

## Brief report

## Verification of the susceptibility loci on 7p12.2, 10q21.2, and 14q11.2 in precursor B-cell acute lymphoblastic leukemia of childhood

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**Recent genome-wide association data have implicated genetic variation at 7p12.2 (*IKZF1*), 10q21.2 (*ARIDB5*), and 14q11.2 (*CEBPE*) in the etiology of B-cell childhood acute lymphoblastic leukemia (ALL). To verify and further examine the relationship between these variants and ALL risk, we genotyped 1384 cases of precursor B-cell childhood ALL and 1877 controls**

**from Germany and the United Kingdom. The combined data provided statistically significant support for an association between genotype at each of these loci and ALL risk; odds ratios (OR), 1.69 ( $P = 7.51 \times 10^{-22}$ ), 1.80 ( $P = 5.90 \times 10^{-28}$ ), and 1.27 ( $P = 4.90 \times 10^{-6}$ ), respectively. Furthermore, the risk of ALL increases with an increasing numbers of variant**

**alleles for the 3 loci (OR<sub>per-allele</sub> = 1.53, 95% confidence interval, 1.44-1.62;  $P_{trend} = 3.49 \times 10^{-42}$ ), consistent with a polygenic model of disease susceptibility. These data provide unambiguous evidence for the role of these variants in defining ALL risk underscoring approximately 64% of cases. (Blood. 2010;115:1765-1767)**

## Introduction

Two genome-wide association (GWA) studies provided evidence that common germline variation influences the risk of precursor B-cell childhood acute lymphoblastic leukemia (ALL).<sup>1,2</sup> The strongest association was attained at 7p12.2 with the single nucleotide polymorphism (SNP) rs4132601 located 3' to the *ikaros family zinc finger 1* (*IKZF1*) gene. Ikaros proteins are master regulators of lymphocyte development and differentiation directing the CD4 versus CD8 T-cell lineage commitment.<sup>3</sup> Mutant mice expressing only non-DNA-binding Ikaros isoforms develop aggressive lymphoblastic leukemia.<sup>4,5</sup> *IKZF1* somatic deletions are common at diagnosis in high-risk/poor prognosis B-cell precursor ALL and in ALL with BCR-ABL1 fusions.<sup>6,7</sup> Functional basis for the association between rs4132601 and ALL was provided by the correlation between reduced *IKZF1* expression and risk genotype in lymphocytes.<sup>1</sup> The second locus identified identified was at 10q21.2 with rs7089424 located in intron 3 of the *AT-rich interactive domain 5B* (*ARIDB5*) gene. *ARIDB5* is a member of the AT-rich interaction domain family of transcription factors, important in embryogenesis and growth regulation.<sup>8,9</sup> The third association seen in only one of the GWA studies was at 14q11.2 with rs2239633 in the vicinity of *CEBPE*, encoding CCAAT/enhancer-binding protein,  $\epsilon$  a regulator of myelopoiesis.

Association studies are often capricious; therefore, replication is highly desirable. Replication across populations can also augment generalization of findings. Estimates of the impact of risk loci derived from GWA studies have a tendency to be overinflated because of "winner's curse." To verify and further explore the relationship between the 7p12.2, 10q21.2, and 14q11.2 variants and ALL risk, we genotyped 2 case-control series from Germany and the United Kingdom totaling 1384 patients and 1877 controls.

## Methods

The German case-control study comprised 1193 patients (663 male and 530 female; mean age, 6 years) with childhood ALL ascertained through the Berlin-Frankfurt-Münster trials between 1993 and 2004. A total of 1516 controls (762 male and 754 female; mean age, 58 years) were ethnically matched healthy persons of German origin recruited in 2004 at the Institute of Transfusion Medicine, Mannheim, Germany.<sup>10</sup> The United Kingdom series was composed of 191 patients (101 male and 90 female) not previously analyzed as part of our GWA study: 114 prevalent cases from the United Kingdom Childhood Cancer Study, an epidemiologic study of childhood malignancies conducted between 1991 and 1998, 24 incident cases from the United Kingdom MRC ALL 97 (99) trial, and 53 cases from Royal Marsden NHS Hospitals Trust. Mean age at diagnosis of patients was

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**Table 1. Association between SNPs and ALL risk**

SNP (gene)	Population	Genotype	Cases (%)	Controls (%)	OR (95% CI)	P
rs4132601 ( <i>IKZF1</i> )	German	AA	471 (39.6)	811 (54.0)	1.0 (referent)	
		AC	552 (46.4)	574 (38.2)	1.7 (1.4-2.0)	$1.2 \times 10^{-9}$
		CC	166 (14.0)	116 (7.8)	2.5 (1.9-3.2)	$7.6 \times 10^{-12}$
	United Kingdom	AA	60 (31.9)	206 (57.2)	1.0 (referent)	
		AC	96 (51.1)	133 (36.9)	2.5 (1.7-3.7)	$3.8 \times 10^{-6}$
		CC	32 (17.0)	21 (5.8)	5.2 (2.7-10.2)	$2.9 \times 10^{-8}$
	Overall	AA	531 (38.6)	1017 (54.6)	1.0 (referent)	
		AC	648 (47.0)	707 (38.0)	1.8 (1.5-2.0)	$1.4 \times 10^{-13}$
		CC	198 (14.4)	137 (7.4)	2.8 (2.2-3.6)	$2.9 \times 10^{-17}$
rs7089424 ( <i>ARID5B</i> )	German	AA	346 (29.0)	683 (45.3)	1.0 (referent)	
		AC	595 (50.0)	673 (44.6)	1.8 (1.5-2.1)	$1.1 \times 10^{-10}$
		CC	249 (21.0)	152 (10.1)	3.2 (2.5-4.1)	$1.0 \times 10^{-22}$
	United Kingdom	AA	46 (25.0)	153 (43.0)	1.0 (referent)	
		AC	99 (53.8)	165 (46.3)	2.0 (1.3-3.0)	$9.5 \times 10^{-4}$
		CC	39 (21.2)	38 (10.7)	3.4 (1.9-6.1)	$8.8 \times 10^{-6}$
	Overall	AA	392 (28.5)	836 (44.9)	1.0 (referent)	
		AC	694 (50.5)	838 (44.9)	1.8 (1.5-2.1)	$8.7 \times 10^{-13}$
		CC	288 (21.0)	190 (10.2)	3.2 (2.6-4.0)	$7.1 \times 10^{-27}$
rs2239633 ( <i>CEBPE</i> )	German	AA	197 (16.5)	307 (20.3)	1.0 (referent)	
		AG	559 (46.9)	773 (51.2)	1.1 (0.9-1.4)	$2.6 \times 10^{-1}$
		GG	437 (36.6)	430 (28.5)	1.6 (1.3-2.0)	$5.1 \times 10^{-5}$
	United Kingdom	AA	26 (14.2)	64 (18.2)	1.0 (referent)	
		AG	95 (51.9)	183 (52.0)	1.3 (0.7-2.2)	$3.5 \times 10^{-1}$
		GG	62 (33.9)	105 (29.8)	1.5 (0.8-2.6)	$1.8 \times 10^{-1}$
	Overall	AA	223 (16.2)	371 (20.0)	1.0 (referent)	
		AG	654 (47.5)	956 (51.3)	1.1 (0.9-1.4)	$1.9 \times 10^{-1}$
		GG	499 (36.3)	535 (28.7)	1.6 (1.3-1.9)	$2.8 \times 10^{-5}$

6 years. Controls were healthy persons collected through a national study of colorectal cancer being conducted in the United Kingdom (117 male and 244 female; mean age, 59 years).<sup>11</sup>

Collection of blood samples and clinicopathologic information from patients and controls was undertaken with informed consent and relevant ethical review board approval from the Institute for Cancer Research in accordance with the Declaration of Helsinki. DNA extractions were performed using standard methodologies. SNP genotyping was conducted using the Kaspar allele-specific polymerase chain reaction (KBiosciences) and analyzed in an Applied Biosystem ABI 7900HT system. Primer sequences and polymerase chain reaction conditions are available on request. Quality control was monitored through blind genotyping of 15 DNA samples with 100% concordance and inclusion of 6% of DNA samples as replicates in each round of genotyping.

For the 3 SNPs, risk of ALL was quantified by calculating odds ratios (OR) with 95% confidence intervals using the STATA statistical package (Version 10.0; Stata Corporation). All statistical tests were 2-sided. To provide increased power to demonstrate a relationship between SNP genotype and risk, we pooled data from the 2 case-control series. Meta-analysis was performed under a fixed effects model, estimating Cochran's Q statistic to test for heterogeneity and the I<sup>2</sup> statistic to quantify the proportion of the total variation between studies. Combined effect of pairs of the 3 loci with risk was investigated by logistic regression modeling, and evidence for interactive effects between SNPs was assessed by a likelihood ratio test. The population attributable fraction was estimated from  $1 - \Pi(1 - (x - 1)/x)$ , where  $x = (1 - p)^2 + 2p(1 - p)OR_1 + p^2OR_2$ , p is the population allele frequency, and OR<sub>1</sub> and OR<sub>2</sub> are the ORs associated with heterozygosity and homozygosity, respectively.

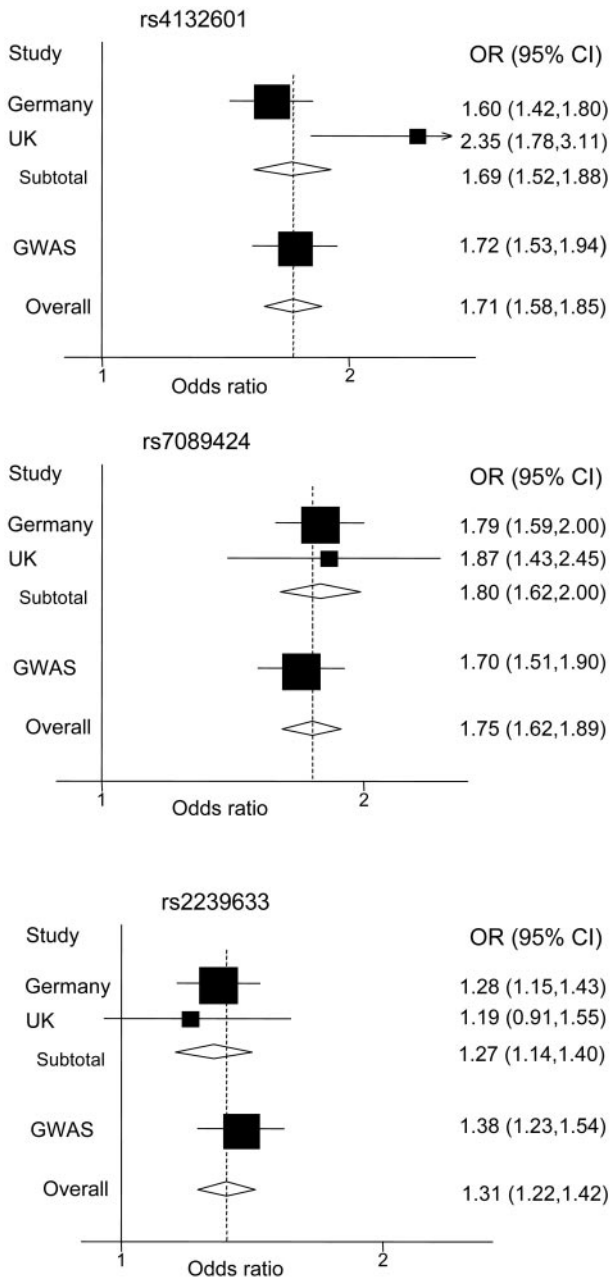
## Results and discussion

Genotypes were obtained for 3572 of 3579 for DNA samples from the German cases (99.8%) and 4519 of 4548 controls (99.4%). The call rate for United Kingdom series was 555 of 578 (96.0%) in the cases and 1068 of 1086 (98.3%) in the controls. SNP call rates were

not significantly different between all cases and controls or between each of the 2 case-control studies. Population stratification was ruled out as the genotype distribution satisfied the criterion for Hardy-Weinberg equilibrium in both control series. The genotype frequencies for each of the 3 SNPs in both case-control series showed a strong relationship with ALL risk in the German series (Table 1). Although a similar relationship between genotype and risk was observed in the United Kingdom series, the association was not statistically significant for rs2239633 (Table 1). In both datasets, the pattern of risk associated with each SNP was compatible with an additive model of disease susceptibility.

The per allele OR of ALL associated with rs4132601, rs7089424, and rs2239633 was 1.69 ( $P = 7.51 \times 10^{-22}$ ), 1.80 ( $P = 5.90 \times 10^{-28}$ ), and 1.27 ( $P = 4.90 \times 10^{-6}$ ), respectively (Figure 1). The associated measures of heterogeneity for the same SNPs were  $P_{het} = .026$ ,  $I^2 = 83\%$ ;  $P_{het} = .76$ ,  $I^2 = 0\%$ ; and  $P_{het} = .63$ ,  $I^2 = 0\%$ , respectively. Further pooling with data from the United Kingdom GWA study provided unambiguous evidence for a relationship between variations at these loci and ALL risk<sup>1</sup> (Figure 1).

No evidence of interactive effects between any of the 3 pairs of loci ( $P > .05$ ) was observed, suggesting that each locus has an independent role in defining the risk. Although the risks of ALL associated with the 3 variants are individually modest, the carrier frequencies of the risk alleles are high in the European population, and the loci make a major contribution to the development of ALL. The estimated population attributable fraction was compatible with approximately 64% of ALL being defined by these variants. Risk of ALL increases with an increasing number of risk alleles for the 3 loci, counting 2 for a homozygote and one for a heterozygote assuming equal weights ( $OR_{per-allele} = 1.53$ , 95% confidence interval, 1.44-1.62;  $P_{trend} = 3.49 \times 10^{-42}$ ). Persons with 5 or more risk alleles have a more than 4-fold increase in risk compared with



**Figure 1.** Forest plots of ORs of ALL for the 3 SNPs. Boxes represent allelic OR point estimates, their areas being proportional to the inverse variance weight of the estimate. Horizontal lines represent 95% confidence intervals. Diamond (and broken line) represents the summary OR computed under a fixed effects model, with 95% confidence interval given by its width. The unbroken vertical line is at the null value (OR = 1.0).

## References

- Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet.* 2009;41(9):1006-1010.
- Trevino LR, Yang W, French D, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. *Nat Genet.* 2009;41(9):1001-1005.
- Harker N, Naito T, Cortes M, et al. The CD8alpha gene locus is regulated by the Ikaros family of proteins. *Mol Cell.* 2002;10(6):1403-1415.
- Georgopoulos K, Bigby M, Wang JH, et al. The Ikaros gene is required for the development of all lymphoid lineages. *Cell.* 1994;79(1):143-156.
- Klug CA, Morrison SJ, Masek M, Hahn K, Smale ST, Weissman IL. Hematopoietic stem cells and lymphoid progenitors express different Ikaros isoforms, and Ikaros is localized to heterochromatin in immature lymphocytes. *Proc Natl Acad Sci U S A.* 1998;95(2):657-662.
- Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature.* 2008;453(7191):110-114.
- Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med.* 2009;360(5):470-480.
- Wiilsker D, Patsialou A, Dallas PB, Moran E. ARID proteins: a diverse family of DNA binding proteins implicated in the control of cell growth, differentiation, and development. *Cell Growth Differ.* 2002;13(3):95-106.
- Lahoud MH, Risteovski S, Venter DJ, et al. Gene targeting of Desrt, a novel ARID class DNA-binding protein, causes growth retardation and abnormal development of reproductive organs. *Genome Res.* 2001;11(8):1327-1334.
- Gast A, Bermejo JL, Flohr T, et al. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. *Leukemia.* 2007;21(2):320-325.
- Penegar S, Wood W, Lubbe S, et al. National study of colorectal cancer genetics. *Br J Cancer.* 2007;97(9):1305-1309.

those with a median number of risk alleles. These ORs may be underestimates because the additive model assumes equal weighting across SNPs. The present data provide only estimates of the effect on susceptibility attributable to the variations. The effect of the actual causal variant responsible for the association is probably larger.

The assertion that ALL may have a genetic basis has long been pursued through association studies based on candidate genes without clear insight into the causal basis of ALL. Many of the studies have been based on small sample series with inconsistent findings. As with the previously published GWA studies, the strongest associations identified were for the SNPs mapping to *IKZF1* and *ARIDB5*. However, our study provides independent evidence that variation in *CEBPE* plays a significant role in ALL. Collectively, these data provide robust evidence that variations in *IKZF1*, *ARIDB5*, and *CEBPE* influence the risk of ALL and that these variants collectively play a major role in the development of this hematologic malignancy.

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## Authorship

Contribution: R.B.P., J.V., E.P., A.G., and R. Koehler performed experiments; C.R.B., R.S.H., R. Kumar, and K.H. designed research; F.J.H. analyzed data; R. Koehler, M.G., E.S., S.E.K., T.L., E.R., M.T., K.P.-J., M. Stanulla, and M. Schrappe provided DNA for analysis; R.S.H., R. Kumar, and K.H. wrote the paper; M.G., K.P.-J., M. Stanulla, M. Schrappe, and C.R.B. contributed to the paper; and all authors approved the paper.

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