

May 22, 2008

Dr. Julie A. Ross, PhD
University of Minnesota Cancer Center
Dept of Pediatrics Epidemiology
Clinical Research, MMC 422
420 Delaware St. S.E.
Minneapolis, MN 55455

Re: COG Scientific Council Concept Re-Review of AEPI08N1: Feasibility of obtaining neonatal blood spots for AADM01P1- CCRN Pilot study participants.

Dr. Ross,

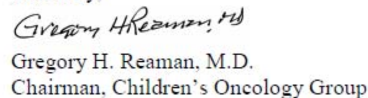
Thank you for your efforts and those of your committee in developing the above-referenced study concept. Your concept was re-reviewed by the Scientific Council on May 13, 2008. The concept was re-reviewed for its significance, innovation, conceptual framework, statistical methodology and feasibility as well as resources required. The re-review and recommendations represent a consensus opinion of the Scientific Council.

Based on the review, which is attached, the Scientific Council recommends:

Approval__X___
Approval with binding recommendations______
Deferral________
Disapproval______

Please send all correspondence concerning this review to the Study Development Office and respective Protocol Coordinator.

Thank you for your prompt attention.
Sincerely,



Gregory H. Reaman, M.D.
Chairman, Children's Oncology Group

Cc: Scientific Council, Judy Everett

Group Chair
Gregory Reaman, M.D.

Group Vice Chair
Frank Smith, M.D.

Administrative Officer
Maura O'Leary, M.D.

Group Statistician
James Anderson, Ph.D.

Associate Group Statistician
Mark Krailo, Ph.D.

Chief Operating Officer
Joseph Woelkers, M.A.

Group Chair's Office
4600 East West Highway, Suite 600
Bethesda, MD 20814-3457
Phone: (240) 235-2220
Fax: (301) 718-0047
greaman@childrensoncologygroup.org

Albert Roy
Director of Finance and Planning

Group Operations Center
440 E. Huntington Drive
Arcadia, CA 91006
Phone: (626) 447-0064
Fax: (626) 445-4334

Statistics & Data Center Headquarters
440 E. Huntington Drive
Arcadia, CA 91006
Phone: (626) 447-0064
Fax: (626) 445-4334

Gainesville Office
104 N. Main Street
Suite 600
Gainesville, FL 32601
Phone: (352) 273-0550
Fax: (352) 392-8162

Omaha Office
University of Nebraska Medical Center
984375 Nebraska Medical Center
Omaha, NE 68198-4375
Phone: (402) 559-4112
Fax: (402) 559-7259

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Children's Oncology Group
4600 East West Highway, Suite 600, Bethesda, MD 20814
240-235-2200 tel. 301-718-0047 fax www.curesearch.org

UNIVERSITY OF MINNESOTA

Cancer Center

Cancer Protocol Review Committee
Non-Therapeutic interventional (NTI)
B405 Mayo

February 21, 2008

Julie A. Ross, MD
Pediatric Epidemiology
554 CCRB

Amy M. Linabery, MS, MPH
Pediatric Epidemiology
554 CCRB

CPRC #2008NT033
"Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study"
Also *"Feasibility of Obtaining Neonatal Blood Spots for AADM01P1 – CCRN Pilot Study Participants"*

Dear Dr. Ross and Ms. Linabery,

The Cancer Protocol Review Committee/NTI reviewed the above study at the February 21, 2008 CPRC/NTI meeting and approved it.

Please send a copy of all correspondence pertaining to your study to Sue Julson, Clinical Trials Office, CPRC, MMC 806, 8806.

Sincerely,

DeAnn Lazovich, PhD
Cancer Protocol Review Committee/NTI

UNIVERSITY OF MINNESOTA

Twin Cities Campus

Research Subjects' Protection Programs
Institutional Review Board: Human Subjects Committee (IRB)
Institutional Animal Care and Use Committee (IACUC)
Institutional Biosafety Committee (IBC)

Mayo Mail Code 820
D-528 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455

612-626-5654
Fax: 612-626-6061
irb@umn.edu
iacuc@umn.edu
ibc@umn.edu
www.research.umn.edu/subjects

04/21/2008

Amy M Linabery
Department of Pediatrics
MMC 422
Minneapolis Campus

RE: "Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study"
"Feasibility of Obtaining Neonatal Blood Spots for AADM01P1 - CCRN Pilot Study Participants"
IRB Code Number: **0803M28241**

Dear Dr. Linabery

The Institutional Review Board (IRB) received your response to its stipulations. Since this information satisfies the federal criteria for approval at 45CFR46.111 and the requirements set by the IRB, final approval for the project is noted in our files. Upon receipt of this letter, you may begin your research.

IRB approval of this study includes the consent forms dated April 10, 2008 and HIPAA Authorization received April 10, 2008.

The IRB would like to stress that subjects who go through the consent process are considered enrolled participants and are counted toward the total number of subjects, even if they have no further participation in the study. Please keep this in mind when calculating the number of subjects you request. This study is currently approved for 2000 subjects. If you desire an increase in the number of approved subjects, you will need to make a formal request to the IRB.

For your records and for grant certification purposes, the approval date for the referenced project is April 1, 2008 and the Assurance of Compliance number is FWA00000312 (Fairview Health Systems Research FWA00000325, Gillette Children's Specialty Healthcare FWA00004003). Research projects are subject to continuing review and renewal; approval will expire one year from that date. You will receive a report form two months before the expiration date. If you would like us to send certification of approval to a funding agency, please tell us the name and address of your contact person at the agency.

As Principal Investigator of this project, you are required by federal regulations to inform the IRB of any proposed changes in your research that will affect human subjects. Changes should not be initiated until written IRB approval is received. Unanticipated problems or serious unexpected adverse events should be reported to the IRB as they occur.

The IRB wishes you success with this research. If you have questions, please call the IRB office at 612-626-5654.

Sincerely,

Felicia Mroczkowski, CIP
Research Compliance Supervisor
FM/cgk
CC: Erica Langer, Julie Ross, Logan Spector

Driven to Discover℠

Amy Linabery

From: irb@umn.edu
Sent: Thursday, February 26, 2009 11:14 PM
To: devr0053@umn.edu
Subject: 0803M28241 - PI Linabery - IRB - APVD Continuing Review

TO : rossx014@umn.edu, rossx014@umn.edu, spect012@umn.edu, kaste007@umn.edu, devr0053@umn.edu, slate074@umn.edu,

The IRB: Human Subjects Committee renewed its approval of the referenced study listed below:

Study Number: 0803M28241

Principal Investigator: Amy Linabery

Expiration Date: 02/24/2010

Approval Date: 02/25/2009

Title(s):
Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study

Feasibility of Obtaining Neonatal Blood Spots for AADM01P1 - CCRN Pilot Study Participants

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This e-mail confirmation is your official University of Minnesota RSPP notification of continuing review approval. You will not receive a hard copy or letter.
This secure electronic notification between password protected authentications has been deemed by the University of Minnesota to constitute a legal signature.

You may go to the View Completed section of http://eresearch.umn.edu/ to view or print your continuing review submission.

For grant certification purposes you will need this date and the Assurance of Compliance number, which is FWA00000312 (Fairview Health Systems Research FWA00000325, Gillette Childrens Specialty Healthcare FWA00004003). Approval will expire one year from that date. You will receive a report form two months before the expiration date.

In the event that you submitted a consent document with the continuing review form, it has also been reviewed and approved. If you provided a summary of subjects' experience to include non-UPIRTSO events, these are hereby acknowledged.

As Principal Investigator of this project, you are required by federal regulations to inform the IRB of any proposed changes in your research that will affect human subjects. Changes should not be initiated until written IRB approval is received. Unanticipated problems and adverse events should be reported to the IRB as they occur. Research projects are subject to continuing review.

If you have any questions, please call the IRB office at (612) 626-5654.

The IRB wishes you continuing success with your research.

1

May 8, 2009 *Protecting, maintaining and improving the health of all Minnesotans*

Amy M. Linabery
University of Minnesota
Department of Pediatrics
420 Delaware St. SE, MMC 422
Minneapolis, MN 55455

Dear Ms. Linabery:

Thank you for your letter dated April 17, 2009, responding to the stipulations and suggestions made by the IRB regarding your study titled "Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study".

The Board has reconsidered all aspects of your study. We find that review by the Minnesota Department of Health Institutional Review Board is **not** required because MDH staff have no role as principal investigator, co-investigator or any other role in directing the study. The fact that a program of the department is asked to provide data is not sufficient to require a separate review by the IRB. The appropriate body to protect human subjects involved in this research is the University of Minnesota's IRB, and we have noted their approval of the study.

In your study, the Department of Health will be asked to provide newborn screening blood spots for cases in which informed consent has been obtained. The Newborn Screening Program has specific policies and procedures for the release of data and samples. You should apply directly to the Newborn Screening Program if you would like to request blood spots for your study.

Please feel free to contact me if you have any questions.

Sincerely,

Peter Rode
MDH IRB Administrator
85 East Seventh Street, PO Box 64882
St. Paul, MN 55164
651-201-5942

Cc:
Ann Kowski

General Information: 651-201-5000 • Toll-free: 888-345-0823 • TTY: 651-201-5797 • www.health.state.mn.us
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DEPARTMENT OF HEALTH

Corning Tower The Governor Nelson A. Rockefeller Empire State Plaza Albany, New York 12237

Richard F. Daines, M.D.
Commissioner

Wendy E. Saunders
Executive Deputy Commissioner

August 14, 2009

Michele Caggana, Sc.D.
NYS Department of Health
Wadsworth Center, Rm. E117
Albany NY 12201

RE: Approval of Study # 09-053
Protocol Title: Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study

Dear Dr. Caggana:

The New York State Department of Health Institutional Review Board (IRB) has reviewed your request for expedited approval of the new study referenced above. Your study is eligible for expedited review under DHHS (OHRP) regulation.

This is to confirm that the IRB has approved your application. The protocol is approved through August 14, 2010.

You are granted permission to conduct your study as described in your application effective immediately. The study is subject to continuing review before it expires on **August 14, 2010**, unless closed before that date.

Any changes to the study must be promptly reported and approved by the IRB prior to implementation. Please feel free to contact me at (518) 474-8539 if you have any questions regarding this approval or require further information.

Sincerely,

Tony M. Watson, M.B.A., C.I.P.
Administrative Coordinator
Institutional Review Board
For the Protection of Human Subjects

Version Date: 1/6/2005

Institutional Review Board Authorization Agreement

Institution Providing IRB Review (Institution A): University of Minnesota
IRB Registration #: 00000438 (IRB #1 Biomedical)   Federalwide Assurance: FWA00000312

Institution Relying on the Designated IRB (Institution B): Washington State Department of Health
Federalwide Assurance: FWA00000327

The Officials signing below agree that the Department of Health may rely on the designated IRB for review and continuing oversight of its human subjects research described below: (*check one*)

☐ This agreement applies to all human subjects research covered by Institution B's FWA.

☒ This agreement is limited to the following specific protocol(s):

Name of Research: Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study
Name of Principal Investigator: Amy M. Linabery, M.S., M.P.H.
Sponsor or Funding Agency: Children's Cancer Research Fund

☐ Other: Describe

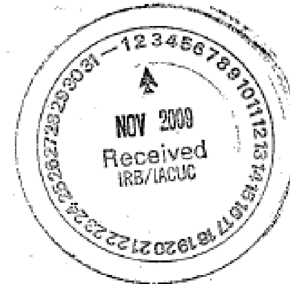
The review and continuing oversight performed by the designated IRB will meet the human subject protection requirements of the Department of Health Services' OHRP-approved FWA. The University of Minnesota IRB will follow written procedures for reporting its findings and actions to appropriate officials at Department of Health (DOH). Relevant minutes of IRB meetings will be made available to the DOH upon request. DOH remains responsible for ensuring compliance with the University of Minnesota's IRB's determinations and with the terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

Signature of Signatory Official (Institution A): R. Timothy Mulcahy Date: 11/4/09

R. Timothy Mulcahy, Vice President for Research
University of Minnesota
419 Joh H
101 Pleasant St SE
Minneapolis, Minnesota 55455
612-624-5054
mulcahy@umn.edu

Signature of Signatory Official (Institution B): Margaret Chederich Date: Oct 27, 2009

Michael A. Garrick, Ph.D., Human Protections Administrator
Washington State Department of Social & Health Services
1115 Washington Street / P.O. Box 45205
Olympia, Washington 98504-5205
(360) 902-8075
mike.garrick@dshs.wa.gov



Michigan Department of Community Health
Institutional Review Board Approval Form
Authority: Code of Federal Regulations Title 45 part 46

To:	Harry Hawkins	"Responsible MDCH Employee"
From:	Harry McGee	MDCH IRB Chair/Administrator
CC:	Frances Pouch-Downes	MDCH Bureau/Center/Office Director

MDCH IRB Log#: 704-PHALAB **Date Received:** 06/03/09

Study Title: Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS)

Investigator(s): Amy M. Linabery and Julie A. Ross (U of Minn)

Funding Source(s): Children's Cancer Research Fund (Minneapolis, MN)

Committee Action/Recommendation:

- [ ] Decision Deferred *
- [x] Exempt from review *
- [ ] Exempt from approval*
- [ ] Approved by expedited review without modifications
- [ ] Approved by expedited review with modifications*
- [ ] Approved by full committee without modifications
- [ ] Approved by full committee with modifications*
- [ ] Disapproved*

Comments: * MDCH is not engaged in the research

Signature (Chair): H. McGee **Approval Date:** 06/04/09

****Expiration Date for the Approval of the Project:** N/A

****Prior to this expiration date the project must be re-approved in order for human subject's research to continue.**

The MDCH IRB must approve any change to this study protocol. Approval must precede implementation, unless the change is necessary to eliminate an apparent immediate hazard to the subject. The Responsible MDCH Employee must see that any unexpected change or problem in the research is reported immediately to the MDCH IRB Chair at 517-241-0806 or mcgeeh@michigan.gov.

Michigan Department of Community Health FWA00007331 IRB00000421

The Department of Community Health is an equal opportunity employer, services and programs provider.

DCH 1280 (10/05)

TEXAS DEPARTMENT OF STATE HEALTH SERVICES

P.O. Box 149347
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www.dshs.state.tx.us

DAVID L. LAKEY, M.D.
COMMISSIONER

January 29, 2010

Amy Linabery, MS, MPH
Department of Pediatrics
Division of Epidemiology/Clinical Research
University of Minnesota
420 Delaware Street SE, MMC 422
Minneapolis, Minnesota 55455-0308

Re: Conditional Approval of Feasibility of Retrospectively Obtaining Guthrie Spots (FROGS) Study, IRB# 09-089

Dear Ms. Linabery:

The Department of State Health Services Institutional Review Board #1 (IRB) met on January 21, 2010, to consider your application for approval of the above named project. This request was reviewed by the entire Department of State Health Services IRB #1, as required by agency policy and Texas Health and Safety Code §33.017 and found compliant with Health and Safety Code §33.017 (b)(2), because it is with consent, of each identified individual or an individual authorized to consent on behalf of an identified child. The IRB voted to approve your project with the following limitations and conditions.

> For purposes of this request, we are approving preservation of those bloodspots where you have consent. However, because of the ambiguity of the consent statement, we will require from you, for any analysis, another submission specifically stating what that or those analyses will be prior to the utilization of those bloodspots.

If you have questions, please contact the IRB Administrator, Steven Lowenstein, at (512) 458-7111, extension 2202, or toll-free at 1-888-777-5037, or by E-mail at steven.lowenstein@dshs.state.tx.us. You may also visit our website at www.dshs.state.tx.us/irb.

In any future correspondence concerning this project, please reference the IRB number noted above.

Sincerely,

John F. Villanacci, Ph.D., NREMTI
Chair, DSHS Institutional Review Board #1
FWA00008616/IRB00004733

:sl.

cc: file (09-089)

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Appendix G. Response rates for the questionnaire, written consent and assent, and adult contact information forms, and NBS retrieval rates overall and by age at diagnosis, year of birth, and years since diagnosis.

	Total	**Age at diagnosis**			
		0-4 years	**5-9 years**	**10-14 years**	**15-20 years**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Questionnaire					
Completed	366 (41.3)	138 (38.5)	65 (33.7)	69 (40.8)	94 (56.3)
Refused, active	27 (3.0)	14 (4.3)	4 (2.1)	6 (3.6)	3 (1.8)
Refused, passive (contact made)	156 (17.6)	60 (16.8)	43 (22.3)	32 (18.9)	21 (12.6)
Unsure if contact made	291 (32.8)	121 (33.8)	73 (37.8)	56 (33.1)	41 (24.6)
Returned to sender	47 (5.3)	25 (7.0)	8 (4.1)	6 (3.6)	8 (4.8)
Consent					
Completed	310 (37.1)	136 (36.3)	62 (30.5)	51 (36.2)	61 (52.6)
Wrong party signed consent	1 (0.1)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Refused, active	26 (3.1)	14 (4.1)	6 (3.0)	4 (2.8)	2 (1.7)
Refused, passive (contact made)	200 (24.0)	69 (18.4)	48 (23.6)	39 (27.7)	44 (37.9)
Unsure if contact made	253 (30.3)	127 (33.9)	78 (38.4)	41 (29.1)	7 (6.0)
Returned to sender	45 (5.4)	28 (7.5)	9 (4.4)	6 (4.3)	2 (1.7)
Assent					
Completed	148 (32.0)	70 (36.3)	56 (29.6)	21 (26.3)	1 (100)
Wrong party signed assent	7 (1.5)	4 (2.1)	2 (1.1)	1 (1.3)	0 (0.0)
Refused, active	13 (2.8)	5 (2.6)	5 (2.6)	3 (3.8)	0 (0.0)
Refused, passive (contact made)	100 (21.6)	35 (18.1)	47 (24.9)	18 (22.5)	0 (0.0)
Unsure if contact made	169 (36.5)	66 (34.2)	70 (37.0)	33 (41.3)	0 (0.0)
Returned to sender	26 (5.6)	13 (6.7)	9 (4.8)	4 (5.0)	0 (0.0)

	Total	Age at diagnosis			
		0-4 years	5-9 years	10-14 years	15-20 years
	N (%)	N (%)	N (%)	N (%)	N (%)
Adult child information form					
Completed	132 (54.1)	0 (0.0)	0 (0.0)	39 (49.4)	93 (56.7)
Refused, active	9 (3.7)	0 (0.0)	0 (0.0)	4 (5.1)	5 (3.0)
Refused, passive (contact made)	31 (12.7)	0 (0.0)	0 (0.0)	14 (17.7)	17 (10.4)
Unsure if contact made	61 (25.0)	0 (0.0)	0 (0.0)	21 (26.6)	40 (24.4)
Returned to sender	11 (4.5)	0 (0.0)	1 (100)	1 (1.3)	9 (5.5)
NBS					
Received	46 (4.9)	23 (6.1)	10 (4.9)	5 (2.8)	8 (4.3)
State not responded	67 (7.1)	34 (9.1)	10 (4.9)	7 (3.9)	16 (8.6)
State stores spots <1 year	72 (7.6)	30 (8.0)	15 (7.4)	14 (7.7)	13 (7.0)
State stores spots 1-5 years, not age-eligible	33 (3.5)	15 (4.0)	4 (2.0)	7 (3.9)	7 (3.7)
State stores spots >5 years, not age-eligible	19 (2.0)	5 (1.3)	4 (2.0)	3 (1.7)	7 (3.7)
Insufficient information to release	2 (0.2)	1 (0.3)	0 (0.0)	1 (0.6)	0 (0.0)
State does not release NBS	60 (6.3)	23 (6.1)	16 (7.8)	11 (6.1)	10 (5.4)
NBS not requested*	648 (68.4)	244 (65.1)	145 (71.1)	133 (73.5)	126 (67.4)

* Includes cases with no consent/assent, lost to follow up, and not born in U.S.

	Year of birth					Years since diagnosis	
	1982-1986	1987-1991	1992-1996	1997-2001	2002-2006	2-4 years	5-10 years
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Questionnaire							
Completed	36 (50.7)	104 (54.2)	57 (30.5)	122 (39.0)	47 (37.9)	158 (42.5)	208 (40.4)
Refused, active	0 (0.0)	8 (4.2)	3 (1.6)	12 (3.8)	4 (3.2)	10 (2.7)	17 (3.3)
Refused, passive (contact made)	11 (15.5)	27 (14.1)	45 (24.1)	47 (15.0)	26 (21.0)	65 (17.5)	91 (17.7)
Unsure if contact made	20 (28.2)	45 (23.4)	75 (40.1)	112 (35.8)	39 (31.5)	121 (32.5)	170 (33.0)
Returned to sender	4 (5.6)	8 (4.2)	7 (3.7)	20 (6.4)	8 (6.5)	18 (4.8)	29 (5.6)
Consent							
Completed	23 (43.4)	69 (53.5)	55 (27.9)	116 (35.6)	47 (36.2)	131 (36.8)	179 (37.4)
Wrong party signed consent	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
Refused, active	2 (3.8)	1 (0.8)	4 (2.0)	15 (4.6)	4 (3.1)	11 (3.1)	15 (3.1)
Refused, passive (contact made)	21 (39.6)	46 (35.7)	49 (24.9)	56 (17.2)	28 (21.5)	85 (23.9)	115 (24.0)
Unsure if contact made	5 (9.4)	10 (7.8)	80 (40.6)	116 (35.6)	42 (32.3)	113 (31.7)	140 (29.2)
Returned to sender	2 (3.8)	3 (2.3)	9 (4.6)	22 (6.7)	9 (6.9)	16 (4.5)	29 (6.1)
Assent							
Completed	0 (0.0)	3 (27.3)	51 (28.3)	94 (34.6)	0 (0.0)	44 (28.4)	104 (33.8)
Wrong party signed assent	0 (0.0)	1 (9.1)	0 (0.0)	6 (2.2)	0 (0.0)	3 (1.9)	4 (1.3)
Refused, active	0 (0.0)	0 (0.0)	3 (1.7)	10 (3.7)	0 (0.0)	4 (2.6)	9 (2.9)
Refused, passive (contact made)	0 (0.0)	3 (27.3)	47 (26.1)	50 (18.4)	0 (0.0)	36 (23.2)	64 (20.8)
Unsure if contact made	0 (0.0)	3 (27.3)	70 (38.9)	96 (35.3)	0 (0.0)	60 (38.7)	109 (35.4)
Returned to sender	0 (0.0)	1 (9.1)	9 (5.0)	16 (5.9)	0 (0.0)	8 (5.2)	18 (5.8)

	Year of birth					Years since diagnosis	
	1982-1986 N (%)	1987-1991 N (%)	1992-1996 N (%)	1997-2001 N (%)	2002-2006 N (%)	1992-1996 N (%)	1987-1991 N (%)
Adult child information form							
Completed	38 (53.5)	94 (54.3)	0 (0.0)	0 (0.0)	0 (0.0)	50 (62.5)	82 (50.0)
Refused, active	2 (2.8)	7 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.8)	6 (3.7)
Refused, passive (contact made)	7 (9.9)	24 (13.9)	0 (0.0)	0 (0.0)	0 (0.0)	5 (6.3)	26 (15.9)
Unsure if contact made	19 (26.8)	42 (24.3)	0 (0.0)	0 (0.0)	0 (0.0)	18 (22.5)	43 (26.2)
Returned to sender	5 (7.0)	6 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	4 (5.0)	7 (4.3)
NBS							
Received	3 (3.5)	7 (3.4)	9 (4.6)	16 (4.9)	11 (8.5)	25 (6.5)	21 (3.7)
State not responded	5 (5.8)	14 (6.7)	8 (4.1)	24 (7.4)	16 (12.3)	38 (9.8)	29 (5.2)
State stores spots <1 year	5 (5.8)	17 (8.2)	14 (7.1)	30 (9.2)	6 (4.6)	18 (4.7)	54 (9.6)
State stores spots 1-5 years, not age-eligible	4 (4.7)	6 (2.9)	9 (4.6)	12 (3.7)	2 (1.5)	8 (2.1)	25 (4.5)
State stores spots >5 years, not age-eligible	3 (3.5)	6 (2.9)	3 (1.5)	5 (1.5)	2 (1.5)	10 (2.6)	9 (1.6)
Insufficient information to release	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	2 (0.4)
State does not release NBS	3 (3.5)	15 (7.2)	12 (6.1)	20 (6.1)	10 (7.7)	26 (6.7)	34 (6.1)
NBS not requested*	63 (73.3)	142 (68.3)	142 (72.1)	218 (66.9)	83 (63.8)	261 (67.6)	387 (69.0)

* Includes cases with no consent/assent, lost to follow up, and not born in U.S.

Appendix H. Studies examining prenatal vitamin and/or iron supplementation and childhood leukemia

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
INFANT LEUKEMIA									
Pombo-de-Oliveira, Brazil (2006)[151]	1999-2005	0-21 months	Age, region of residence, sex, income, maternal age, birthweight	202	440	Maternal consumption of vitamins/iron supplements during pregnancy	IAL	0.90 (0.63-1.28)	
CHILDHOOD LEUKEMIA									
Schüz, Germany (2007)[39]	1992-1994	0-14 years	Age (± 1 year), sex, community of residence, year of birth, degree of urbanization, SES	650	2057	Maternal vitamin, folate and/or iron supplement use during pregnancy	ALL	**0.84 (0.69-1.01)**	Maintaining the original matching moved OR closer to null (OR = 0.96, 95% CI: 0.75-1.22). Upon stratification by child's age, $OR_{<5\ years}$ = 0.98 (95% CI: 0.76-1.25) and $OR_{\geq 5\ years}$ = 0.67 (0.50-0.90).
				105	2057	Maternal vitamin, folate and/or iron supplement use during pregnancy	AML	1.13 (0.74-1.72)	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Dockerty, New Zealand (2007)[93]	1990-1993	0-14 years	Age, sex, marital status, maternal education level	97	303	Mother's use of folic acid during pregnancy	ALL	1.1 (0.5-2.7)	No association for 3 months before pregnancy or while breastfeeding (data not shown).
						Mother's use of multivitamins during pregnancy	ALL	0.8 (0.2-3.1)	
						Mother's use of other vitamin or mineral supplements during pregnancy	ALL	1.5 (0.7-3.1)	
						Mother’s use of iron (with or without folic acid) during pregnancy	ALL	1.2 (0.7-2.1)	
						Mother’s use of iron without folic acid during pregnancy	ALL	1.3 (0.8-2.3)	
Kwan, USA (2007)[154]	1995-2002	0-14 years	Date of birth, sex, Hispanic ethnicity, maternal race, age, and education, household income, country of residence at birth	365	460	Overall: Iron supplement use from 3 months prior to pregnancy to end of breastfeeding	Leukemia	**0.70 (0.51-0.97)**	Case participation rate: 86%, control participation rate: 85%

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Kwan, USA (2007)[154], con't						Peripregnany: Iron supplement use from 3 months prior to pregnancy to end of pregnancy	Leukemia	0.76 (0.52-1.11)	
						Overall iron supplement use	ALL	**0.67 (0.47-0.94)**	
						Peripregnancy iron supplement use	ALL	0.72(0.47-1.09)	
Ross, USA (2005)[40]	1997-2002	0-18 years	Age, sex, maternal age, maternal race, maternal education level	97	173	Maternal vitamin use in the year before and throughout pregnancy	ALL	**0.49 (0.29-0.84)**	Study conducted in children with Down syndrome. No evidence of an interaction with advanced maternal age (≥35 years vs. <35 years). No differences observed upon stratification by child's age.
						Maternal vitamin use during periconceptional period (year before pregnancy and during pregnancy before knowledge of pregnancy)	ALL	**0.51 (0.30-0.89)**	
						Maternal vitamin use only after knowledge of pregnancy	ALL	**1.70 (0.98-2.92)**	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Ross, USA (2005)[40], con't						Number of vitamins taken during year before pregnancy = 1	ALL	**0.56 (0.32-0.98)**	
						Number of vitamins taken during year before pregnancy = 2	ALL	0.55 (0.20-1.49)	
						Iron supplements taken in addition to multi-vitamin	ALL	No association	Results not provided; text indicates no association with time periods examined.
				61	173	Maternal vitamin use in the year before and throughout pregnancy	AML	0.98 (0.51-1.86)	Reduced risk in those <2 years at diagnosis (OR = 0.48, 95% CI: 0.21-1.07) and no association in those 2-5 years at diagnosis (OR = 1.02, 95% CI: 0.21-5.04).
						Maternal vitamin use during periconceptional period (year before pregnancy and during pregnancy before knowledge of pregnancy)	AML	0.92 (0.48-1.76)	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Ross, USA (2005)[40], con't						Maternal vitamin use only after knowledge of pregnancy	AML	1.45 (0.75-2.78)	
						Number of vitamins taken during year before pregnancy = 1	AML	0.83 (0.43-1.60)	
						Number of vitamins taken during year before pregnancy = 2	AML	1.07 (0.36-3.20)	
						Iron supplements taken in addition to multi-vitamin	AML	No association	Results not provided; text indicates no association with time periods examined.
Shaw, Canada (2004)[94]	1980-2000	0-14 years	Age, sex, maternal age, maternal education level	789	789	Maternal use of vitamins (with folic acid) during pregnancy	ALL	1.0 (0.8-1.2)	
						Maternal use of vitamins (with folic acid) during pregnancy, <50 times	ALL	1.1 (0.6-2.1)	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Shaw, Canada (2004)[94], con't						Maternal use of vitamins (with folic acid) during pregnancy, 50-100 times	ALL	1.4 (0.9-2.3)	
						Maternal use of vitamins (with folic acid) during pregnancy, >100 times	ALL	0.9 (0.7-1.1)	
Jensen, USA (2004)[156]	1995-1999	0-14 years	Date of birth, sex, maternal race, parental Hispanic ethnicity, county of residence, energy intake, income, prior fetal loss, hours child in preschool, indoor pesticide exposure during pregnancy, proportion of portion sizes reported as large or extra large	138	138	Total folic acid in the maternal diet (1 year prior to index pregnancy)	ALL	0.78 (0.33-1.81)	Authors note that supplement use increases during pregnancy and therefore, pre-pregnancy diet may not be an adequate surrogate.
						Iron intake in year before pregnancy	ALL	0.89 (0.51-1.53)	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Wen, USA (2002)[41]	1989-1993	0-14 years	Age (±2 years), race, telephone area code and exchange, immunophenotype, gender, household income, maternal race, maternal education, maternal smoking before or during pregnancy, maternal drinking before or during pregnancy, other medication use	1842	1986	Ever used vitamins during year before pregnancy through pregnancy and nursing	ALL	**0.7 (0.5-1.0)**	99% CIs reported
						Ever used vitamins during year before pregnancy through pregnancy	ALL	**0.7 (0.5-1.0)**	
						Ever used vitamins during year before pregnancy	ALL	1.2 (0.4-3.0)	
						Ever used vitamins during pregnancy	ALL	**0.7 (0.5-1.0)**	
		<1 year		64	81	Ever used vitamins during year before pregnancy through pregnancy	ALL	0.9 (0.1-7.1)	
		0-14 years		1842	1986	Ever used iron supplements during year before pregnancy through pregnancy and nursing	ALL	**0.9 (0.7-1.0)**	Also looked at paternal iron supplement use

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Wen, USA (2002)[41], con't						Ever used iron supplements during year before pregnancy through pregnancy	ALL	1.5 (0.8-2.6)	
						Ever used iron supplements during year before pregnancy	ALL	1.4 (0.6-3.6)	
						Iron supplement use only during pregnancy	ALL	**0.8 (0.7-1.0)**	
		<1 year		64	81	Iron supplement use only during pregnancy	ALL	1.1 (0.3-3.9)	
Thompson, Australia (2001)[42]	1984-1992	0-14 years	Sex, date of birth (± 6 months), region of residence	83	166	Maternal use of folate (with or without iron) during pregnancy	ALL	**0.40 (0.21-0.73)**	OR robust to adjustment by potential confounders examined separately and in combination. No effect modification observed across strata of sex or age at diagnosis (<5 vs. ≥5 years).
						First use of folate (with or without iron) at ≤7 weeks	ALL	**0.18 (0.04-0.80)**	
						First use of folate (with or without iron) at 8-11 weeks	ALL	0.39 (0.13-1.16)	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
Thompson, Australia (2001)[42], con't						First use of folate (with or without iron) at >11 weeks	ALL	**0.33 (0.14-0.81)**	
						Duration of use of folate (with or without iron) in pregnancy of 1-26 weeks	ALL	**0.28 (0.10-0.76)**	
						Duration of use of folate (with or without iron) in pregnancy of 27-33 weeks	ALL	0.39 (0.15-1.02)	
						Duration of use of folate (with or without iron) in pregnancy of ≥34 weeks	ALL	**0.21 (0.04-0.98)**	
						Maternal iron use (without folate) during pregnancy	ALL	0.75 (0.37-1.51)	
						Maternal use of iron and folate during pregnancy	ALL	**0.41 (0.22-0.75)**	

Author, Location (Year)	Diagnosis dates included	Ages	Matching/ adjustment variables	N_{cases}	$N_{controls}$	Exposure	Outcome	OR (95% CI)	Comments
McKinney, Scotland (1999)[155]	1991-1994	0-14 years	Age (+/- 1 month), sex, area of residence	144	271	Iron preparations taken in pregnancy	Leukemia	0.95 (0.62-1.45)	
				124	236	Iron preparations taken in pregnancy	ALL	0.80 (0.51-1.25)	
Sarasua, USA (1994)[92]	1976-1983	0-14 years	Age (± 3 years), gender, telephone area and exchange	56	206	Maternal vitamin intake during pregnancy	ALL	0.50 (0.22-1.13)	
Robison, USA (1989)[95]	1980-1984	0-17 years	Date of birth, race, telephone area code and exchange	204	204	Maternal vitamins/iron use (≥5 days) during or in the year before the index pregnancy	ANLL	1.00 (0.51-1.96)	

OR = odds ratio, 95% CI = 95% confidence interval, IAL = infant acute leukemia, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, ANLL = acute non- lymphoblastic leukemia

Appendix I. Questionnaire items regarding prenatal vitamin and iron supplementation

INFANT LEUKEMIA STUDY
SECTION I

For these next questions, please turn to the blue page in your interview guide.
Thinking about your pregnancy with ____(INDEX CHILD)____________, did you take any of the following medications in the year before you were pregnant or during pregnancy.
This would be from about __(MO/YR)________ to about __(MO/YR)_________.

[REPEAT Q I1 – I4 FOR EACH VITAMIN, UP TO 2 TIMES]

I1. During any of that time, did you take vitamins? (Did you take any **other** vitamins?)

1 YES → I2. What type of vitamin was that?

____

____ [ ][ ]

I3. Were they prescribed by a doctor?

1 YES 5 NO 8 DK 9 REF

I4. Did you take them

a) In the year before your pregnancy?

1 YES 5 NO 8 DK 9 REF

b) In early pregnancy, before you knew you were pregnant?

1 YES 5 NO 8 DK 9 REF

c) From the time you found out you were pregnant until ____(INDEX CHILD)____ was born?

1 YES 5 NO 8 DK 9 REF

GO TO Q. I5, NEXT PAGE

5 NO

8 DK

9 REF

GO TO Q. I5, NEXT PAGE

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TIME PERIOD FROM __(MO/YR)
TO __(MO/YR)__________.

I5. During any of that time, did you take iron supplements, other than what's in a multivitamin?

1 YES →

I6. For what reason or condition were you taking them?

____

____ [ ][ ]

I7. Were they prescribed by a doctor?

1 YES 5 NO 8 DK 9 REF

I8. Did you take them

a) In the year before your pregnancy?

1 YES 5 NO 8 DK 9 REF

b) In early pregnancy, before you knew you were pregnant?

1 YES 5 NO 8 DK 9 REF

c) From the time you found out you were pregnant until ___(INDEX CHILD)___ was born?

1 YES 5 NO 8 DK 9 REF

GO TO Q. I9, NEXT PAGE

5 NO

8 DK

9 REF

GO TO Q. I9, NEXT PAGE

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Appendix J. Prevalence of atopic disease among children and adolescents

Exposure	Prevalence	Population/Source	Reference
Any atopic disease	31.5%	Controls ages 0-6, medical record review (U.S.)	43
	37.5%	Controls ages 0-11+ (U.S.)	37
	52.3%	NHANES III – ages 6-17	216
Allergic rhinitis (hay fever)	4.7%	NHIS 2006-2008 – ages 0-4	254
	10.4%	NHIS 2006-2008 – ages 5-9	254
	12.3%	NHIS 2006-2008 – ages 10-17	254
	7.5%	Controls ages 0-11+ (U.S.)	37
	10.8%	ISAAC, Phase III (Canada) – ages 6-7	218
	19.1%	ISAAC, Phase III (U.S.) – ages 13-14	218
Asthma	6.2%	NHIS 2006-2008 – ages 0-4	253
	10.6%	NHIS 2006-2008 – ages 5-9	253
	10.4%	NHIS 2006-2008 – ages 10-17	253
	10.9%	Controls ages 0-6, medical record review (U.S.)	216
	9.1%	NHANES III – ages 6-17	
	8.9%	Controls ages 0-11+ (U.S.)	37
	18.2%	ISAAC, Phase III (Canada) – ages 6-7	218
	22.3%	ISAAC, Phase III (U.S.) – ages 13-14	218
			43
Atopic dermatitis (eczema)	10.6%	Controls ages 0-6, medical record review (U.S.)	43
	7.3%	Controls ages 0-11+ (U.S.)	37
	12.0%	ISAAC, Phase III (Canada) – ages 6-7	218
	8.3%	ISAAC, Phase III (U.S.) – ages 13-14	218
Urticaria (hives)	8.4%	Controls ages 0-11+ (U.S.)	37

Appendix K. International experts surveyed to request any other relevant published or unpublished results

Investigator	Location	Institution	E-mail address	Responded
Judith R. Thompson	Australia	Cancer Foundation of Western Australia	judy.thompson@health.wa.gov.au	No
Elizabeth Milne	Australia	University of Western Australia	lizm@ichr.uwa.edu.au	Yes
Maria S. Pombo-De-Oliveira	Brazil	Instituto Nacional de Cancer Divisao de Medicina Experimental Rua Andre Cavalcanti	mpombo@inca.gov.br	No
Mary L. McBride	British Columbia, Canada	University of British Columbia	mmcbride@bccrc.ca	Yes
Claire Infante-Rivarde	Quebec, Canada	McGill University	claire.infante-rivard@mcgill.ca	Yes
Lisa Lyngsie Hjalgrim	Denmark	Statens Serum Institut	LIH@SSI.dk	No
Mads Melbye	Denmark	Statens Serum Institut	mme@ssi.dk	No
Tine Westergaard	Denmark	Statens Serum Institut	twe@ssi.dk	No
Henrik Hjalgrim	Denmark	Statens Serum Institut	hhj@ssi.dk	Yes
Joachim Schüz	Denmark	Danish Cancer Society	joachim@cancer.dk	Yes
Jacqueline Clavel	France	Institut national de la sante et de la recherche medicale (INSERM)/ Universite de Paris-Sud	clavel@vjf.inserm.fr	No

Investigator	Location	Institution	E-mail address	Responded
Florence Menegaux	France	Institut national de la sante et de la recherche medicale (INSERM)/ Universite de Paris-Sud	menegaux@vjf.inserm.fr	No
Peter Kaatsch	Germany	University of Mainz	kaatsch@imbei.uni-mainz.de	No
Eleni Petridou	Greece	Athens University Medical School/Harvard School of Public Health	epetrid@med.uoa.gr	Yes
Corrado Magnani	Italy	Childhood Cancer Registry of Piedmont (CPO Piemonte)	corrado.magnani@cpo.it	No
John Dockerty	New Zealand	University of Otago	john.dockerty@stonebow.otago.ac.nz	No
Estelle Naumburg	Sweden	Uppsala University	estelle.naumburg@kbh.uu.se	No
Sven Cnattingius	Sweden	Karolinska Institute	Sven.Cnattingius@ki.se	Yes
Eve Roman	United Kingdom	University of York	eve.roman@egu.york.ac.uk	No
Tracy Lightfoot	United Kingdom	University of York	tracy.lightfoot@egu.york.ac.uk	No
Mel Greaves	United Kingdom	Institute of Cancer Research	mel.greaves@icr.ac.uk	No
Leo Kinlen	United Kingdom	Oxford University	leo.kinlen@dphpc.ox.ac.uk	No
Jill Simpson	United Kingdom	University of York	jill.simpson@egu.york.ac.uk	No

Investigator	Location	Institution	E-mail address	Responded
Richard McNally	United Kingdom	Newcastle University	richard.mcnally@ncl.ac.uk	No
Patricia McKinney	United Kingdom	University of Leeds	p.a.mckinney@leeds.ac.uk	Yes
Leslie L. Robison	Tennessee, USA	St. Jude Research Hospital	les.robison@stjude.org	No
Patricia A. Buffler	California, USA	University of California, Berkeley	pab@berkeley.edu	Yes
Peggy Reynolds	California, USA	Northern California Cancer Center	preynolds@nccc.org	Yes
Xiaomei Ma	Connecticut, USA	Yale	xiaomei.ma@yale.edu	Yes
Martha Linet	Maryland, USA	National Cancer Institute	linetm@exchange.nih.gov	Yes
Frederica P. Perera	New York, USA	Columbia University	fpp1@columbia.edu	Yes
Paula Rosenbaum	New York, USA	State University of New York Upstate Medical University	rosenbap@upstate.edu	Yes
Andrew Olshan	North Carolina, USA	University of North Carolina, Chapel Hill	Andy_Olshan@UNC.edu	Yes
Greta R. Bunin	Pennsylvania, USA	Children's Hospital of Philadelphia	bunin@email.chop.edu	Yes
Xiao Ou Shu	Tennessee, USA	Vanderbilt University	xiao-ou.shu@vanderbilt.edu	Yes

Investigator	Location	Institution	E-mail address	Responded
Beth A. Mueller	Washington, USA	University of Washington	bmueller@fhcrc.org	Yes

Appendix L. Meta-analysis data abstraction form

The association between atopy and childhood leukemia: A meta-analysis

Data abstraction form - page 1

Study ID 440

Study Identification

Title **A survey of childhood malignancies.**

First Author **Stewart A** Country Year **1958**

Citation **Br Med J. 1958 Jun 28;1(5086):1495-508.**

Eligibility Criteria

- ☐ Outcome of leukemia
- ☐ Children ages 0-18 years
- ☐ Appropriate control group
- ☐ Exposure of atopy
- ☐ OR/data provided in paper

- ☐ Study is eligible

Notes/Comments ______________________________

Abstractor initials ____________ *Date* ____________ *Page 1 of*

The association between atopy and childhood leukemia: A meta-analysis

Data abstraction form - page 2

Study ID 440

Study Description

Study design ______________________________

Participant ages ______________________________

Diagnostic dates included ______________________________

Ncases ____________ RRcases ____________

Source of cases ______________________________

Ncontrols ____________ RRcontrols ____________

Source of controls ______________________________

Source of exposure data ______________________________

Source of outcome data ______________________________

Matching variables ______________________________

____

Statistical method ______________________________

Statistical method appropriate?
- ☐ Yes
- ☐ No

Exposure-disease associations to abstract ____________

Notes/Comments ______________________________

____

____

____

Abstractor initials ____________ *Date* ____________ *Page 2 of* ___

The association between atopy and childhood leukemia: A meta-analysis

Data abstraction form - page 3

Study ID [ ]

Results - Association # [ ]

Exposure variable (*Circle one*) **atopy** **allergies** **asthma** **eczema** **hay fever** **hives**

other: ____________________

Outcome variable (*Circle one*) **leukemia** **ALL** **cALL** **AML** **ANLL**

other: ____________________

Adjustment variables ____________________

	Cases	Controls	
Exposed			
Not Exposed			

OR = ________

95% CI: ________ , ________

SE = ________ p-value = ________

Results - Association # [ ]

Exposure variable (*Circle one*) **atopy** **allergies** **asthma** **eczema** **hay fever** **hives**

other: ____________________

Outcome variable (*Circle one*) **leukemia** **ALL** **cALL** **AML** **ANLL**

other: ____________________

Adjustment variables ____________________

	Cases	Controls	
Exposed			
Not Exposed			

OR = ________

95% CI: ________ , ________

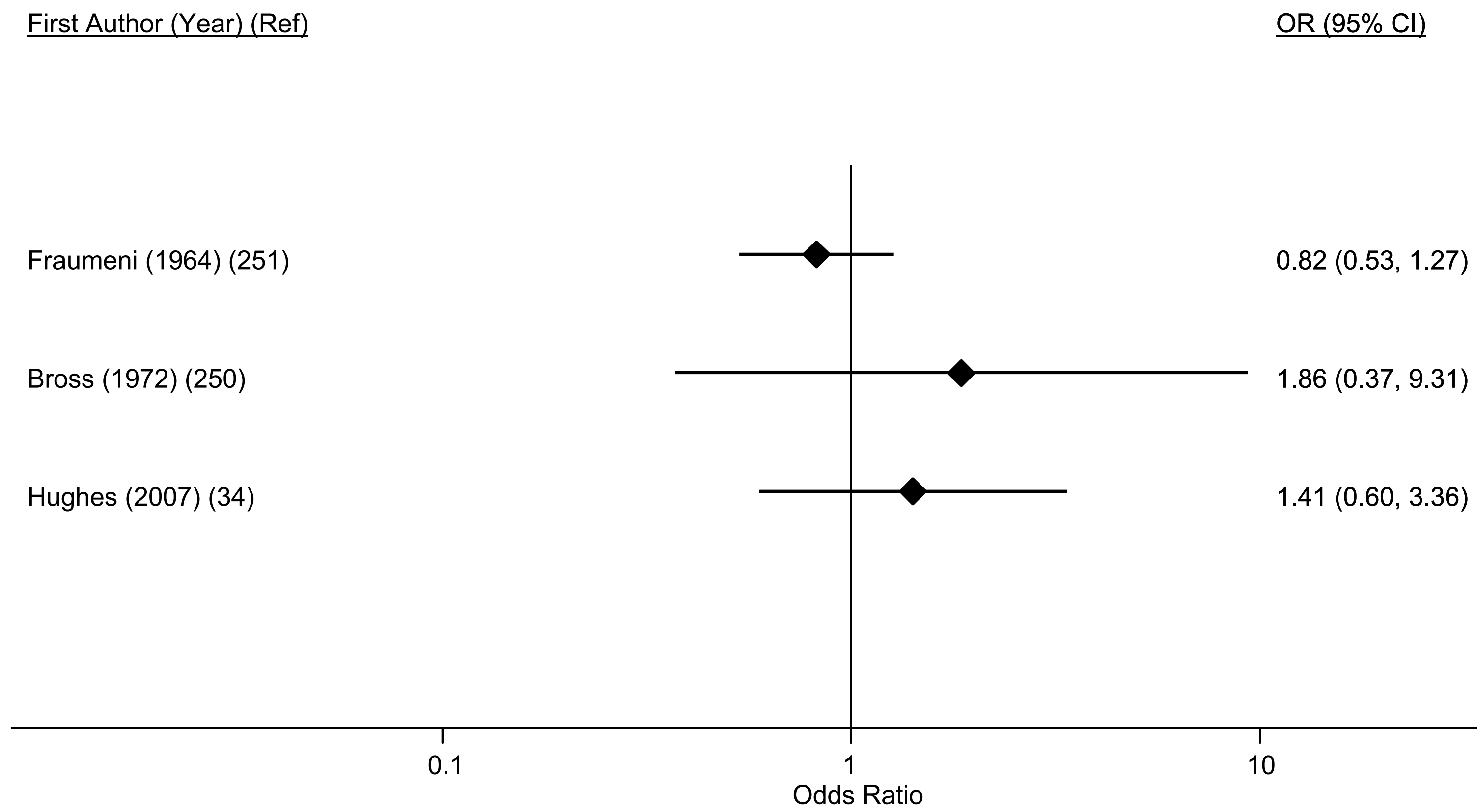
SE = ________ p-value = ________

Abstractor initials ____________ *Date* ____________ *Page* ___ *of* ___

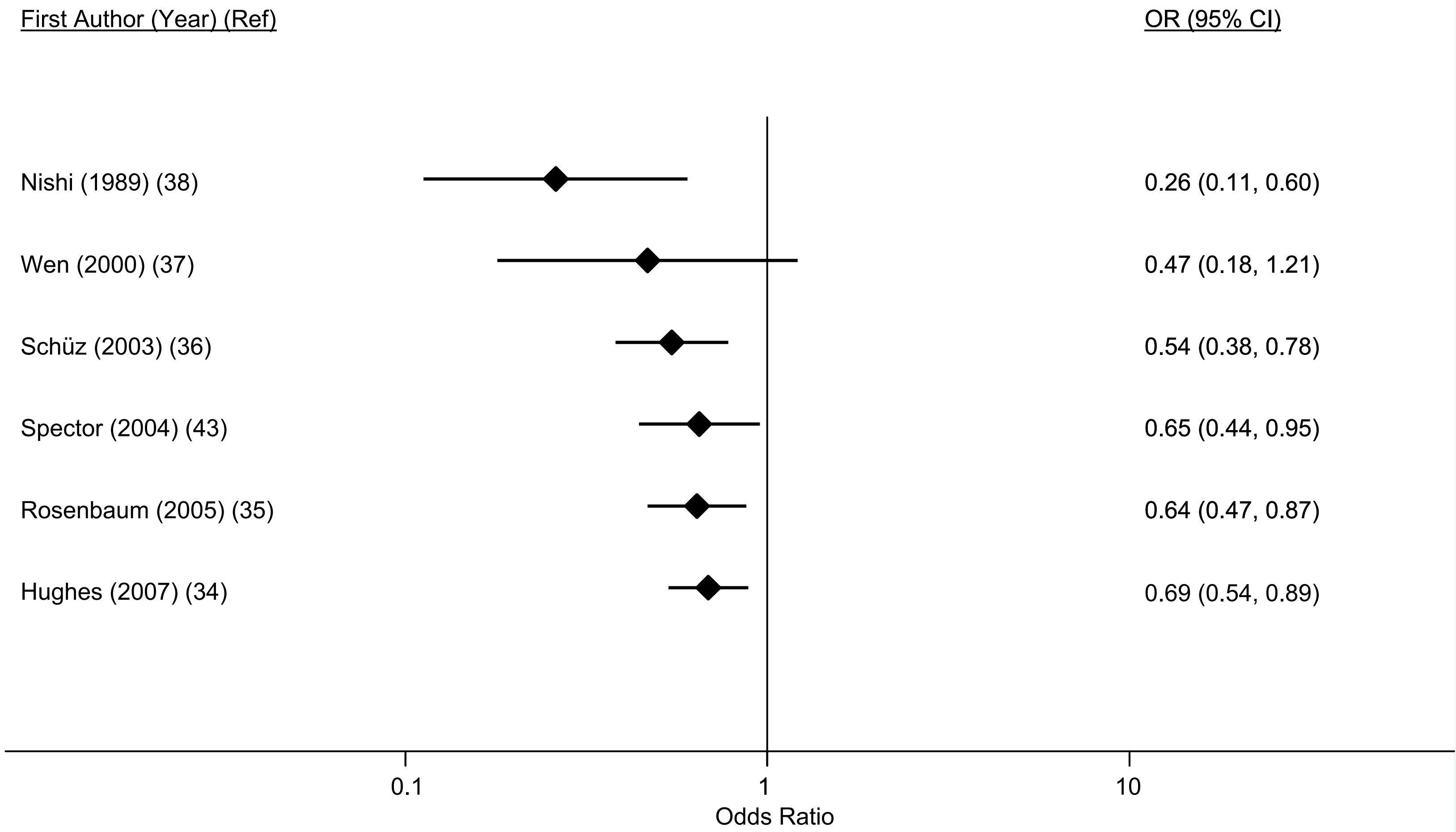
Appendix M. Results of cumulative meta-analysis for A) leukemia overall and atopy or allergies, B) ALL and atopy or allergies, C) ALL and asthma, D) ALL and eczema, E) AML and asthma.

*Results for cases ages 0-18 years obtained from first author (Karin Söderberg, Karolinska Institutet, personal communication, 2009).

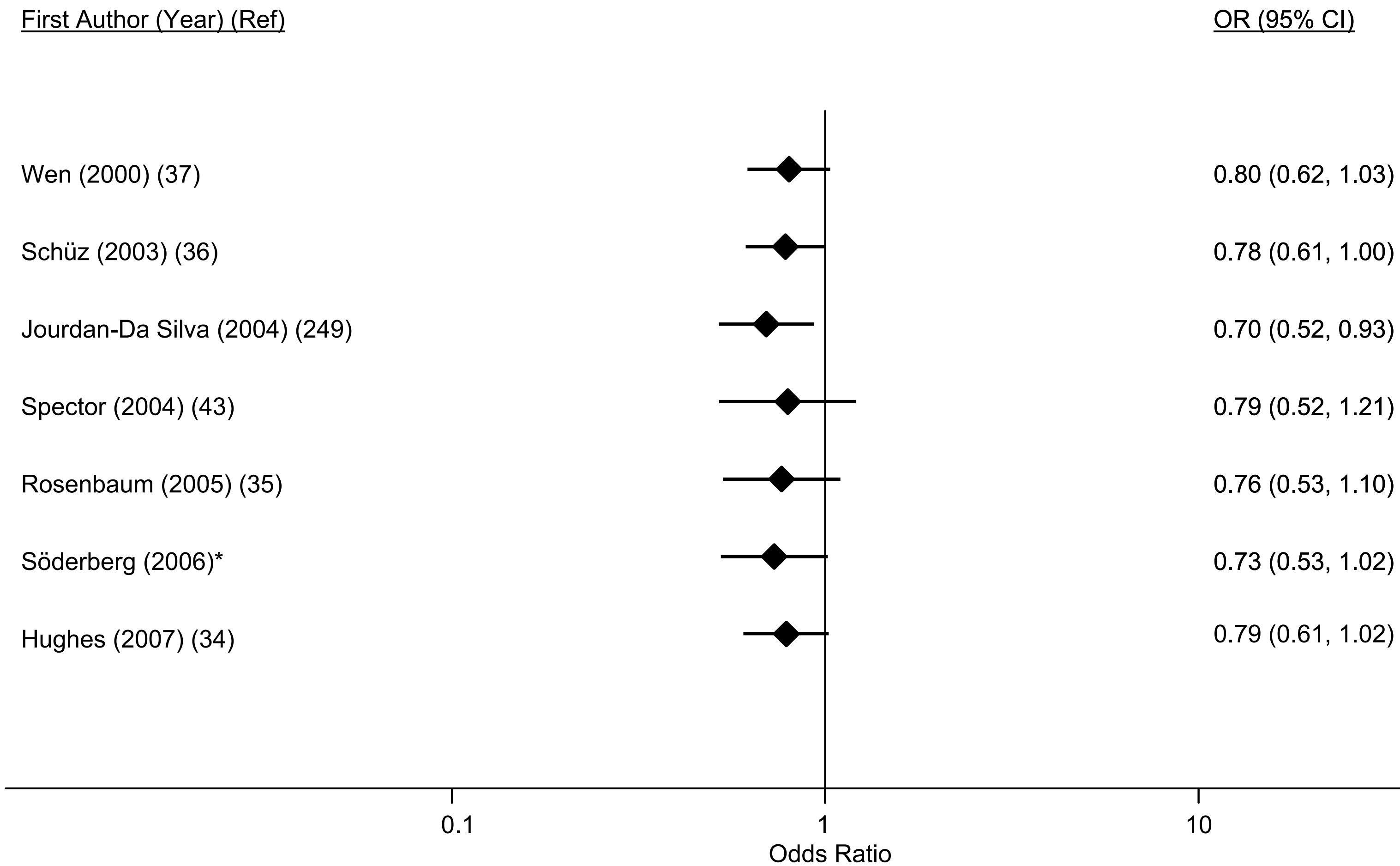
P-A)



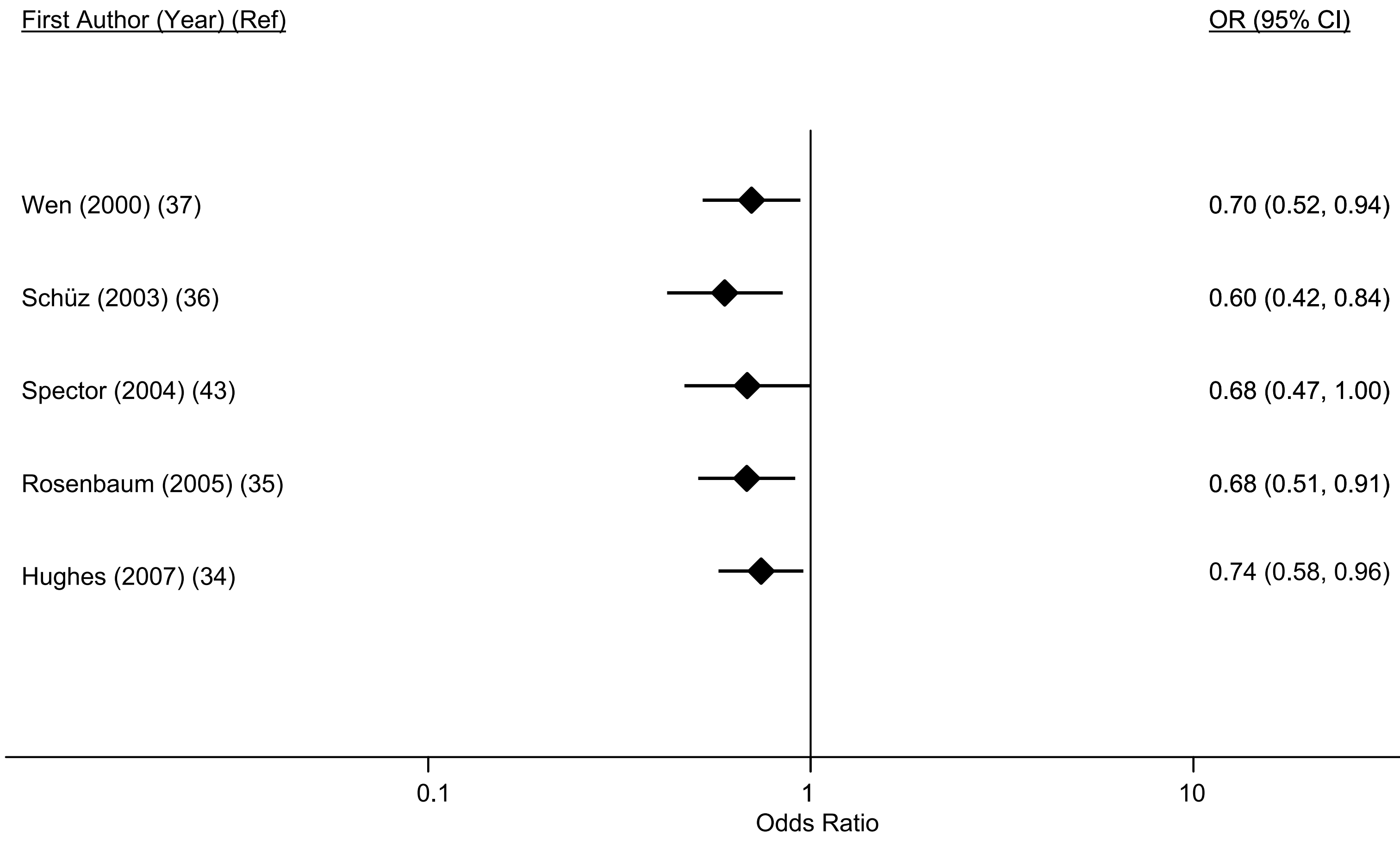
P-B)



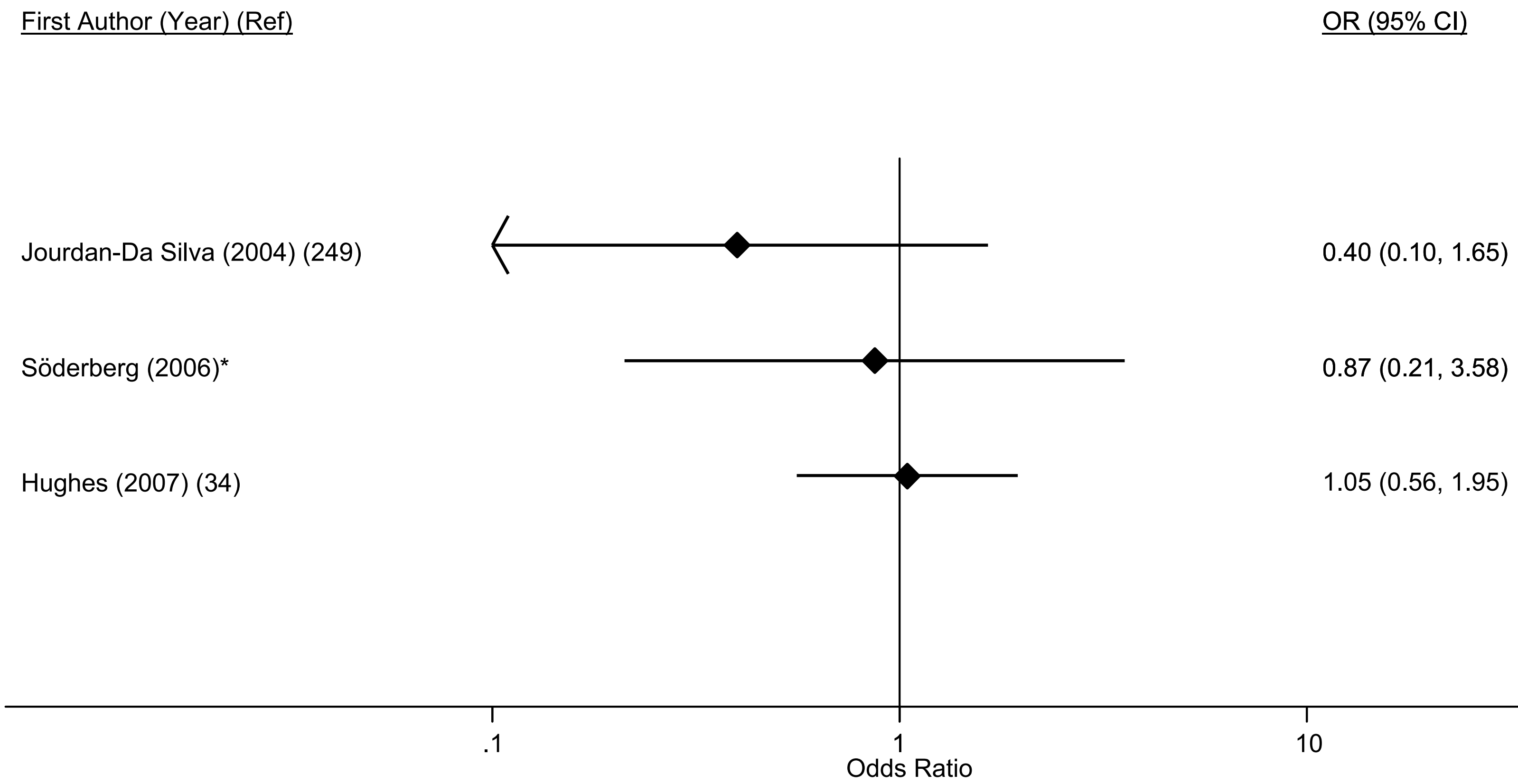
P-C)



P-D)

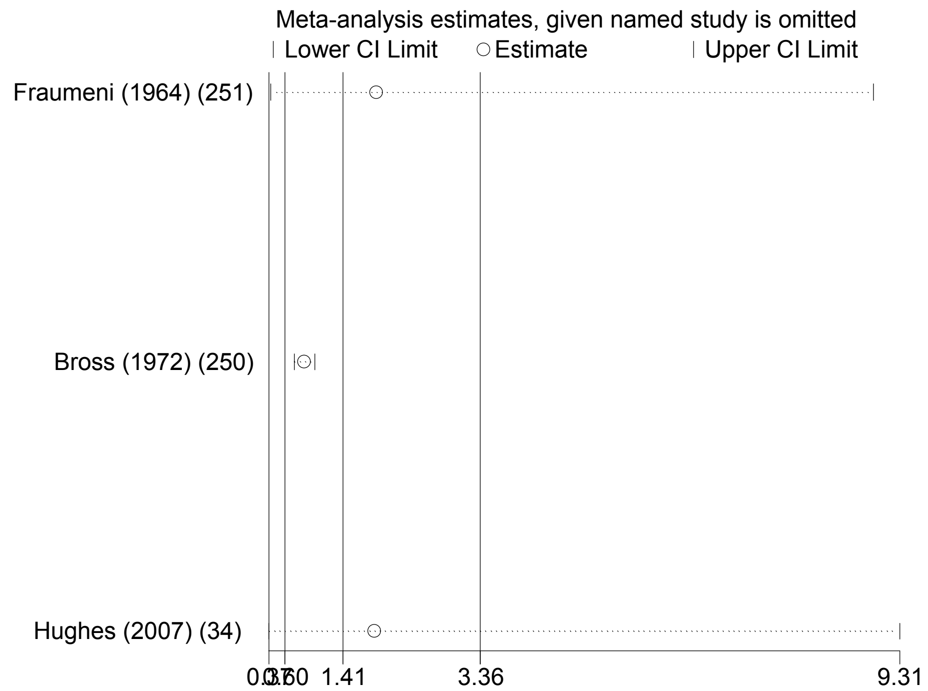


P-E)

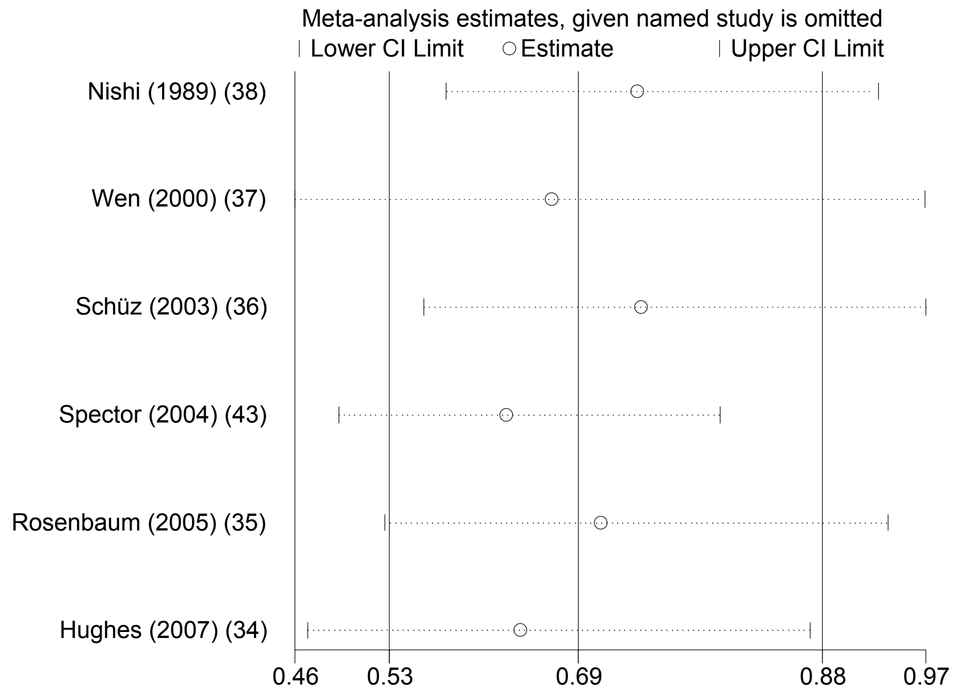


Appendix N. Results of influential analysis for A) leukemia overall and atopy or allergies, B) ALL and atopy or allergies, C) ALL and asthma, D) ALL and eczema, E) AML and atopy or allergies, F) AML and asthma.

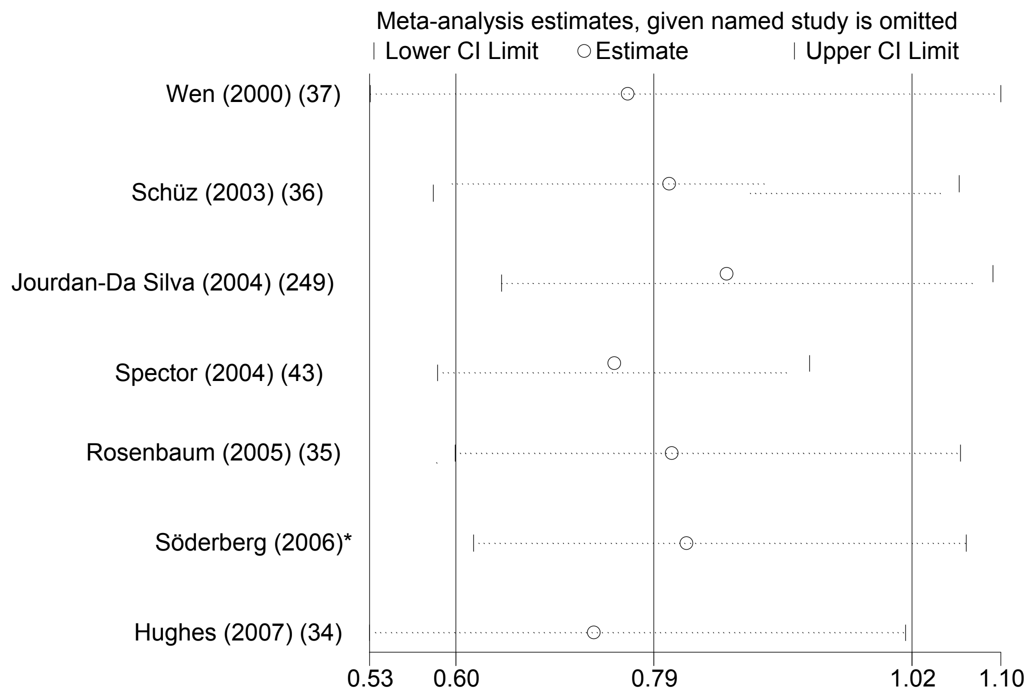
Q-A)



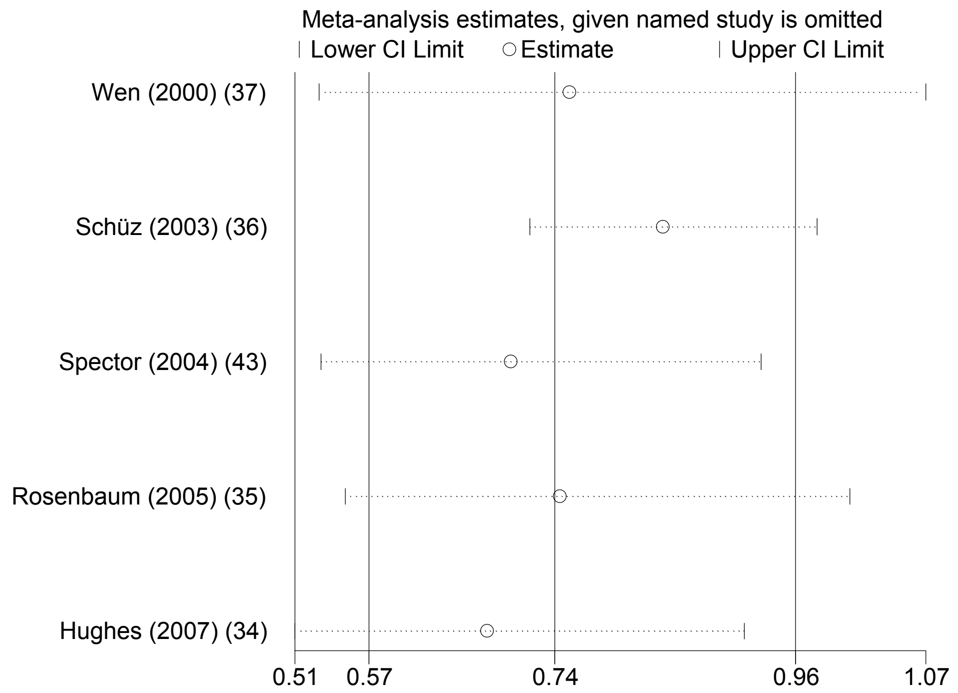
Q-B)



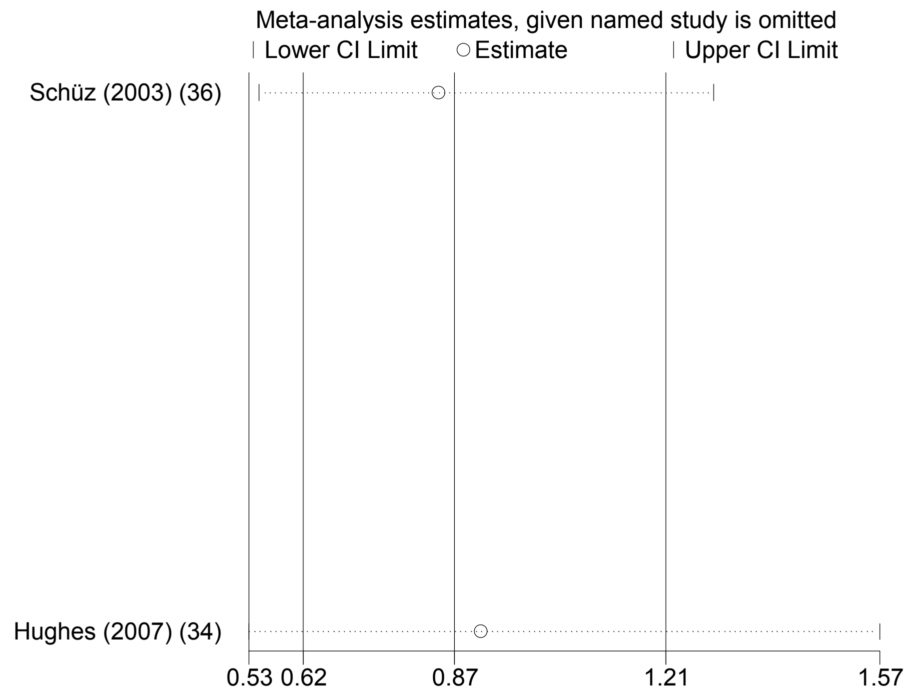
Q-C)



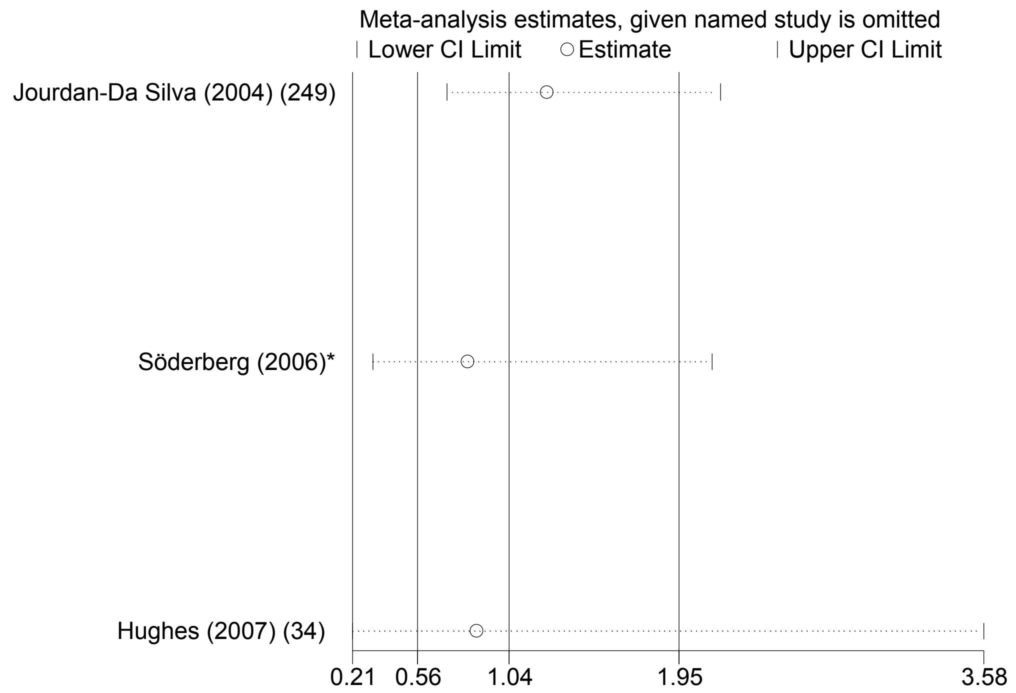
Q-D)



Q-E)



Q-F)



* Results for cases ages 0-18 years obtained from first author (Karin Söderberg, Karolinska Institutet, personal communication, 2009).