Confirmation of Childhood Acute Lymphoblastic Leukemia Variants, **ARID5B** and **IKZF1**, and Interaction with Parental Environmental Exposures

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**Abstract**

Genome wide association studies (GWAS) have established association of **ARID5B** and **IKZF1** variants with childhood acute lymphoblastic leukemia (ALL). Epidemiological studies suggest that environmental factors alone appear to make a relatively minor contribution to disease risk. The polygenic nature of childhood ALL predisposition together with the timing of environmental triggers may hold vital clues for disease etiology. This study presents results from an Australian GWAS of childhood ALL cases (n = 358) and population controls (n = 1192). Furthermore, we utilised family trio (n = 204) genotypes to extend our investigation to gene-environment interaction of significant loci with parental exposures before conception, and child’s sex and age. Thirteen SNPs achieved genome wide significance in the population based case/control analysis; ten annotated to **ARID5B** and three to **IKZF1**. The most significant SNPs in these regions were **ARID5B** rs4245595 (OR 1.63, CI 1.38–1.93, P = 2.13×10⁻⁶), and **IKZF1** rs1110701 (OR 1.69, CI 1.42–2.02, p = 7.26×10⁻⁶). There was evidence of gene environment interaction for risk genotype at **IKZF1**, whereby an apparently stronger genetic effect was observed if the mother took folic acid or if the father did not smoke prior to pregnancy (respective interaction P-values: 0.04, 0.05). There were no interactions of risk genotypes with age or sex (P-values >0.2). Our results evidence that interaction of genetic variants and environmental exposures may further alter risk of childhood ALL; however, investigation in a larger population is required. If interaction of folic acid supplementation and **IKZF1** variants holds, it may be useful to quantify folate levels prior to initiating use of folic acid supplements.

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**Data Availability:** The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. Data are available from the AUS-ALL Institutional Data Access/Ethics Committee for researchers who meet the criteria for access to confidential data. We will provide data but due to restrictions from our ethics committees we are unable to freely provide all data unless an agreement between all parties has been made. Data are from the AUS-ALL study whose authors may be contacted at rodney.scott@newcastle.edu.au, t.evans@newcastle.edu.au or lizm@ichr.uwa.edu.au.

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**Introduction**

Contemporary genome wide association studies (GWAS) have consistently revealed, in the European population, two genetic loci that are associated with childhood acute lymphoblastic leukemia (ALL) risk [1–4]. The genes implicated at these loci, **AT-rich interactive domain 5b (ARID5B)** and **Ikaros family zinc finger 1 (IKZF1)**, encode proteins that regulate normal lymphocyte differentiation. Other genes have been identified from some but not all GWASs including **CEBPE**, **CDKN2A** and **GATA3** [5,6]. A recent report [7] demonstrated a greater sensitivity to detect risk loci by using an ethnically diverse population of childhood ALL patients and identified a new risk locus at 10p12.31 (**BMI1-PIP4K2A**). Also, a graduated scale of ALL risk was demonstrated for: i) increasing number of risk alleles at four of the loci combined (**ARID5A, IKZF1, CEBPE** and **BMI1-PIP4K2A**); and ii) an
**ARID5B** risk allele with odds highest for children younger than five years and lowest for those older than 10 years. This information underscores the polygenic nature of childhood ALL and places some emphasis on the potential for interaction with environmental triggers and the developmental stages at which they occur.

Epidemiological studies have assessed the role of environmental factors in the pathogenesis of childhood ALL however these factors alone appear to make a relatively minor contribution to disease risk [8]. Previous investigations from our own Australian ALL case-control study have focussed on parental exposures before and after conception including: alcohol consumption, folic acid use, and smoking. We found that paternal smoking around time of conception increased the odds of childhood ALL [9]; our meta-analysis for maternal folate demonstrated that supplementation had a protective effect [10] and, while parental alcohol consumption did not alter odds, the quantity of consumption might [11].

Aforementioned GWAS have investigated associations of **IKZF1** and **ARID5B** genotypes with treatment outcomes, and Linabery et al. [12] assessed interaction with age and sex demographics; however, we hypothesise that genetic variants compounded with environmental exposures and the developmental stages at which they occur, may contribute to the background risk in childhood ALL.

This study presents results from an Australian GWAS of childhood ALL cases and population controls. A French case-control study was used to determine the validity of novel findings [3]. Finally, we investigated gene-environment interaction of key loci identified in our GWAS with parental exposures before conception, and child’s sex and age.

**Materials and Methods**

**Study population**

The study population comprised 441 childhood ALL cases from whom blood-derived DNA had been isolated during remission. 324 were participants in The Australian Study of Childhood Acute Lymphoblastic Leukaemia (Aus-ALL) described in Milne et al. [10]. This study involved the collection of environmental exposure data via questionnaire. The remaining 117 cases were from The Tumour Bank at the Children’s Hospital Westmead in Sydney NSW, consecutively collected from 1998–2006. The control series comprised 1229 healthy Caucasian adults sourced from the Hunter Community Study (HCS), Newcastle, Australia [13]. Aus-ALL was approved by the human research ethics committees at all participating hospitals and the Hunter Community study was approved by the University of Newcastle Research Ethics Committee and the Hunter New England Health Service Research Ethics Committee.

**Genome-wide genotyping**

Genome-wide genotyping was undertaken on Illumina (San Diego, USA) BeadChips accordingly: 102 cases were genotyped for the pilot study using HumanCVD370-Duo v.1; 191 cases using HumanCVD370-Quad v.3; 146 cases and 1229 controls using Human610-Quad v.1. Genotype validation for rs7099424 and rs1432601 was carried out for the Aus-ALL cases and 400 HCS controls using Applied Biosystems (Foster City, USA) TaqMan Technology, revealing 100% concordance. Genotyping for these single nucleotide polymorphisms (SNPs) was extended to both parents of Aus-ALL cases for whom DNA and exposure data were available (n = 204 trios).

**Genome-wide association analysis**

PLINK v1.07 [14] was used to apply stringent quality control (QC) first by BeadChip version and again following data merging (Methods S1 in File S1) resulting in 358 cases, 1192 controls and 309 117 SNPs carried forward to genotype imputation (Post-QC case demographics for age, gender and ALL subtype available in Table S1 in File S1). Samples were pre-phased using SHAPEIT, and imputation using the 1000 Genomes phase 1 reference panel was carried out using IMPUTE2 v2.3.0. Case-control association at each SNP was tested under an additive model using a missing data likelihood score test which takes into account genotype uncertainty due to imputation. Models were fitted using SNPTEST v2.4.1, adjusting for the top three principal components defining ancestry. SNPs with a SNPTEST information measure less than 0.4 and a minor allele frequency less than 2% (in cases or controls or cases/controls combined) were filtered out resulting in 7 162 141 SNPs passing QC. Regional association plots for significant loci were produced using LocusZoom [15]. Pritchard Lab and Blood eQTL browsers [16,17] were used to establish whether SNPs are expression quantitative trait loci (eQTLs).

Data from the French ESCALE nationwide registry, comprising 441 cases and 1542 controls [3], were available for replication of a new suggestive association.

**Gene-environment interaction analyses**

For genotyped SNPs at genome-wide significant loci, the method of Cordell [18] was used to investigate gene-environment interactions for 204 cases-parent trios. Interactions with paternal smoking before conception, and maternal use of folic acid prior to pregnancy were investigated because they demonstrated main effects in previous Aus-ALL studies [9,10]. Maternal alcohol consumption before pregnancy was also investigated as adequate numbers in each exposure category was achievable [11]. Interactions with the child’s sex and age were also assessed. The Stata function predict coupled with conditional logistic regression was used for analysis. Genotype-based (rather than allele based) odds ratios were estimated as recommended by Sasieni [20]. This process uses the parents’ genotypes to estimate the theoretical genotypes their offspring could have had, and these are used as matched controls for each case. Thus, only genetic main effects and their interactions with ‘environmental’ (age, sex, folate, etc) effects can be estimated as each case/control set has the same values for the environmental variables.

**Results**

**Genome-wide association study for childhood acute lymphoblastic leukemia**

QQ-plot and genomic inflation factor (λ = 1.0006) indicated an absence of population substructure or other systematic bias between cases and controls (Figure S1 in File S1).

Table 1 annotates 13 SNPs at two loci that were significantly associated with childhood ALL (P < 5 × 10−8), and 259 additional SNPs achieved P < 10−5 (Table S2 in File S1 and Manhattan plot in Figure S2 in File S1). Ten of the 13 significant SNPs map to intron three of **ARID5B** on chromosome 10 and three flank **IKZF1** on chromosome 7 (LocusZoom plots in Figure S3 in File S1). The most significantly associated SNP was rs4245595 at **ARID5B** (OR 1.63, CI 1.38–1.93, P = 2.13 × 10−7) where G is the risk allele, and the association was stronger for B-cell ALL (OR 1.86, CI 1.54–2.25, P = 1.21 × 10−10).
Table 1. SNPs associated with Childhood ALL at $P < 5 \times 10^{-8}$.

| Alleles (A/B) | MAF | Cases (n = 358) | Controls (n = 1192) | Allelic OR (95% CI)$^a$ | P | Allelic OR (95% CI)$^a$ | P |
|---------------|-----|----------------|---------------------|--------------------------|---|--------------------------|---|
| rs4245595     | 0.46| 0.34          | 1.63 (1.38–1.93)     | 2.13E-09                 | 1.86 (1.54–2.25) | 1.21E-10                 |
| rs7090445     | 0.46| 0.34          | 1.63 (1.37–1.93)     | 2.81E-09                 | 1.83 (1.51–2.20) | 2.88E-10                 |
| rs4948492     | 0.46| 0.34          | 1.63 (1.37–1.93)     | 2.84E-09                 | 1.82 (1.51–2.20) | 2.91E-10                 |
| rs7896246     | 0.46| 0.34          | 1.61 (1.36–1.91)     | 4.63E-09                 | 1.81 (1.50–2.19) | 5.11E-10                 |
| rs4245597     | 0.46| 0.35          | 1.6 (1.35–1.9)       | 5.87E-09                 | 1.81 (1.50–2.19) | 5.99E-10                 |
| rs10821937    | 0.46| 0.35          | 1.6 (1.35–1.9)       | 6.36E-09                 | 1.81 (1.50–2.18) | 6.96E-10                 |
| rs10821936    | 0.46| 0.35          | 1.6 (1.35–1.9)       | 6.36E-09                 | 1.81 (1.50–2.18) | 6.95E-10                 |
| rs7087507     | 0.46| 0.35          | 1.62 (1.37–1.92)     | 6.96E-09                 | 1.80 (1.50–2.18) | 6.99E-10                 |
| rs1110701     | 0.39| 0.28          | 1.69 (1.42–2.02)     | 7.26E-09                 | 1.91 (1.57–2.32) | 8.27E-11                 |
| rs9415635     | 0.46| 0.34          | 1.6 (1.35–1.89)      | 2.17E-08                 | 1.76 (1.46–2.12) | 2.96E-09                 |

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*Genotyped SNPs indicated in this column, dashed entries indicate imputed SNPs.

*Allele B is the minor allele for all SNPs in this table.

Odds ratios represent the effect of additional copies of the B (minor allele).

Abbreviations: MAF, Minor Allele Frequency; Chr, Chromosome; OR, Odds Ratio; CI, Confidence Interval; P, P-value; B-ALL, B-cell Acute Lymphoblastic Leukemia.

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Table 2. Estimates for genetic main effects of variation at rs4132601 and interaction with parental exposures, age and sex for 204 case-trios.

| Variable Name                        | Genotype | P  | Environmental Exposure | Present | Cases: Controls | OR[^b] | 95% CI     | P    |
|--------------------------------------|----------|----|------------------------|---------|----------------|--------|------------|------|
| Restricted to non-missing questionnaires |          |    |                        |         |                |        |            |      |
| rs4132601                            | (GG)     |    |                        |         | 25:41:00       | 2.53   | 1.31–4.91  | 0.006|
|                                      | (GT)     | 0.02[^c] |                        |         | 84260          | 1.2    | 0.82–1.77  | 0.35  |
|                                      | (TT) (REF) |    |                        |         | 95311          | 1      |            |      |
| Mother alcohol use before pregnancy  |          |    |                        |         |                |        |            |      |
|                                      | GG       |    |                        | Yes     | 1723           | 3.72   | 1.63–8.50  | 0.002|
|                                      | GT       |    |                        |         | 58168          | 1.5    | 0.92–2.45  | 0.1   |
|                                      | TT       |    |                        |         | 58208          | 1      |            |      |
|                                      | GG       |    |                        | No      | 8.18           | 1.2    | 0.38–3.71  | 0.46  |
|                                      | GT       |    |                        |         | 2692           | 0.78   | 0.40–1.51  | 0.76  |
|                                      | TT       |    |                        |         | 37103          | 1      |            |      |
| Mother folic acid use before pregnancy |          |    |                        |         |                |        |            |      |
|                                      | GG       |    |                        | Yes     | 1214           | 7.34   | 2.23–24.2  | 0.001|
|                                      | GT       |    |                        |         | 2672           | 2.39   | 1.06–5.40  | 0.04  |
|                                      | TT       |    |                        |         | 31121          | 1      |            |      |
|                                      | GG       |    |                        | No      | 1327           | 1.46   | 0.63–3.37  | 0.38  |
|                                      | GT       |    |                        |         | 57189          | 0.89   | 0.57–1.41  | 0.63  |
|                                      | TT       |    |                        |         | 63183          | 1      |            |      |
| Father smoked during 2 years before pregnancy |          |    |                        |         |                |        |            |      |
|                                      | GG       |    |                        | Yes     | 6:11           | 1.35   | 0.42–4.36  | 0.61  |
|                                      | GT       |    |                        |         | 2389           | 0.68   | 0.36–1.29  | 0.24  |
|                                      | TT       |    |                        |         | 40107          | 1      |            |      |
|                                      | GG       |    |                        | No      | 1828           | 4.07   | 1.73–9.57  | 0.001|
|                                      | GT       |    |                        |         | 55151          | 1.84   | 1.08–3.15  | 0.03  |
|                                      | TT       |    |                        |         | 47181          | 1      |            |      |
| Patient sex                          |          |    |                        |         |                |        |            |      |
|                                      | GG       |    | Male                   |         | 1323           | 2.24   | 0.93–5.40  | 0.07  |
|                                      | GT       |    |                        |         | 45137          | 1.2    | 0.71–2.02  | 0.49  |
|                                      | TT       |    | Female                 |         | 51167          | 1      |            |      |
|                                      | GG       |    |                        | Female  | 1218           | 2.98   | 1.08–8.19  | 0.03  |
|                                      | GT       |    |                        |         | 39123          | 1.21   | 0.67–2.16  | 0.53  |
|                                      | TT       |    |                        |         | 44144          | 1      |            |      |
Association at the \textit{IKZF1} locus was most significant for rs1110701 (OR 1.69, CI 1.42–2.02, \(p = 7.26 \times 10^{-10}\)) where G is the risk allele and this association was also stronger when analysis was confined to the B-cell subtype (OR 1.91, CI 1.57–2.32, \(p = 8.27 \times 10^{-11}\)).

For the third most significant genotyped loci, located on chromosome 8 in the vicinity of the \textit{ZNF704} gene, borderline significance was achieved (\(p = 10^{-5}\)). There were three genotyped SNPs in the region with \(10^{-8} < p < 10^{-5}\): rs7000234, rs7018449 and rs6992620. This result, however, was not replicated when interrogated in the French dataset (Table S3 in File S1 and Figure S4 in File S1).

### Interaction analyses of environmental variables and \textit{IKZF1} and \textit{ARID5B} variants

Main effects of genotyped SNPs at \textit{ARID5B} and \textit{IKZF1} loci (rs7089424 and rs4132601 respectively) and interactions with environmental variables were conducted using a reduced population of 204 case-parent trios for whom environmental exposure data were available. Genetic main effects demonstrated significant risk increases with homozygosity for the minor alleles (GG for both) versus the referent homozygous major alleles (TT) (Table 2 and Table S4 in File S1).

An interaction was observed between the genotyped SNP at the \textit{IKZF1} locus (rs4132601) and maternal use of folic acid pre-pregnancy (Table 2). There was a significantly increased risk of ALL among children with the homozygous minor genotype (GG) whose mothers used supplements; the OR was less elevated if they did not (\(p = 0.04\)).

We also observed interaction of rs4132601 genotype and father’s smoking prior to conception, whereby risk of ALL associated with the GG genotype was greater among children of non-smoking fathers (\(p = 0.05\)) (Table 2). In addition, there was some evidence that risk associated with the GG genotype at this SNP was elevated only if the mother drank alcohol before pregnancy (OR: 7.34 CI: 2.23–24.2); however, the interaction \(p\)-value was 0.18 (Table 2).

No interaction was evident for rs7089424 with parental exposure variables (Table S4 in File S1). No interaction was observed for variables sex and age with either SNP (\textit{IKZF1} \(p = 0.90/0.31\), and \textit{ARID5B} \(p = 0.96/0.72\)) (Table 2 and Table S4 in File S1).

### Discussion

Our current study has demonstrated that variants in \textit{ARID5B} and \textit{IKZF1} are associated with childhood ALL in an Australian Caucasian cohort. This is consistent with former GWAS publications in similar cohorts [1–4]. Also in agreement with these studies is our finding that the strength of association was increased when analysis was confined to B-cell ALL versus controls. Our study was not large enough to reveal any other associations such as those previously reported at \textit{CEBPE}, \textit{CDKN2A}, \textit{GATA3} and \textit{BMI1-PIP4K2A} - loci which have not consistently been identified across multiple GWAS but which meta-analyses have validated [3,21,22].

The frequencies of the \textit{ZNF704} SNPs in our case population were lower than that reported in the 1000 GENOMES phase 1 database but they were not replicated in the French data. This result could be due to population differences thereby suggesting a real effect; however, considering our cases were from an Anglo-Celtic background, and these SNPs were not identified in...
other such populations [1], it remains to be categorically defined as a risk variant.

The ARID5B transcription factor is important in embryogenesis and B-cell development [23] and ARID5B deletion mutations occur in leukemic cells. None of the significantly associated SNPs we identified at this locus are expression quantitative trait loci (eQTLs) according to Pritchard Lab and Blood eQTL browsers [16,17], nor did they overlap enhancer binding sites annotated by ENCODE regulatory segmentation. Despite the four years since publication of the first childhood ALL GWAS, a dearth of information persists for the mechanisms responsible for ARID5B variants predisposing to childhood ALL.

The Ikaros transcription factor is restricted to the hemopoietic system and is a key regulator of lymphocyte differentiation via chromatin remodelling. Our strongest associated SNP rs1110701, and other IKZF1 SNPs identified in this study (rs10272724 and rs17133807), annotate to enhancer binding sites in the GM12878-B-Lymphocyte cell line according to ENCODE regulatory segmentation. These SNPs were identified as eQTLs acting in cis [16,17] consistent with Papaemmanuil’s et al. [1] demonstrated attenuation of IKZF1 gene expression with variant allele dosage at rs4132601 (which is in linkage disequilibrium with rs1110701 r² = 0.802 D’ = 1; P in the current study 8.51 x 10⁻¹⁶). Interestingly, Meyer’s et al. [24] recent sequence analysis of IKZF1 deletion breakpoints in leukemic cells revealed four recombination hotspots, one of which was observed in 20% of deletion breakpoints in leukemic cells revealed four IKZF1 genotyped, rather than imputed, at potential for interaction of parental exposures and child’s age and rearrangements [25]. In the current study, we investigated the potential for interaction of parental exposures and child’s age and sex with child’s genotype. The most significant SNPs that were genotyped, rather than imputed, at ARID5B (rs7089424) and IKZF1 (rs4132601) loci were used for the interaction analysis. This was to facilitate genotyping validation of the Illumina array technology and the extension of the study in a single step. Paternal smoking, maternal folate and alcohol use (each before conception) were investigated; however no interaction was apparent for ARID5B variant rs7089424 with any of these exposures. There were no interactions observed for the two variants with child’s sex and age, validating the findings of Linabery et al. [12].

A meta-analysis of maternal folic acid supplementation and risk of childhood ALL conducted by our affiliated Aus-ALL consortium, verified a protective effect for supplementation [10]. When we assessed interaction of maternal folic acid supplementation with IKZF1 SNP rs4132601 (the most significant genotyped SNP at the locus), we observed elevated odds ratios for children with the risk (GG) genotype whose mother took supplements. This observation is contrary to expectations, and biologically plausible explanations for this direction of effect are lacking. Nonetheless, potential interaction between folate levels and genotype at IKZF1 warrants further investigation since: i) transcription of the reduced folate carrier (SLC19A1) can be modulated by the balance of Ikaros activating and dominant-negative isoforms [26] and ii) increased circulating un-metabolised folic acid has demonstrated association with reduced natural killer cell cytotoxicity [27] which is in turn associated with cancer [28]. Additionally, our measure of folic acid exposure (supplementation-Yes/No) is without knowledge of possible dietary or metabolic folate sufficiency which could have influenced the observations. Implications of this finding suggest that folate quantification may be valuable prior to initiating supplementation.

We observed a similarly unexpected interaction whereby risk of childhood ALL was greater for children with rs4132601 risk (GG) genotype and non-smoking fathers (Table 2) despite a previous Aus-ALL epidemiological study [9] demonstrating that paternal smoking was a risk factor for childhood ALL. An interesting trend was observed whereby risk associated with IKZF1 was elevated only for the GG genotype if the mother drank alcohol before pregnancy. While this may be a chance finding, it corroborates health recommendations to avoid alcohol during pregnancy.

Given the limited numbers of cases homozygous for the minor alleles for all interactions, it is possible that our findings for SNP rs4132601 occurred due to chance, nonetheless investigation in a larger population is required to elucidate whether any true interactions exist.

Conclusions
We have replicated associations of IKZF1 and ARID5B variants with childhood ALL in an Australian Caucasian population. The IKZF1 variants identified were eQTLs and impacted enhancer sequences however, such was not the case for ARID5B variants. Furthermore, there was some evidence for gene-environment interaction for an IKZF1 variant, with an apparently stronger genetic effect if the mother took folic acid or drank alcohol, or if the father did not smoke prior to pregnancy, but these may be explained by chance. These findings warrant further investigation in larger samples and may indicate that folate quantification may be valuable prior to initiating supplementation.

Supporting Information
File S1 Contains Methods S1, Tables S1–S4, Figures S1–S4. (DOCX)

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Author Contributions
Conceived and designed the experiments: RJS JRA EM BKA DC. Performed the experiments: T-JE DA SJ JR LO ER LL. Analyzed the data: T-JE NDK SJ EGH JC EM JRA. Contributed reagents/materials/analysis tools: BAT-P NAB LO JR DA EM NDK RJS. Contributed to the writing of the manuscript: T-JE EM NDK BAT-P NAB EGH DC JRA BKA JC RJS.

References
1. Papaemmanuil E, Hosking F, Vijayakrishnan J, Price A, Oliver B, et al. (2009) Loci on 7p12.2, 10q21.2 and 1q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet 41: 1006–1010.
2. Treviño L, Yang W, French D, Hunger S, Carroll W, et al. (2009) Genetic polymorphisms and childhood acute lymphoblastic leukemia: GWAS of the ESCALe study (SFCE). Leukemia 26: 2361–2364.
3. Orsi L, Rudant J, Bonaventure A, Gougon-Bellec S, Corda E, et al. (2012) Gene Environment Interactions Act on ARID5B and IKZF1. Genet 41: 1001–1005.
4. Healy J, Richer C, Bourgey M, Kritikos EA, Sinnott D (2010) Replication analysis confirms the association of ARID5B with childhood B-cell acute lymphoblastic leukemia. Haematologica 95: 1608–1611.
5. Migliorini G, Fiege B, Hosking EJ, Ma Y, Kumar R, et al. (2013) Variation at 10p12.2 and 10p14 influences risk of childhood B-cell acute lymphoblastic leukemia and phenotype. Blood 122: 3298–3307.

6. Sherborne AL, Hosking EJ, Prasad RB, Kumar R, Koehler R, et al. (2010) Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet 42: 492–494.

7. Xu H, Yang W, Perez-Andreu D, Devidas M, Fan Y, et al. (2015) Novel susceptibility variants at 10p12.12-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. J Natl Cancer Inst 105: 735–742.

8. Belson M, Kingsley B, Holmes A (2007) Risk Factors for Acute Leukemia in Children: A Review. Environmental Health Perspectives 115: 138–145.

9. Milne E, Greenop KR, Scott RJ, Bailey HD, Attia J, et al. (2012) Parental prenatal smoking and risk of childhood acute lymphoblastic leukemia. Am J Epidemiol 175: 43–53.

10. Milne E, Royle JA, Miller M, Bower C, de Klerk NH, et al. (2010) Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. Int J Cancer 126: 2690–2699.

11. Milne E, Greenop KR, Scott RJ, de Klerk NH, Bower C, et al. (2013) Parental alcohol consumption and risk of childhood acute lymphoblastic leukemia and brain tumors. Cancer Causes Control 24: 391–402.

12. Linabery AM, Blommer CN, Spector LG, Davies SM, Robison LL, et al. (2013) ARID5B and IKZF1 variants, selected demographic factors, and childhood acute lymphoblastic leukemia: a report from the Children’s Oncology Group. Leuk Res 37: 936–942.

13. McEvoy M, Smith W, D’Estey C, Duke J, Peel R, et al. (2010) Cohort profile: The Hunter Community Study. Int J Epidemiol 39: 1452–1463.

14. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.

15. Purcell R, Welch RP, Sanna S, Tedovitch TM, Chines PS, et al. (2010) LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 26: 2336–2337.

16. Veyrioner JB, Kudaravalli S, Kim SY, Dermitzakis ET, Gilad Y, et al. (2008) High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS Genet 4: e1000214.

17. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, et al. (2013) Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 45: 1238–1243.

18. Cordell HJ, Barratt BJ, Clayton DG (2004) Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. Genet Epidemiol 26: 167–185.

19. Clayton DG genassoc: Stata module for analysis of genetic association studies.

20. Sasieni PD (1997) From genotypes to genes: doubling the sample size. Biometrics 53: 1235–1261.

21. Chokkalingam AP, Hsu L, Metayer C, Hansen HM, Mount SR, et al. (2013) Genetic variants in ARID5B and CEBPE are childhood ALL susceptibility loci in Hispanics. Cancer Causes Control 24: 1789–1795.

22. Prasad R, Hosking F, Vijayakrishnan J, Papanemanul E, Koehler R, et al. (2010) Verification of the susceptibility loci on 7p12.2, 10q21.2, and 1q11.2 in precursor B-cell acute lymphoblastic leukemia of childhood. Blood 115: 1765–1767.

23. Lahoud MH, Ristic D, Venter DJ, Jermiin LS, Bertoccelli I, et al. (2001) Gene targeting of Desr, a novel ARID class DNA-binding protein, causes growth retardation and abnormal development of reproductive organs. Genome Res 11: 1327–1334.

24. Meyer C, Zur Stadt U, Esherich G, Hofmann J, Binato R, et al. (2013) Refinement of IKZF1 recombination hotspots in pediatric BCP-ALL patients. Am J Blood Res 3: 163–173.

25. Krivtsov A, Armstrong S (2007) MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer 7: 825–833.

26. Liu M, Whetstone JR, Payton SG, Ge Y, Flatley RM, et al. (2004) Roles of USF, Ikaros and Sp proteins in the transcriptional regulation of the human reduced folate carrier B promoter. Biochem J 383: 249–257.

27. Imai K, Matsuyama S, Miyake S, Saga K, Nakachi K (2000) Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356: 1795–1799.

28. Tsen AM, Mitchell B, Norris B, Wener MH, Johnston A, et al. (2006) Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 136: 189–194.