Research Article

Association of the independent polymorphisms in CDKN2A with susceptibility of acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most common cancer in children, and alterations in CDKN2A were considered to play an important role on leukemogenesis. Two single nucleotide polymorphisms (SNPs) at CDKN2A locus were identified to impact on ALL susceptibility via genome wide association studies, and followed by multiple subsequent replication studies at the specific hits. Here, we conducted a systematic review and meta-analysis to re-evaluate the association of both SNPs (rs3731217 and rs3731249) with ALL susceptibility by gathering the data from 24 independent studies, totally containing 7922 cases/21503 controls for rs3731217 and 6295 cases/24191 controls for rs3731249. Both SNPs were significantly associated with ALL risk (odds ratio [OR] = 0.72 and 2.26 respectively), however, exhibit race-specific pattern. In summary, our meta-analysis indicated that two SNPs at CDKN2A locus are associated with ALL susceptibility independently mainly in Caucasians. Future large-scale studies are required to validate the associations in other ethnicities.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer worldwide [1]. Genetic susceptibility basis of such deadly malignancy has been largely investigated, identifying somatically acquired genomic aberrations, which is hallmark of the majority ALL cases [2, 3]. For instance, focal or whole-arm deletion of CDKN2A gene, which encodes two important tumor proteins (p14ARF and p16INK4A), is commonly observed in leukocytes of ALL patients [1, 4]. Recently, inherited predispositions to ALL susceptibility have also been recognized by conducting genome-wide association studies (GWASs) across diverse ethnicities, identifying common variants at several genetic loci, including single nucleotide polymorphisms (SNPs) in ARID5B, IKZF1, CEBPE, CDKN2A, PIP4K2A-BMI1, and GATA3 [5-9]. However, variations even inconsistence were observed in the replication studies, possibly because of the diverse clinical characteristics, including ethnicity, age and subtypes [10-13]. Besides the top SNPs identified in intronic or intergenic regions, exome-array based GWAS was also conducted to systematically investigate the relationship between coding variants and ALL susceptibility in Caucasian population with 1773 cases and 10,448 controls [14]. Only one common missense variant at CDKN2A (rs3731249) reaches genome-wide significance. By using imputation or array-based approaches, association of this SNP with ALL susceptibility has been validated among multiethnic populations in another three independent studies [15-17]. Further investigation on the interaction between germline variants and somatic alterations, we and other group found that patients with heterozygous genotype of rs3731249 were tend to loss the expression of wild-type CDKN2A in their leukemia cells, either through loss of heterozygosity or allele-specific post-transcriptional inactivation [14]. Moreover, we conducted in vitro experiments to further test the
influence of variants at rs3731249 on leukemogenesis, and found p14ARF encoded by CDKN2A gene with variant allele of rs3731249 loses its ability to suppress leukemic transformation compared with the wild-type p14ARF, indicating rs3731249 is a potential causal variant to ALL susceptibility [14]. Therefore, two SNPs in CDKN2A were identified as ALL-associated GWAS signals, highlighting the importance of CDKN2A in leukemogenesis in both germline and somatic levels.

In the present study, we incorporated all the relevant publications, and collected the information from 24 independent studies [9, 14-29], 7922 cases/21503 controls for rs3731217 and 6295 cases/24181 controls for rs3731249 were gathered. Finally, meta-analysis was conducted with a large sample size with diverse ethnicities to investigate the effects of CDKN2A SNPs (including rs3731217 and rs3731249) on ALL risk.

Methods

Literature and study acquisition
We systematically retrieved all the related papers in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Google Scholar (http://scholar.google.com/) date to November 5, 2015 utilizing the following retrieve phrases: “rs3731217” or “rs3731249” or “acute lymphoblastic leukemia” and “polymorphisms” and “CDKN2A” or “childhood acute lymphoblastic leukemia” and “susceptibility” or “genetic polymorphism” and “acute lymphoblastic leukemia” and “GWAS”, or “germline variants” and “acute lymphoblastic leukemia”. All papers (N=131) were confined to English. After that, initial screening of the titles along with the abstracts was executed by two independent reviewers, overlapped studies as well as papers that do not meet our motif were discarded (N=87). Following, full-text based filtering was conducted among the left studies and reserved eligible papers according to the criteria listed below: (1) studies adopted case–control design; (2) assessed the effect of rs3731217 or rs3731249 polymorphisms on ALL risk; (3) offering the sample size; (4) giving the genotype counts or detail information to infer the genotypes; and (5) data from each study without overlap. When repeated data encountered, only the latest publication or most detailed data were included.

Data extraction and verification
Two independent reviewers extracted information from each study based on the following contents: first author, publication date, country, ethnicity, study design, sample size, genotyping platform, and genotypes. When datasets were not accessible or partial for the requisite data, corresponding authors were connected for additional information. Gathering information from the included studies was listed in Tables 1 and 2.

Choice of genetic model
The rs3731249 polymorphism owns variant T allele and wild-type C allele. We plan to investigate the relationship between rs3731249 polymorphism and childhood acute lymphoblastic leukemia risk by utilizing the allele model (T allele vs. C allele), the dominant model (TT + TC vs. CC), and the recessive model (TT vs. TC + CC). Identically, the rs3731217 polymorphism contains wild-type allele T and variant allele C. We also employed the similar genetic model to inquiry the association between rs3731217 polymorphism and childhood acute lymphoblastic leukemia predisposition.

Heterogeneity test
Heterogeneity among studies was assessed through Q statistic and I^2 statistic [30], where Q approximately obeys a \( \chi^2 \) distribution with \( k - 1 \) degrees of freedom, while \( k \) is the number of studies. Specially, \( P \) value can be employed to test the significance level of heterogeneity; \( I^2 = (Q - (k - 1))/Q \times 100\% \), varying from 0 to 100\%. Generally, \( I^2 = 50\% \) is a threshold value, when \( I^2 < 50\% \) and \( P \) value >0.1, heterogeneity among studies were acceptable and fixed-effect model is more suitable to compute the merge OR and 95\% CI. On the contrary, if \( I^2 > 50\% \) and \( P \) value <0.1, indicating highly heterogeneous, were existed in those studies and random-effect model was utilized to compute the merge OR and 95\% CI. When necessary, subgroup analyses can be employed.

Sensitivity analysis
Sensitivity test carried out by omitting each of the studies discussed the association of rs3731217 (or rs3731249) with ALL susceptibility, pooled OR and 95\% CI are not of significant difference (Supplementary Tables S5 and S6), which in turn certified the robustness of the relationship between rs3731217 or rs3731249 and ALL predisposition.
### Table 1 Principle characteristics of the studies included in the meta-analysis for rs3731217 polymorphism at CDKN2A locus

| Author                  | Year | Country or Institution | Ethnicity        | Study design | No. of cases | No. of control | Genotyping platform | Kind of genotypes |
|-------------------------|------|------------------------|------------------|--------------|--------------|-------------------|-------------------|-------------------|
| Sherborne et al.        | 2010 | U.K.                   | Caucasian        | GWAS         | 504          | 1438             | Illumina array     | TT/TC/CC          |
| Vijayakrishnan et al.   | 2010 | U.K.                   | Asian            | Replication  | 190          | 182             | KASP               | TT/TC/CC          |
| Orsi et al.             | 2012 | France                 | Caucasian        | GWAS         | 441          | 1984             | Illumina array     | TT/TC/CC          |
| Burmeister et al.       | 2014 | Berlin                 | Caucasian        | Replication  | 322          | 1516            | TaqMan             | T/G               |
| Peyrouze et al.         | 2012 | France                 | Caucasian        | Replication  | 150          | 180             | TaqMan             | T/G               |
| Pastorczak et al.       | 2011 | Poland                 | Caucasian        | Replication  | 398          | 731             | KASP               | TT/TC/CC          |
| Walsh et al.            | 2015 | U.S.A.                 | Caucasian        | Replication  | 980          | 2624            | Affymetrix 6.0     | T/G               |
|                         |      |                        | African-American| Replication  | 163          | 201             | TaqMan             | T/G               |
|                         |      |                        |                  |              |              |                  |                   |                   |
| Vijayakrishnan et al.   | 2015 | U.K.                   | Caucasian        | GWAS         | 824          | 5200            | Illumina array     | TT/TC/CC          |
| Chokkalingam et al.     | 2013 | U.S.A.                 | Caucasian        | GWAS         | 834          | 2024            | Illumina array     | TT/TC/CC          |
|                         |      |                        | Hispanic         | Replication  | 300          | 406             | Sequenom iPLEX     | T/G               |
| Hungate et al.          | 2016 | U.S.A.                 | Caucasian        | Replication  | 1406         | 1399            | Array based Imputation | TT/TC/CC          |
|                         |      |                        | African-American| Replication  | 203          | 1363            | Array based Imputation | TT/TC/CC          |
|                         |      |                        | Hispanic         | Replication  | 391          | 1008            | Array based Imputation | TT/TC/CC          |
| Kreile et al.           | 2016 | Latvia                 | Caucasian        | Replication  | 76           | 121             | PCR-RFLP           | T/C               |
| Gharbi et al.           | 2016 | Tunis                  | African          | Replication  | 58           | 150             | PCR                | TT/TC/CC          |
| Al-absi et al.          | 2017 | Yemen                  | Asian            | Replication  | 136          | 153             | Fluidigm 192.24 Dynamic Array | TT/TC/CC          |

Abbreviations: GWAS, genome-wide association study; KASP, Kompetitive Allele Specific PCR; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

### Table 2 Principle characteristics of the studies included in the meta-analysis for rs3731249 polymorphism at CDKN2A locus

| Author                  | Year | Country or Institution | Ethnicity        | Study design | No. of cases | No. of controls | Genotyping platform | Kind of genotypes |
|-------------------------|------|------------------------|------------------|--------------|--------------|-------------------|-------------------|-------------------|
| Heng et al.             | 2015 | U.S.A.                 | Caucasian        | GWAS         | 1773         | 10448            | Illumina array     | TT/CT/CC          |
|                         |      |                        |                  | Replication  | 410          | 1599             | Illumina array     | TT/CT/CC          |
|                         |      |                        |                  |              | 240          | 277             | PCR               | TT/CT/CC          |
|                         |      |                        |              |              | 824          | 5200            | Illumina array     | TT/CT/CC          |
|                         |      |                        |                  |              |              |                  |                   |                   |
| Healy et al.            | 2007 | U.S.A.                 | Caucasian        | Replication  | 834          | 2024            | Illumina array     | TT/CT/CC          |
|                         |      |                        |                  |              |              |                  |                   |                   |
| Vijayakrishnan et al.   | 2015 | U.K.                   | Caucasian        | GWAS         | 519          | 1016            | KASP              | TT/CT/CC          |
|                         |      |                        |                  | Replication  | 321          | 454             | Illumina array     | T/C               |
|                         |      |                        |                  |              | 980          | 2624            | Affymetrix array   | T/C               |
|                         |      |                        |                  |              | 163          | 201             | TaqMan             | T/C               |
| Walsh et al.            | 2015 | U.S.A.                 | African-American | Replication  | 231          | 338             | PCR               | TT/CT/CC          |
| Gutierrez-Camino et al. | 2017 | Spain                  | Caucasian        | Replication  | 880          | 2624            | TaqMan             | T/C               |

Abbreviations: GWAS, genome wide association study; KASP, Kompetitive Allele Specific PCR; PCR, polymerase chain reaction.
Paper quality assessment
The quality estimation of the researches was based on the methodological quality assessment scale, which was adjusted from the Newcastle–Ottawa assessment scale for case–control studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). The scales evaluated study qualities in several aspects (Supplementary Table S10): (1) inclusion and exclusion criteria of patients, (2) source of controls, (3) comparability of cases and controls, (4) sample size, (5) quality control of genotyping methods, and (6) HWE. Strictly obey the principles, two investigators separately evaluated and scored each study and the final results reached an agreement with a third author participated in the discussion. The final score showed in Supplementary Tables S11 and S12, and the higher the score, the better is the quality of the study.

Publication bias analysis and Hardy–Weinberg equilibrium (HWE) test
According to the Cochrane Handbook for Systematic Reviews for Interventions, we examined the potential publication bias for all the rs3731217 studies (or rs3731249) by Begg’s funnel plot and Egger’s test (Supplementary Figures S1 and S2). Further, HWE was assessed by Chi-square test among the included studies in both SNPs studies respectively (Supplementary Tables S8 and S9).

Results
Study characteristics
Through literature search with keywords (see Methods), 24 independent researches manifested in 16 literatures met the inclusion criteria, and were selected for meta-analyses (Figure 1), describing association between ALL susceptibility and the SNPs at CDKN2A locus. For rs3731217, association of this SNP with ALL susceptibility was first reported in 2010 through GWAS approach, and investigated in other three GWASs or follow-up candidate studies. For the missense SNP rs3731249 (A148T), its influence on ALL risk was first reported in 2015, and was validated in another three studies. Since rs3731249 and rs36228834 are in perfect linkage disequilibrium (LD) with each other (r² = 1) in Caucasians, an earlier study can thus be included in the meta-analysis for rs3731249. The characteristics of 19 studies on rs3731217 and 10 studies on rs3731249 were summarized in Tables 1 and 2 respectively.

Meta-analysis of the rs3731217 polymorphism and ALL susceptibility
Nineteen studies assessed the association between rs3731217 and ALL susceptibility with a total of 7922 cases and 21503 controls. As no significant heterogeneity was observed in the allele model (P=0.87 and I² = 0%, Figure 2A), we applied fixed-effect model to conduct the meta-analysis, and found that C allele significantly exhibited a 0.72-fold (odds ratio [OR] = 0.72, 95% confidence interval [CI]: 0.68–0.77) increased risk to develop ALL (P < 0.0001, Figure 2A and Supplementary Table S2) compared with T allele. In addition, no heterogeneity was observed in the dominant model (P=0.51 and I² = 0%) and recessive model (P=0.86 and I² = 0%) among 12 out of 19 studies that individual genotypes can be got (Figure 2B and Supplementary Table S1). Not surprisingly, results consistently exhibited that both genotypes CC and CT could lower the ALL predisposition. Next, we examined the effect of rs3731217 across ethnicities, age and ALL subtypes, no association was observed in Asian or T-lineage ALL patients (Figure 3A–C), possibly because of the small sample size for patients with these clinical characteristics in the selected reports.

Meta-analysis of the rs3731249 polymorphism and ALL susceptibility
Five articles assessed the association between rs3731249 and ALL susceptibility with a total of 6295 cases and 24181 controls. As rs36228834 is in prefect LD with rs3731249 in Caucasians, another study was also included to conduct this meta-analysis. Since no significant heterogeneity was observed in the allele model (P=0.35 and I² = 10%, Figure 4A and Supplementary Table S4), we used fixed model to estimate the influence of the variant allele, and found that the minor allele (T) was significantly augment the ALL risk (P < 0.0001, OR = 2.26, and 95% CI: 2.06–2.48). In addition, no heterogeneity was observed in the dominate model (P=0.19 and I² = 32%) and recessive model (P=0.68 and I² = 0%) among 7 out of 10 studies that individual genotypes can be got (Figure 4B and Supplementary Table S3). Given rs3731249 was located in the coding region of an important tumor suppressor gene CDKN2A, and induced alanine to threonine alteration in 148 position (A148T), allelic imbalance was evaluated in somatic leukemia cells from individuals with heterozygous genotype of rs3731249 in 15 and 35 cases in two independent studies and indicated the variant allele preferentially retained significantly through either copy number variation or post-transcriptional inactivation during leukemogenesis.
Figure 1. Flow chart of included studies for analyzing the association between rs3731217 and rs3731249 polymorphisms with ALL susceptibility
Abbreviation: ALL, acute lymphoblastic leukemia.

Publication bias analysis and sensitivity analysis
We utilized Begg's test and Egger's test to measure the publication bias for the all models for both SNPs, no evidence of obvious asymmetry was observed (Supplementary Figures S1 and S2). The result of sensitivity analysis showed that the association between rs3731217 (or rs3731249) and ALL risk doesn’t significantly fluctuate when removing each of the studies (Supplementary Tables S5 and S6).

Ethnic diversity and LD pattern of SNPs at CDKN2A locus
Variant allele of rs3731217 was common across all ethnicities, while variant allele of rs3731249 was only common in Caucasians and Hispanics, rare in Africans, even not observed in Asians. It could be the reason that no association between rs3731217 with ALL risk if rs3731249 was tagged by this SNP, we next investigated the genetic characteristic of CDKN2A locus across ethnicities, and identified the similar LD pattern between Caucasians and Hispanics, much less extensive in Africans and Asians. However, rs3731217 and rs3731249 were not in the same LD region, with $r^2 < 0.01$ in Caucasians and Hispanics based on 1000 genome data (Supplementary Figure S3), which was consistent with the fact that these two SNPs associated with ALL risk independently in conditional model.

Causal variant candidate determination
Variant allele of rs3731249 induced amino acid change in p16INK4A, protein product of CDKN2A, and reduced the tumor suppression effect of p16INK4A, suggesting rs3731249 act as a causal variant for ALL susceptibility. We next investigated the potential causal variant tagged by rs3731217 by using webtool (Haploreg), because association of this SNP with ALL can’t explained by rs3731249. Totally 17 known SNPs showed moderate LD ($r^2 > 0.4$) with rs3731217.
Figure 2. Forest plots of ALL predisposition associated with rs3731217 polymorphism under genetic models
(A) Allelic model analysis (C vs. T) of rs3731217 and ALL risk. (B) Dominant (TC + CC vs. TT) and recessive (CC vs. TC + TT) model analysis of rs3731217 and ALL risk. Studies were plotted refer to the first author followed by publication year. For each research, the estimates of OR and its 95% CI are exhibited with square and a horizontal line. The area of the squares reflects the weight. The diamond represents the summary OR and 95% CI. Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; OR, odds ratio.

Figure 3. Forest plots of ALL susceptibility associated with rs3731217 polymorphism across ethnicity, age, and immunophenotype subtypes under the allelic model
Forest plots of ALL susceptibility associated with rs3731217 polymorphism across ethnicity (A), age (B), and immunophenotype (C) subtypes under the allelic model (C vs. T). For each study, the estimates of OR and its 95% CI are plotted with square and a horizontal line. The area of the squares reflects the weight. The diamond represents the summary OR and 95% CI. Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; OR, odds ratio.
in Caucasians (Chr9:21956078-22039426) (Supplementary Table S7). Among these SNPs, rs2811711 is located within the region marked by activated promoter or enhancer histones in multiple tissue types, especially in B cells, based on the chromatin state information from Roadmap and ENCODE database (Figure 5A), and DNA-binding proteins (i.e. POL2, TBP, and GATA1) can also bind the rs2811711-located spot strongly (Figure 5B), suggesting the potential important role of rs2811711 on CDKN2A regulation (e.g. gene expression level). Moreover, variant allele frequency of rs2811711 is high in Caucasians, but no detected in Asians, which could explain the different association status of rs3731217 in diverse ethnic populations. Although further functional experiments are needed, rs2811711 could be considered as a causal variant candidate for ALL susceptibility.

Discussion

A series of GWAS approaches had identified at least several loci that are significantly associated with ALL susceptibility. After the extensive replication studies, some loci can be validated in all independent multiethnic cohorts (e.g. ARID5B), whereas inconsistent associations were noticed in others (e.g. CDKN2A). Considering CDKN2A acts as an important tumor suppressor gene and is frequently inactivated in somatic leukemic cells [31], germline variants could also impact CDKN2A function during leukemogenesis through either inducing amino acid alteration or expression decreasing. To systematically investigate the influence of SNPs at CDKN2A locus on ALL risk, we conducted a meta-analysis by pooling the ALL-related GWAS and replication studies. Both rs3731217 and rs3731249 exhibits significant associations with ALL risk in this large-scale sample size analysis with 7922 cases/21503 controls and 6295 cases/24181 controls respectively. Individuals carrying risk alleles of these two SNPs have 0.72-fold and 2.26-fold increase in disease susceptibility respectively. What’s more, influence of rs3731217 risk allele diverse among ages and ethnicities, possibly because the ALL-associated causal variants are ethnic-specific, or the causal variants can’t be tagged by rs3731217 due to different LD pattern among diverse ethnicities. Similarly, rs3731249 is tend to be an ethnic specific GWAS variant, because the risk allele of rs3731249 were not detected in Asians (MAF = 0, N = 4254), rare in Africans (MAF = 0.42%, N = 4879), common in Hispanics (MAF = 1.41%, N = 5739) and Caucasians (MAF = 3.52%, N = 32549) according to the largest exome database (ExAC Browser, http://exac.broadinstitute.org/) [32], indicating the different value of these SNPs on ALL risk assessment across ethnicities. Lack of association for both CDKN2A SNPs in Asian population raises a possibility that these two SNPs are in the same LD region, and can be explained by the same causal variant. However, rs3731249 poorly links to rs3731217 across all ethnicities ($r^2 < 0.01$ (Supplementary Figure S3), and is associated with ALL susceptibility independently according to conditional analysis, suggesting multiple causal variants located within the CDKN2A locus. Moreover, we combined the recent studies and noticed that wild-type allele of rs3731249 is tend to be lost through either loss of heterozygosity or post-transcriptional inactivation during the leukemogenesis process significantly.
By the end of 2017, ~2110 GWAS had published, reporting around 18000 genome-wide significant ($P < 5 \times 10^{-8}$) SNPs associated with >1000 traits [33], but only a few SNPs identified were considered to be functional and causal, such as rs11685232, a missense SNP induced NUDT15 deficient for 6-mercaptopurine metabolism [34], while the vast majority of GWAS signals were intronic or intergenic SNPs, and considered to tag the nearby causal variants [35]. Great effort has been taken to search the causal variants through fine mapping, or alternatively by exome-array based GWAS, followed by functional analyses. Missense variants are easier to be determined as causal variants by functional analyses or experiments, such as rs1127354 and rs7270101 in TTPA, which can predict the ribavirin-induced anemia [36], and rs3731249 in this study, which induce loss of function of the tumor suppressor p16<sup>INK4A</sup>. Interestingly, we and another independent group also observed that the risk allele is enriched in the leukemic cells through either loss of heterozygosity in DNA level or allele-specific epigenetic modification [14]. In another hand, however, more and more evidence indicate that causal variants are located in the noncoding regions (e.g. promoter, enhancer etc.), and possibly affect the phenotypes by regulating the expression level of the nearby genes, such as rs1427407 in <i>BCL11A</i>, which altered the GATA1 and TAL binding motifs of <i>BCL11A</i> enhancer region respectively [37]. The systematic analyses have been done to estimate or screen these expression quantitative trait loci (eQTL), including the recently released big project of Genotype-Tissue Expression (GTex) [38]. Actually, more and more causal GWAS signals were considered to be eQTLs rather than directly affecting the protein coding. In another hand, a recent report demonstrated that rs3731217 was associated with the usage of <i>CDKN2A</i> exon 3 [39], which requires more experimental determination but provides an alternative mechanism for GWAS explanation.
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Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution
X.Z. and F.L. searched the literature for selection of relevant studies. J.Z. and Y.Q. verified the selection and extracted the required data from the articles. X.Z, F.L., Z.D., and Y.Z. performed the analysis. H.X. and F.Z. wrote the manuscript. All the authors reviewed and approved the final manuscript.

Abbreviations
ALL, acute lymphoblastic leukemia; eQTL, expression quantitative trait loci; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

References
1. Pui, C.H., Robison, L.L. and Look, A.T. (2008) Acute lymphoblastic leukaemia. Lancet 371, 1030–1043. https://doi.org/10.1016/S0140-6736(08)60457-2
2. Yang, J.J. et al. (2008) Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. Blood 112, 4178–4183. https://doi.org/10.1182/blood-2008-06-165027
3. Mullighan, C.G. et al. (2008) Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 322, 1377–1380. https://doi.org/10.1126/science.1164266
4. Okuda, T. et al. (1995) Frequent deletion of p16INK4a/MTS1 and p15INK4b/MTS2 in pediatric acute lymphoblastic leukemia. Blood 85, 2321–2330
5. Xu, H. et al. (2013) Novel susceptibility variants at 10p12.31-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. J. Natl. Cancer Inst. 105, 733–742. https://doi.org/10.1093/jnci/djt042
6. Trevino, L.R. et al. (2009) Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat. Genet. 41, 1001–1005. https://doi.org/10.1038/ng.432
7. Perez-Andreu, V. et al. (2015) A genome-wide association study of susceptibility to acute lymphoblastic leukemia in adolescents and young adults. Blood 125, 680–686. https://doi.org/10.1182/blood-2014-09-595744
8. Perez-Andreu, V. et al. (2013) Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. Nat. Genet. 45, 1494–1508. https://doi.org/10.1038/ng.2803
9. Sherborne, A.L. et al. (2010) Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat. Genet. 42, 492–494. https://doi.org/10.1038/ng.585
10. Xu, H. et al. (2012) ARID5B genetic polymorphisms contribute to racial disparities in the incidence and treatment outcome of childhood acute lymphoblastic leukemia. J. Clin. Oncol. 30, 751–757. https://doi.org/10.1200/JCO.2011.38.0345
11. Liao, F. et al. (2016) Association between PIP4K2A polymorphisms and acute lymphoblastic leukemia susceptibility. Medicine 95, e3542. https://doi.org/10.1097/MD.0000000000003542
12. Guo, L.-M. et al. (2014) ARID5B gene rs10821936 polymorphism is associated with childhood acute lymphoblastic leukemia: a meta-analysis based on 39,116 subjects. Tumor Biol. 35, 709–713. https://doi.org/10.1007/s13277-013-1097-0
13. Prasad, R.B. et al. (2010) Verification of the susceptibility loci on 7p12.2, 10q21.2, and 14q11.2 in precursor B-cell acute lymphoblastic leukemia. J. Clin. Oncol. 28, 751–757. https://doi.org/10.1200/JCO.2011.38.0345
14. Walsh, K.M. et al. (2015) A heritable missense polymorphism in CDKN2A confers strong risk of childhood acute lymphoblastic leukemia and is preferentially selected during clonal evolution. Cancer Res. 75, 4884–4894. https://doi.org/10.1158/0008-5472.CAN-15-1105
15. Vijayakrishnan, J. et al. (2015) The 9p21.3 risk of childhood acute lymphoblastic leukaemia is explained by a rare high-impact variant in CDKN2A. Sci. Rep. 5, 15065. https://doi.org/10.1038/srep15065
16. Gutierrez-Camino, A. et al. (2017) Confirmation of involvement of new variants at CDKN2A/B in pediatric acute lymphoblastic leukemia susceptibility in the Spanish population. PLoS One 12, e0177421. https://doi.org/10.1371/journal.pone.0177421
17. Vijayakrishnan, J. et al. (2010) Variation at 1p12.2 and 10q21.2 influences childhood acute lymphoblastic leukemia risk in the Thai population and may contribute to racial differences in leukemia incidence. Leuk. Lymphoma 51, 1870–1874. https://doi.org/10.3109/10428194.2010.511356
19 Orsi, L. et al. (2012) Genetic polymorphisms and childhood acute lymphoblastic leukemia: GWAS of the ESCALE study (SFCE). *Leukemia* **26**, 2561–2564, https://doi.org/10.1038/leu.2012.148

20 Peyrouze, P. et al. (2012) Genetic polymorphisms in ARID5B, CEBPB, IKZF1 and CDKN2A in relation with risk of acute lymphoblastic leukaemia in adults: a Group for Research on Adult Acute Lymphoblastic Leukaemia (GRAALL) study. *Br. J. Haematol.* **159**, 599–602

21 Pastorczak, A. et al. (2011) Role of 657del5 NBN mutation and 7p12.2 (IKZF1), 9p21 (CDKN2A), 10q21.2 (ARID5B) and 14q11.2 (CEBPE) variation and risk of childhood ALL in the Polish population. *Leuk. Res.* **35**, 1534–1536, https://doi.org/10.1016/j.leukres.2011.07.034

22 Chokkalingam, A.P. et al. (2013) Genetic variants in ARID5B and CEBPE are childhood ALL susceptibility loci in Hispanics. *Cancer Causes Control.* **24**, 1789–1795, https://doi.org/10.1007/s10552-013-0256-3

23 Hungate, E.A. et al. (2016) A variant at 9p21.3 functionally implicates CDKN2B in paediatric B-cell precursor acute lymphoblastic leukaemia aetiology. *Nat. Commun.* **7**, 10635, https://doi.org/10.1038/ncomms10635

24 Kreile, M. et al. (2016) Analysis of possible genetic risk factors contributing to development of childhood acute lymphoblastic leukemia in the Latvian population. *Arch. Med. Sci.* **12**, 479–485, https://doi.org/10.5114/ams.2016.59920

25 Vijayakrishnan, J. et al. (2016) A genome-wide association study identifies risk loci for childhood acute lymphoblastic leukaemia at 10q26.13 and 12q23.1. *Leukemia* **3**, 573–579

26 Gharbi, H. et al. (2016) Association of genetic variation in IKZF1, ARID5B, CDKN2A, and CEBPE with the risk of acute lymphoblastic leukaemia in Tunisian children and their contribution to racial differences in leukaemia incidence. *Pediatr. Hematol. Oncol.* **7**, 1–17, https://doi.org/10.3109/08880018.2016.1161685

27 Burmeister, T. et al. (2014) Germline variants in IKZF1, ARID5B, and CEBPE as risk factors for adult-onset acute lymphoblastic leukaemia: an analysis from the GMALL study group. *Haematologica* **99**, e23–e25, https://doi.org/10.3324/haematol.2013.090720

28 Al-Abasi, B. et al. (2017) Contributions of IKZF1, DDC, CDKN2A, CEBPE, and LM01 Gene Polymorphisms to Acute Lymphoblastic Leukemia in a Yemeni Population. *Genet. Test Mol. Biomarkers* **10**, 592–599, https://doi.org/10.1089/gtmb.2017.0084

29 Healy, J. et al. (2007) Promoter SNPs in G1/S checkpoint regulators and their impact on the susceptibility to childhood leukemia. *Blood* **109**, 683–692, https://doi.org/10.1182/blood-2006-02-003236

30 Laliman, V. and Roiz, J. (2014) Frequentist approach for detecting heterogeneity in meta-analysis pair-wise comparisons: enhanced q-test use by using I2 and H2 statistics. *Value Health* **17**, A576, https://doi.org/10.1016/j.jval.2014.08.1942

31 Iacobucci, I. and Mullighan, C.G. (2017) Genetic basis of acute lymphoblastic leukaemia. *J. Clin. Oncol.* **35**, 975–983, https://doi.org/10.1200/JCO.2016.60.7836

32 Lek, M. et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 265, https://doi.org/10.1038/nature19057

33 MacArthur, J. et al. (2016) The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Res.* **45**, D896–D901, https://doi.org/10.1093/nar/gkw1133

34 Moriyama, T. et al. (2015) NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat. Genet.* **47**, 367

35 Do, C. et al. (2017) Genetic–epigenetic interactions in cis: a major focus in the post-GWAS era. *Genome Biol.* **18**, 120, https://doi.org/10.1186/s13059-017-1250-y

36 Fellay, J. et al. (2010) ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* **464**, 405, https://doi.org/10.1038/nature08825

37 Bauer, D.E. et al. (2013) An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science* **342**, 253–257, https://doi.org/10.1126/science.1242088

38 Brown, A.A. et al. (2017) Predicting causal variants affecting expression by using whole-genome sequencing and RNA-sequencing from multiple human tissues. *Nat. Genet.* **49**, 1747, https://doi.org/10.1038/ng.3979

39 Hungate, E.A. et al. (2016) A variant at 9p21.3 functionally implicates CDKN2B in paediatric B-cell precursor acute lymphoblastic leukaemia aetiology. *Nat. Commun.* **7**, 10635, https://doi.org/10.1038/ncomms10635