Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis

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ABSTRACT

To evaluate the contribution of candidate gene association studies to the understanding of genetic susceptibility to childhood acute lymphoblastic leukemia we conducted a systematic review and meta-analysis of published studies (January 1996-July 2009). Studies had to meet the following criteria: be case-control design, be studied by two or more studies, not be focused on HLA antigen genetic markers and be published in English. We identified 47 studies of polymorphic variation in 16 genes and acute lymphoblastic leukemia risk. To clarify the impact of individual polymorphisms on risk, pooled analyses were performed. Of the 25 polymorphic variants studied, significant associations (P<0.05) were seen in pooled analyses for eight variants: *GSTM1* (OR =1.16; 95%CI: 1.04-1.30), *MTRR* A66G (OR=0.73, 95%CI:0.59-0.91), *SHMT1* C1420T (OR=0.79, 95%CI: 0.65-0.98), *RFC1* G80A (OR=1.37, 95%CI: 1.11-1.69), *CYP1A1*2A* (OR=1.36, 95%CI:1.11-1.66), *CYP2E1*5B* (OR=1.99, 95%CI:1.32-3.00) *NQO1* C609T (OR=1.24, 95%CI:1.02-1.50) and *XRCC1* G28152A (OR=1.78, 95%CI:1.32-2.42). These findings should, however, be interpreted with caution as the estimated false-positive report probabilities (FPRP) for each association were not noteworthy (i.e. FPRP>0.2). While candidate gene analyses are complementary to genome-wide association studies, future analyses should be based on sample sizes commensurate with the detection of small effects and attention needs to be paid to study design.

Key words: polymorphisms, acute lymphoblastic leukemia, risk, meta-analysis, false positive report probabilities.

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Introduction

Acute leukemia is the major pediatric cancer in developed countries, affecting between 30-45 per 1,000,000 children each year.[1] Dysregulated immune response to infection may be a cause of childhood acute lymphoblastic leukemia (ALL)[2] and epidemiological data are consistent with transplacental carcinogen exposure as a basis for infant leukemia associated with MLL gene fusion,[3] but the role of environmental carcinogenesis in ALL is currently undefined. It is, however, likely that the risk of ALL from environmental exposure is influenced by co-inheritance of multiple low-risk variants.

The commonest method for identifying common low-risk variants is through association studies. These are based on comparing the frequency of polymorphic genotypes in cases and controls. Alleles positively associated with the disease are analogous to risk factors in epidemiology and may be causally related to disease risk or in linkage disequilibrium with disease-causing variants. There are a number of different methods of analyzing the risk associated with a specific variant. For simple bi-allelic polymorphisms, the odds ratio of disease can be derived by comparing allele frequencies in cases and controls. This approach is, however, less powerful than a comparison of frequencies of the three genotypes among cases and controls using homozygosity of the "wild-type allele" as the reference group. Where homozygotes are rare, it is common to combine the heterozygotes and homozygotes together; but this is only appropriate if a dominant model can be presumed. Similarly, combining heterozygotes with wild-type homozygotes is only appropriate if alleles act recessively.

Excluding HLA, the genetic candidates that have been evaluated as susceptibility genes for childhood ALL to date can be broadly delineated into those coding for carcinogen metabolism enzymes, folate metabolism enzymes, DNA repair proteins, and others. Table 1 details the candidate gene polymorphisms that have been reviewed. For clarity the position and standard nomenclature of each polymorphism is provided.

Carcinogen metabolism genes

Children may be particularly vulnerable to environmental toxins because of their greater relative exposure, immature metabolism and higher rate of cell division and growth.[4] In

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this context, functional polymorphisms in xenobiotic metabolizing enzymes have been postulated to be of relevance in determining susceptibility to ALL.[4]

The first and obligatory step in the activation of arylamines is N-hydroxylation by phase-I enzymes that include cytochrome P4501A1 (*CYP1A1*; MIM 108330).[5,6] The glutathione S-transferases are a family of phase-II enzymes responsible for the detoxification of mutagenic electrophiles including polyaromatic hydrocarbons (PAHs). Homozygotes for null alleles (deletion) of *GSTM1* (MIM 138350) and *GSTT1* (MIM 600436) have absent activity of the respective enzyme. DNA-adduct formation and rates of somatic mutation have been reported to be increased in carriers of null alleles.[7]

Other metabolic gene variants that have been investigated as risk factors for ALL include polymorphisms in *NQO1* (NADPH:quinone oxidoreductase; MIM 125860) a cytosolic enzyme catalyzing reduction of quinones and prevention of their participation in redox cycling and thus in oxidative stress.[8] Variation in *MDR1* (MIM 171050) which encodes the P-glycoprotein (P-gp) has also been studied as a possible risk factor for childhood ALL on the basis that it provides a cellular defense against toxic xenobiotic compounds.[9]

Folate metabolism genes

Leukemia commonly arises as a result of DNA translocation, inversions or deletions in genes regulating lymphocyte development. The formation of translocations in leukemia are thought to involve DNA double-strand break formation by means other than aberrant V(D)J recombinase activity.[10] Folate deficiency has been associated with uracil misincorporation into DNA and double strand breaks during uracil excision repair thus potentially increasing the risk of chromosomal aberration.[11] Pregnancy is a time of extreme folate requirement and supplementation in pregnancy has been reported to afford protection against childhood ALL.[12] Dysfunctional folate metabolism is, therefore, an attractive candidate in the etiology of ALL as the most common translocations in infant leukemia (MLL-AF4) and in common childhood ALL (TEL-AML1) occur *in utero* in most, if not all, patients.[13] Central to folate metabolism are the enzymes 5,10-methylenetetrahydrofolate reductase (*MTHFR*; MIM 607093), methionine synthase (*MTR* alias *MS*; MIM 156570) and methionine synthase reductase (*MTRR*; MIM 602568). These genes are characterized by functional polymorphisms and it has been postulated that these variants may impact on ALL risk through affecting folate metabolism. A similar rationale for evaluating vari-

Table 1. Polymorphism studies as risk factors for childhood acute lymphoblastic leukemia.

Class/Gene	Polymorphism	Change	Effect	Method of detection
Carcinogen metabolism				
CYP1A1	CYP1A1*2A,T6235C (rs4646903) CYP1A1*2B/*2C, A4889G (rs1048943)	None I462V	Increased enzymatic activity Increased activity?	Creates *Msp*I site *BsrD*I RFLP
CYP2D6	CYP2D6*4, G1934A (rs3892097) CYP2D6*3, del2637 (rs35742686)	None Frameshift	Abnormal splicing Premature termination	*BstN*I RFLP *Hpa*II RFLP
CYP2E1	CYP2E1*5B, G-1293C/C-1053T, (rs3813867/rs2031920)	None	Altered expression *in vitro*?	*Pst*I/*Rsa*I RFLP
GSTT1	Deletion	None	Absent activity	Allele specific PCR
GSTM1	Deletion	None	Absent activity	Allele specific PCR
GSTP1	A1578G (rs1695) C2293T (rs1138272)	I105V A114V	Altered activity Altered activity	Fluorescent allele specific PCR Fluorescent allele specific PCR
NQO1	C609T (rs1800566) C465T (rs1131341)	P187S R139W	Abolishes activity Alters mRNA splice site	Allele specific oligonucleotide (ASO) *Hpa*II RFLP
MDR1	C3435T (rs1045642) G2677T/A (rs2032582)	Silent A893S/T	Lower expression? Altered activity?	PCR, *Mbo*I RFLP Single base extension method
Folic acid pathway				
MTHFR	A1298C (rs1801131) C667T (rs1801133)	Q429A A222V	Altered activity? Decreased activity	Abolishes *Mbo*II RE site Creates *Hinf*I RE site
MTRR	A66G (rs1801394)	I22M	Altered activity?	Real time PCR/HinfI digestion.
MTR	A2756G (rs1805087)	D919G	Reduced activity with GG.	PCR, *Hae*III RFLP
SHMT	C1420T (rs1979277)	L474F	Reduced red blood cell folate levels.	PCR, RFLP
RFC1	G80A (rs1051266)	R27H	Unknown	PCR, RFLP
TS	Tandem repeat polymorphism in the 5-prime untranslated region of TYMS. 2R>3R	None	Triple-repeat allele results in higher TYMS expression	PCR
DNA repair pathway				
ERCC2	A35931C (rs13181) G23591A (rs1799793)	K751G D312N	Low DNA repair capacity? Low DNA repair capacity?	multiplex PCR-RFLP, *Pst*I multiplex PCR-RFLP, *Sty*I
XRCC1	C26304T (rs1799782) G27466A (rs25489) G28152A (rs25487)	R194W R280H R399Q	Defective polymerase β Defective polymerase β Defective polymerase β	multiplex PCR-RFLP, *Pvu*II multiplex PCR-RFLP, *Rsa*II multiplex PCR-RFLP, *Msp*I

RFLP: restriction fragment length polymorphism; RE: restriction enzyme.

ants of serine hydroxymethyltransferase (*SHMT*; MIM 182144), thymidylate synthetase (*TS*; MIM 188350) and reduced folate carrier 1 (*RFC1*, alias *SLC19A1*; MIM 600424) as risk factors for ALL has also been advanced.

DNA repair genes

XRCC1 (X-Ray repair-cross complementing group 1; MIM 194360) is believed to play a role in DNA single strand repair by forming protein complexes with DNA repair associated proteins.[14] Polymorphic variants in *XRCC1* may, therefore, play a role in ALL through aberrant DNA repair. Polymorphic variants in other DNA repair genes, including *ERCC2* (excision repair-complementing group 2; MIM 126340), mutations which cause xeroderma pigmentosum (MIM 278700), trichothiodystrophy (MIM 601675) and Cockayne syndrome (MIM 216400)[15] have also been evaluated as risk factors for ALL for similar reasons.

Although some polymorphic variants have only been examined once, most have been evaluated as risk factors in several studies but often with discordant findings. Furthermore, many of the studies have been based on small sample sizes with limited power to robustly demonstrate a relationship with ALL risk. To gain better insight into the impact of polymorphic variants on risk of childhood ALL, we have undertaken a systematic review of published studies and used standard meta-analysis techniques according to Cochrane[16] and PRISMA[17] guidelines to derive a more precise estimate of the individual variants. The implications of findings for future research on predisposition to ALL are discussed.

Design and Methods

Study identification

A literature search for studies reporting on the association between polymorphic variants and risk of childhood ALL was conducted using the electronic database PubMed from January 1996 up to the end of June 2009 (*www.ncbi.nml.nih.gov/pubmed*). The search strategy used the key words "acute lymphoblastic leukemia, genetic, association, case, control, polymorphism, risk". We searched for any additional studies in the bibliographies of identified publications, including previous review articles and meta-analyses.[18-22]

Selection criteria

Studies were eligible if they were based on unrelated individuals and examined the association between childhood ALL and polymorphic genotype. Only studies published as full-length articles or letters in peer-reviewed journals in English were included in the analysis.

Data extraction

Data for analyses, including study design, sample size, ethnicity, as well as allele and genotype frequencies, were extracted from the published articles and summarized in a consistent manner to aid comparison. When a study reported results on different subpopulations according to ethnicity, we considered each sub-population as a separate study in the meta-analyses.

Statistical analysis

Raw data of genotype frequencies, without adjustment, were used for calculation of the study-specific estimates of odds ratio (OR) and 95% confidence interval (CI). Cochran's Q statistic was used to test for heterogeneity, and the percentage variability of the pooled OR attributable to heterogeneity between studies was quantified using the I^2 statistic; large heterogeneity is typically defined as $I^2 \geq 75\%$. A *P* value of >0.05 for the Q test was considered to indicate a lack of heterogeneity across studies and for these analyses the pooled estimation of ORs of each study was calculated by the fixed effects model (Mantel-Haenszel methods).[23] For results showing high heterogeneity, the random effects model (DerSimonian and Laird's method)[24] was used. The significance of the pooled OR was determined by the z-test and $P<0.05$ was considered as statistically significant. An estimate of the potential publication bias was conducted by examination of funnel plots. An asymmetric plot is reflective of publication bias. The funnel plot symmetry was assessed by Egger's test[25] based on inverse-variance weighted regression of the standardized effect sizes (OR/standard error (SE) of OR) on their precision (1/SE) to test whether the intercept deviates significantly from zero; $P<0.05$ was considered indicative of statistically significant publication bias.

To test for population stratification, the distribution of genotypes in control subjects of each individual population was tested for departure from Hardy-Weinberg equilibrium [HWE] by means of the χ^2 test.[26]

For each statistically significant association identified, we estimated the false positive reporting probability (FPRP).[27] The FPRP value is determined by the *P* value, the prior probability for the association, and statistical power. We calculated FPRP assuming a prior of 0.001 as previously proposed for candidate gene analyses.[28] Statistical power was based on the ability to detect an OR of 1.2 and 1.5 (or reciprocal), with α equal to the observed *P* value. To evaluate whether the association was noteworthy, we imposed an FPRP cut-off value of 0.2, advocated for summary analyses.[27] Hence, FPRP values less than 0.2 were considered to indicate robust associations.

All statistical analyses were undertaken using STATA version 10.0 (Stata Corporation, College Station, TX, USA).

Results

Characteristics of published studies

We retrieved 267 published studies using our search criteria (Figure 1). Forty-seven of these studies met our predetermined criteria for inclusion. These 47 publications detailed 25 polymorphisms in 16 genes (Table 1). All the studies were essentially of similar design, although different types of controls have been analyzed (*Online Supplementary Table S1*). In the final group, three studies[29-31] sub-grouped cases and controls according to ethnicity and seven studies[32-38] grouped subjects according to ethnicity but did not present data on genotype and risk for the different subgroups. We did not identify any overlapping studies. The ORs of ALL associated with each polymorphism in individual studies are detailed in the *Online Supplementary Table S2*.

Meta-analysis

Pooling data from the 17 studies[29,33,38-51] which have examined *MTHFR* C677T provided no evidence for a relationship between genotype and risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). There was evidence of population stratification in the studies reported by Wiemels *et al.*[38] and Balta *et al.*,[40] as controls showed evidence of departure from HWE (P=0.02, 0.05). The pooled ORs were, however, unchanged excluding these studies from the meta-analy-

sis. *MTHFR* A1298C has been evaluated in 14 studies.[29,33,38,39,42,44-51] Pooled analysis provided no evidence for a relationship between A1298C and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). Similarly pooling data from the four studies of the *MTR* A2756G polymorphism[42,44,47,52] provided no support for a relationship between A2756G genotype and risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

The *MTRR* A66G polymorphism had been evaluated in three studies (*Online Supplementary Figures S1 and S2*; *Online Supplementary Table S2*).[42,47,52] Pooling data from the three studies (Table 2) there was a significant association between A66G genotype and decreased risk; ORs for heterozygosity 0.76 (95% CI: 0.60-0.96, *P*=0.02; *P* heterogeneity (P_{het}) =0.85, I^2=0%), homozygosity 0.67 (95% CI: 0.52-0.88, *P*=0.003; P_{het}=0.84, I^2=0%) and carrier status 0.73 (95% CI: 0.59-0.91, *P*=0.005; P_{het}=0.82, I^2=0%). Genotype frequency of controls in the study reported by Gast *et al.*,[52] however, showed deviation from HWE (*P*=0.05) and excluding this study provided no evidence for an association between *MTRR* A66G and ALL risk (*data not shown*).

SHMT1 C1420T has been studied as a risk factor for ALL in two studies.[42,52] Pooling data from both studies provided evidence for an association between the heterozygosity and risk (OR=0.79, 95% CI: 0.65-0.98, *P*=0.028; P_{het}=0.46, I^2=0%). There was, however, no evidence for an association with homozygosity or carrier status (Table 2; *Online Supplementary Figures S1 and S2*; *Online Supplementary Table S2*).

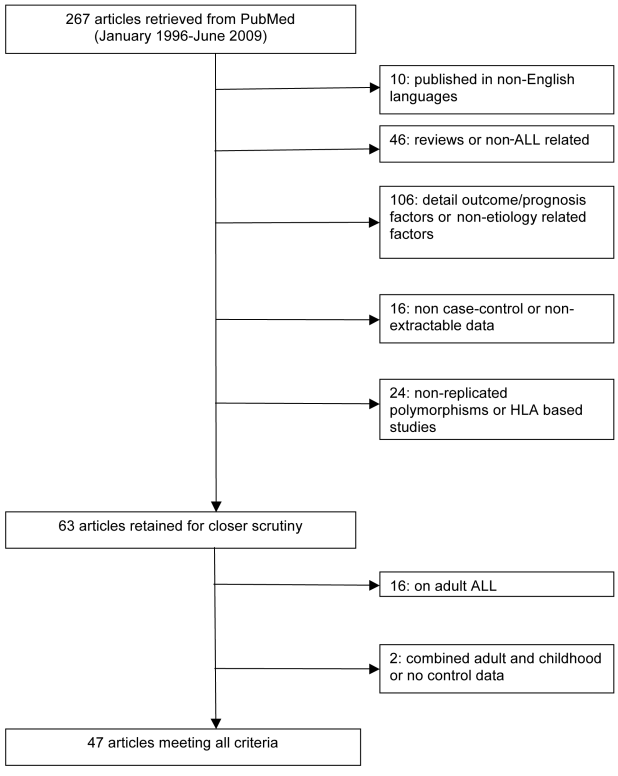

Figure 1. Inclusion and exclusion criteria for studies.

RFC1 G80A has also been evaluated in two studies (*Online Supplementary Figure S2*; Table 2; *Online Supplementary Table S2*).[42,52] One study showed heterozygosity, homozygosity and carrier status to be associated with an increased ALL risk (Table 2; *Online Supplementary Table S2*). Pooling all studies, ORs were: 1.37 (95% CI: 1.10-1.72, *P*=0.005; P_{het}=0.87, I^2=0%), 1.36 (95% CI: 1.02-1.81, *P*=0.04; P_{het}=0.02, I2=81%) and 1.37 (95% CI: 1.11-1.69, *P*=0.003; P_{het}=0.49, I^2=0%), respectively (*Online Supplementary Figures S1 and S2*). Evidence for an association between homozygosity and risk was not apparent under a random effects model (OR=1.44, 95% CI: 0.74-2.8).

The four studies[42,43,47,52] of the TS 2R>3R provide no evidence for a relationship between this polymorphism and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Seven studies[35,36,53-57] have evaluated *NQO1* C609T as a risk factor for ALL (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S1*). The study by Lanciotti *et al.*[53] only provided data on 609T carrier status. One study[57] showed a significant relationship between heterozygosity and risk while two studies showed a significant association for carrier status[36,57] (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). Pooling data from all seven studies, however, provided no evidence of a relationship between *NQO1* C609T genotype and risk (*Online Supplementary Figure S1*). Controls in the study by Sirma *et al.*[56] showed evidence of population stratification (*P*=0.01). In pooling data from the six other studies (Table 2; *Online Supplementary Figure S2*), there was evidence for a relationship between carrier status and risk (1.24, 95% CI: 1.02-1.50, *P*=0.03; P_{het}=0.49, I2=0%) but no relationship with either hetero- or homozygosity (*data not shown*).

Two studies[54,57] have examined the relationship between NQO1 C465T and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*); pooled analyses provided no evidence for a relationship between genotype and risk (*Online Supplementary Figure S1*).

Fifteen studies[30-32,35,37,40,58-64] have examined the relationship between *GSTM1* and *GSTT1* and ALL risk (Table 2; *Online Supplementary Figures S1 and S2*; *Online Supplementary Table S2*). Five of the studies reported a statistically significant increased risk associated with *GSTM1* deletion. The pooled OR for *GSTM1* was significant (*Online Supplementary Figure S2*), under both fixed (OR=1.16, 95%CI: 1.04-1.30, *P*=0.008; P_{het}<0.01, I^2=13%) and random effects models (OR=1.26, 95% CI: 1.05-1.51). There was, however, evidence of publication bias (*P*<0.01), with four of the smallest studies showing a significant association but with no effect being observed in the larger studies (*Online Supplementary Figure S3*). Across all studies no evidence for a relationship between *GSTT1* null genotype and ALL risk was shown (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Seven studies[32,35,37,40,53,65,66] have examined *GSTP1* A1578G and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). Pooling data from the seven studies provides no evidence of a relationship between genotype and risk (*Online Supplementary Figure S1*; Table 2). Similarly, pooling of data from the two studies which have examined *GSTP1* C2293T as a risk factor provides no support for an association between this variant and ALL (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Table 2. Pooled odds ratios (ORs) of all the polymorphisms analyzed together with the false positive report probabilities (FPRP).

Gene-variant	Studies n	Cases n	Controls n	OR(95% CI)	P	Phet	I2	PowerFPRP OR=1.2	PowerFPRP OR=1.5	@prior probability of 0.001 OR=1.2	@prior probability of 0.001 OR=1.5
MTHFR C677T	17	2770	4713								
TC *vs*. CC				0.97 (0.87-1.07)	0.54	<0.01	55%	95%	100%	-	-
TT *vs*. CC				0.87 (0.73-1.03)	0.10	0.25	18%	62%	100%	-	-
Carrier				0.95 (0.86-1.05)	0.32	<0.01	59%	96%	100%	-	-
MTHFR A1298C	14	2496	4403								
CA *vs*. AA				1.07 (0.96-1.20)	0.25	<0.01	66%	93%	100%	-	-
CC *vs*. AA				1.05 (0.88-1.25)	0.58	<0.01	58%	57%	100%	-	-
Carrier				1.07 (0.96-1.18)	0.18	<0.01	70%	95%	100%	-	-
MTR A2756G	4	846	1570								
GA *vs*. AA				1.15 (0.96-1.39)	0.15	0.14	5%	49%	99%	-	-
GG *vs*. AA				0.85 (0.52-1.37)	0.50	0.29	20%	8%	29%	-	-
Carrier				1.12 (0.93-1.34)	0.22	0.09	55%	51%	99%	-	-
MTRR A66G	3	766	1306								
GA *vs*. AA				0.76 (0.60-0.96)	0.02	0.85	0%	23%	95%	0.99	0.96
GG *vs*. AA				0.67 (0.52-0.88)	<0.01	0.84	0%	19%	87%	0.98	0.89
Carrier				0.73 (0.59-0.91)	<0.01	0.82	0%	25%	96%	0.97	0.87
SHMT1 C1420T	2	697	1038								
TC *vs*. CC				0.79 (0.65-0.98)	0.03	0.46	0%	40%	97%	0.99	0.97
TT *vs*. CC				1.16 (0.83-1.61)	0.37	0.34	0%	17%	69%	-	-
Carrier				0.86 (0.71-1.04)	0.12	0.37	0%	32%	99%	-	-
RFC1 G80A	2	696	1037								
AG *vs*. GG				1.37 (1.10-1.72)	<0.01	0.87	0%	26%	96%	0.98	0.89
AA *vs*. GG				1.36 (1.02-1.81)	0.03	0.02	81%	22%	78%	0.99	0.98
Carrier				1.37 (1.11-1.69)	<0.01	0.49	0%	30%	98%	0.96	0.80
TS 2R>3R	4	840	1328								
2R/3R *vs*. 2R/2R				1.01 (0.80-1.27)	0.93	0.92	0%	25%	96%	-	-
3R/3R *vs*. 2R/2R				1.23 (0.95-1.60)	0.12	0.59	0%	21%	91%	-	-
Carrier				1.08 (0.87-1.35)	0.50	0.79	0%	30%	98%	-	-
NQO1 C677T	7	1019	4476								
TC *vs*. CC				1.09 (0.90-1.32)	0.38	0.07	50%	54%	100%	-	-
TT *vs*. CC				1.35 (0.86-2.12)	0.19	0.70	0%	44%	98%	-	-
Carrier				1.11 (0.93-1.32)	0.23	0.11	42%	56%	100%	-	-
Carrier#				1.24 (1.02-1.50)	0.03	0.49	0%	26%	97%	0.98	0.97
NQO1 C465T	2	244	508								
TC *vs*. CC				1.63 (0.98-2.73)	0.06	0.27	18%	7%	28%	-	-
Carrier				1.59 (0.95-2.65)	0.08	0.25	26%	7%	28%	-	-
GSTM1	15	2262	3298								
Carrier				1.16 (1.04-1.30)	0.01	0.20	13%	-	-	0.93	0.91
GSTT1	15	2311	3429								
Carrier				0.96 (0.83-1.11)	0.58	<0.01	41%	-	-	-	-
GSTP1 A1578G	7	1224	1585								
GA *vs*. AA				1.08 (0.92-1.27)	0.35	0.90	0%	61%	100%	-	-
GG *vs*. AA				1.07 (0.81-1.43)	0.64	0.94	0%	24%	85%	-	-
Carrier				1.08 (0.93-1.26)	0.33	0.95	0%	65%	100%	-	-
GSTP1 C2293T	2	361	590								
TC *vs*. CC				0.77 (0.50-1.19)	0.24	0.54	0%	14%	57%	-	-
Carrier				0.73 (0.48-1.13)	0.16	0.49	0%	15%	59%	-	-
CYP1A1 T6235C	7	917	1190								
CT *vs*. TT				1.38 (1.12-1.70)	<0.01	0.06	51%	42%	98%	0.96	0.76
CC *vs*. TT				1.10 (0.67-1.79)	0.7	0.03	58%	12%	50%	-	-
Carrier				1.36 (1.11-1.66)	<0.01	0.04	54%	46%	99%	0.95	0.75
CYP1A1 A4889G	3	393	576								
GA *vs*. AA				1.13 (0.80-1.59)	0.48	0.05	68%	18%	71%	-	-
GG *vs*. AA				1.96 (0.92-4.19)	0.08	0.18	42%	5%	14%	-	-
Carrier				1.22 (0.88-1.70)	0.24	0.02	75%	20%	74%	-	-
CYP2D6 G1934A	3	383	530								
AG *vs*. GG				1.01 (0.74-1.38)	0.95	0.23	32%	20%	76%	-	-
AA *vs*. GG				1.81 (0.78-4.21)	0.16	0.69	0%	4%	13%	-	-
Carrier				1.07 (0.79-1.44)	0.65	0.19	39%	22%	79%	-	-

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Table 2. Pooled odds ratios (ORs) of all the polymorphisms analyzed together with the false positive report probabilities (FPRP). *(Continued from the previous page)*

Gene-variant	Studies n	Cases n	Controls n	OR(95% CI)	*P*	Phet	I2	PowerFPRP @prior probability of 0.001 OR=1.2	PowerFPRP @prior probability of 0.001 OR=1.5	FPRP @prior probability of 0.001 OR=1.2	FPRP @prior probability of 0.001 OR=1.5
CYP2D6 del 2637	3	322	582								
Heterozygous				0.64 (0.28-1.49)	0.30	0.63	0%	6%	21%	-	-
Carrier				0.86 (0.41-1.81)	0.70	0.33	0%	7%	26%	-	-
CYP2E1 5B	4	574	870								
Heterozygous				2.03 (1.34-3.06)	<0.01	0.28	22%	10%	40%	0.99	0.90
Carrier				1.99 (1.32-3.00)	<0.01	0.24	29%	10%	41%	0.99	0.92
MDR1 C3435T	5	1010	940								
CT *vs.* TT				1.04 (0.84-1.30)	0.73	0.70	0%	40%	97%	-	-
CC *vs.* TT				1.26 (0.98-1.62)	0.07	0.04	60%	29%	89%	-	-
Carrier				1.12 (0.92-1.38)	0.29	0.51	0%	32%	99%	-	-
MDR1 G2677T/A	2	655	550								
Heterozygous				1.04 (0.78-1.37)	0.78	0.68	0%	18%	86%	-	-
Homozygous				0.98 (0.70-1.36)	0.90	0.31	0%	18%	67%	-	-
Carrier				1.01 (0.78-1.32)	0.94	0.88	0%	20%	91%	-	-
XRCC1 C26304T	3	295	509								
CT *vs.* TT				1.08 (0.78-1.50)	0.64	0.04	68%	20%	74%	-	-
CC *vs.* TT				0.86 (0.41-1.81)	0.69	0.03	73%	7%	24%	-	-
Carrier				1.08 (0.79-1.48)	0.63	<0.01	80%	21%	77%	-	-
XRCC1 G28152A	3	295	509								
AG *vs.* GG				1.78 (1.30-2.46)	<0.01	0.20	37%	19%	72%	0.98	0.76
AA *vs.* GG				1.60 (0.95-2.70)	0.07	0.10	56%	8%	29%	-	-
Carrier				1.78 (1.32-2.42)	<0.01	0.10	37%	22%	77%	0.97	0.63
XRCC1 G27466A	2	225	434								
AG *vs.* GG				1.20 (0.78-1.83)	0.40	0.38	0%	12%	47%	-	-
Carrier				1.17 (0.77-1.78)	0.50	0.28	13%	12%	49%	-	-
ERCC2 G23591A	2	178	392								
AG *vs.* GG				0.78 (0.47-1.29)	0.33	0.72	0%	11%	44%	-	-
AA *vs.* GG				0.94 (0.39-2.30)	0.90	0.72	0%	4%	13%	-	-
Carrier				0.78 (0.48-1.27)	0.32	0.59	0%	12%	49%	-	-
ERCC2 A35931C	2	178	392								
CA *vs.* AA				1.00 (0.64-1.57)	0.99	0.94	0%	12%	47%	-	-
CC *vs.* AA				1.15 (0.48-2.77)	0.76	0.15	52%	4%	12%	-	-
Carrier				1.04 (0.67-1.60)	0.86	0.75	0%	13%	52%	-	-
B-cell ALL subgroup											
MTHFR C677T	2	133	399								
TC *vs.* CC				0.85 (0.57-1.28)	0.44	<0.01	86%	11%	43%	-	-
TT *vs.* CC				0.75 (0.33-1.68)	0.48	0.03	78%	8%	27%	-	-
Carrier				0.84 (0.57-1.24)	0.39	<0.01	89%	12%	46%	-	-
GSTM1	2	694	918								
Carrier				0.81 (0.66-0.99)	0.04	<0.01	92%	-	-	0.99	0.97
GSTT1	2	534	918								
Carrier				0.86 (0.65-1.13)	0.28	0.12	58%	-	-	-	-

 # Carrier status results after one study were excluded from NQO1 due to deviation from HWE.

*CYP1A1*2A* (T6235C) heterozygosity and carrier status was associated with a significant increased risk in two of seven studies[32,35,40,59-61,67] (Table 2; *Online Supplementary Figures S1 and S2*; *Online Supplementary Table S2*). A relationship between genotype and risk was supported by pooled analysis; ORs for heterozygosity and carrier status were: 1.38 (95% CI: 1.12-1.70, *P*=0.002; P_{het}=0.06, I^2=51%), and 1.36 (95% CI: 1.11-1.66, *P*=0.003; P_{het}=0.04, I^2=54%), respectively (*Online Supplementary Figure S2*). While there was evidence of between study heterogeneity, an association was still shown under a random effects model, with respective ORs of 1.38 (95% CI: 1.03-1.40) and 1.36 (95% CI: 1.01-1.84).

Of the three studies[60,61,67] which evaluated CYP1A1*2B (A4889G) as a risk factor, one study showed a significant association between the A4889G and increased risk (*Online Supplementary Figure S1*; *Online Supplementary Table S2*). Pooled data from the three studies, however, provided no evidence for relationship between *CYP1A1*2B* genotype and risk (Table 2).

None of the three studies[59-61] of *CYP2D6*4* (G1934A) showed a relationship with ALL risk (*Online Supplementary Figure S1*; *Online Supplementary Table S2*). Similarly, pooling data from the three studies provided no evidence for an association (Table 2; *Online Supplementary Figure S1*). Two studies of *CYP2D6*3* (del 2637) also found no evi-

dence for a relationship between this polymorphism and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Two of the four studies[32,59,61,68] of *CYP2E1*5B* showed evidence of a statistically significant association between heterozygosity/carrier status and increased risk (*Online Supplementary Figure S1*; *Online Supplementary Table S2*). Pooling data from all four studies (Table 2; *Online Supplementary Figure S2*), respective ORs were 2.03 (95% CI: 1.34-3.06, *P*=0.001; P_{het}=0.28, I^2=22%) and 1.99 (95% CI: 1.32-3.00, *P*=0.001; P_{het}=0.24, I^2=29%).

Five studies[34,69-72] have evaluated the synonymous *MDR1* C3435T polymorphism as a risk factor (*Online Supplementary Figure S1*; *Online Supplementary Table S2*). Homozygosity for TT was associated with a statistically significant increased risk in one study.[69] In the pooled analysis there was, however, no evidence of an association with risk (Table 2). While the controls analyzed by Jamroziak *et al.*[70] showed evidence of departure from HWE (*P*=0.04), findings were unaffected excluding this study from the meta-analysis (*data not shown*).

Pooling data from the two studies[34,70] of the triallelic variant *MDR1* G2677T/A provides no evidence for a relationship between this variant and ALL risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Three studies[73-75] have evaluated *XRCC1* polymorphisms C26304T and G28152A (*Online Supplementary Figure S1*; *Online Supplementary Table S2*). A significant association between *XRCC1* C26304T carrier status/homozygosity and elevated risk was shown in two studies.[74,75] Pooling data from the three studies did not, however, provide evidence of a relationship between the C26304T genotype and risk (Table 2; *Online Supplementary Figure S1*). Two of the three studies showed evidence of an association between *XRCC1* 28152A and increased risk (*Online Supplementary Figure S1*). Pooling data from all three studies (Table 2; *Online Supplementary Figure S2*), respective ORs were 1.78 (95% CI: 1.30-2.46, *P*<0.001; P_{het}=0.20, I^2=37%) for heterozygosity and 1.78 (95% CI: 1.32-2.42, *P*<0.001; P_{het}=0.10, I^2=37%) for carrier status. No significant association between XRCC1 G27466A genotype and ALL risk was shown in the two case-control studies[74,75] and pooling data also provided no evidence of a relationship (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). Similarly no significant association between *ERCC2* G23591A and A35931C genotype and risk was shown in two studies[73,75] (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

Acute lymphoblastic leukemia is genetically heterogeneous, hence some associations may be subtype specific. Only five studies examined the relationship between B-cell ALL and risk.[30,40,41,44,64] Among the three studies[40,41,44] that looked at *MTHFR* C677T and risk, one study had irregularities in the genotype data presented[40] hence data from the study could not be pooled. Combining data from the other two studies showed that C677T was not significantly associated with risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*). Of the three studies[30,40,64] which examined the relationship between *GSTM1* and risk of B-cell ALL, only two[30,40] had extractable genotype data. One of the studies was significantly associated with risk. Pooling data from both studies the OR was 0.80 (95% CI: 0.66-0.99, *P*=0.037; P_{het}<0.001, I^2=92%). However, the association was not significant (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*) under a random effects model (OR=1.22, 95% CI: 0.38-3.89, *P*=0.742). Two studies[30,40] evaluated *GSTT1* and risk of B-cell ALL and pooling data from these showed no evidence of an association with risk (Table 2; *Online Supplementary Figure S1*; *Online Supplementary Table S2*).

To evaluate the robustness of the 16 significant findings from the pooled analyses, we calculated FPRP conditional on a pre-specified prior probability of 0.001. None of the results can be considered noteworthy on the basis of assumptions (Table 2). For example, although the summary OR from the pooled analysis of *XRCC1* G28152A indicated a statistically significant positive association with risk (OR, 1.78; 95% CI, 1.32-2.42), the FPRP was 0.63 which was much higher than our cut off for noteworthiness (<0.2) for pooled analyses.[27]

Discussion

It is clear that substantial research has been carried out evaluating polymorphic variants in a number of specific genes as susceptibility alleles for ALL. Has this been worthwhile? While our meta-analysis provides support for variation at *GSTM1*, *MTRR*, *SHMT1*, *RFC1*, *CYP1A1*, *CYP2E1*, *NQO1* and *XRCC1* as risk determinants for ALL, these data should be interpreted with caution as the associations are not robust on the basis of FPRP estimates.

It is possible to draw a number of conclusions from the current data. Of the 25 variants examined to date, few have been reported as statistically significant, defined by a *P* value of 0.05, in more than one study. Given the issue of multiple testing in many reports, it is clearly advantageous when interpreting any purported association to have been replicated in an independent series. In some studies, the failure to demonstrate a relationship may be due to a lack of power. Genome-wide association (GWA) studies of cancer have demonstrated that the risk associated with individual polymorphic variants is quite modest (point estimates between 1.1 and 1.6 for an additive mode of inheritance).[76] Fewer than 20% of the studies we reviewed had 80% power to demonstrate a 2-fold difference in risk at the 0.05 significance level. To overcome this lack of power, we have undertaken a meta-analysis pooling the data from the published studies. There are, however, caveats to this statistical procedure. In any systematic review, publication bias is clearly of great concern. The most common scenario is that negative findings may go unreported. Furthermore, many studies do not describe the ethnicity of cases or controls, and it is assumed that each polymorphism is functional with respect to risk in each study population. These are likely to be the case with *MTHFR* C667T, and presumably also for *GSTM1* and *GSTT1*, since the functional role of the variants are better characterized. If, however, the polymorphism is a neutral marker for another variant, the assumption may well not apply, since LD is often population dependent.

An important lesson from current studies is that greater attention should be paid to design of future studies. Considering the issues of concomitant disease, a number of the studies were based on a comparison of cases and hospital based controls. The use of healthy population controls is preferable, since it is conceivable that the polymorphism might confer susceptibility to non-cancer diseases. The issue of population stratification in case-control

studies and resulting false positive results is also of great concern. If population subdivisions exist, it is possible that associations will be found between disease and arbitrary markers that are unlinked to causative loci. Such associations occur because of population subdivision and non-random mating, leading to variation in the marker frequency within the population as a result of founder effects and/or genetic drift. The severity of spurious association becomes an increasing problem with increasing study size. To avoid this problem, it is essential that any potential confounding effect of population stratification be allowed for in the design and analysis of the study. This requires the identification of sub-populations in terms of factors that can influence both disease and marker allele frequencies. Provided cases and controls are well matched, differences in the frequency of genotypes will only be seen at predisposition loci. Hence, stratification can be detected by typing a series of unlinked markers chosen from a panel known to exhibit differences in allele frequency between populations.[77]

The focus of this meta-analysis was strictly on results from candidate-gene association studies and did not take into account results from other analyses. We have attempted to review published analyses of the relationship between polymorphic variation and risk of ALL through several iterations of search criterion; it is, however, possible that we have missed some reported studies. As the number of articles on genetic variation on risk of ALL published in the past decade has increased considerably and continues to grow, we fully accept that this review will not long remain current but provides a snapshot of progress to date in the field.

The choice of tissue used in the 47 studies selected for analysis varied widely. The majority of them[29-35,40-44,46-48,51-56,59,60,62,63,65,67,68,70,71,74] were DNA extracted from peripheral blood, bone marrow or buccal samples collected at the time of diagnosis at the study center or with unspecified patient status. Five studies included samples from remission and those collected at diagnosis.[36-39,49,69] Only six studies[45,57,61,72,73,75] clearly stated that the samples were collected when the patients were in remission. The tissue origin for these studies varied from bone marrow, buccal swabs, Guthrie cards and peripheral blood. Four studies[50,58,64,66] gave no clarification pertaining to sample origin. Studies that are based on DNA extracted from peripheral blood or bone marrow tissue obtained from non-remission patient samples may potentially contain somatic mutations that have the potential to confound association studies. Hence the results of such studies should be reassessed and accepted with caution. Due to the small number of studies that only used remission samples, such a screening criterion was not used in our current study selection.

Acute lymphoblastic leukemia is heterogeneous with respect to its underlying cellular and molecular biology, acquired genetic abnormalities and associated clinical responses to combination chemotherapy.[78,79] It is, therefore, suspected that subtypes of B- or T-cell precursor ALL may not share a common etiology. To date only a very few studies have examined the relationship between variants and risk by subtype. Additionally these studies invite the issue of *post hoc* analysis.

All of the studies we have reviewed have been based on a candidate gene approach. It is becoming increasing clear from studies of cancer that without a clear understanding of tumor causalities the definition of what constitutes a candidate gene is inherently problematic, making an unbiased approach to loci selection highly desirable.

The availability of high resolution LD maps and comprehensive sets of tagging SNPs, coupled with the development of highly efficient analytical platforms, allow GWA studies for disease associations to be conducted cost effectively. This approach is unbiased and does not depend upon prior knowledge of function or presumptive involvement of any gene in disease causation. Moreover, it avoids the possibility of missing the identification of important variants in hitherto unstudied genes. Very recently, results from the first GWA studies of ALL have become available and these studies have identified risk loci for ALL at *IKZF1*, *ARID5B* and *CEBPE*.[80,81] The substantial evidence supporting these variants, including sizeable power and replication in large samples, indicates that the associations are highly robust. These data thus provide the first unambiguous evidence that common low-penetrance susceptibility alleles contribute to the risk of developing childhood ALL. Furthermore, they provide novel insight into disease causation of ALL; notably all of the risk variants map to genes involved in transcriptional regulation and differentiation of B-cell progenitors. Given sub-optimal LD between many polymorphisms with tagging SNPs, especially those with low minor allele frequencies [MAF], the findings provide a strong rationale for directly evaluating variation in other B-cell developmental genes as risk factors.

Of the eight associations from the current meta-analysis which were nominally significant at the 5% threshold, one was a deletion. Of the seven SNPs one was directly genotyped as part of our GWA study of ALL (rs1051266; *RFC1* G80A) and the *CYP2E1*5B* SNP rs3813867 was tagged by rs10857733 (R^2=0.88). Both SNPs showed no association with risk (respective *P* values =0.81 and *P*=0.84).[81]

The search for polymorphic variants influencing the risk of ALL is a worthy enterprise; however, the studies that have been conducted to date have important lessons for the design and execution of future studies. Candidate gene analyses should be viewed as complementary to GWA studies, as they offer considerable advantages both in terms of statistical power and an ability to identify low frequency risk variants. Furthermore, many functional variants, such as the small scale insertion and deletions in carcinogen metabolism genes, are poorly captured by the tagging SNPs used in GWA studies. It is, however, clear that in addition to conducting studies using sample sizes commensurate with the detection of polymorphic risk factors, attention should be paid to study design to avoid problems of population stratification and other sources of potential bias in order to maximize the output of any study.

Authorship and disclosures

RSH study conception, drafting of manuscript; JV literature review, statistical analysis and drafting of manuscript.

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