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Genetic Variants Modify Susceptibility to Leukemia in Infants: A Children's Oncology Group Report

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Abstract

Background—The mixed lineage leukemia (*MLL*) gene is commonly rearranged in infant leukemia (IL). Genetic determinants of susceptibility to IL are unknown. Recent genome wide association studies for *childhood* acute lymphoblastic leukemia (ALL) have identified susceptibility loci at *IKZF1*, *ARID5B*, and *CEBPE*.

Procedure—We genotyped these loci in 171 infants with leukemia and 384 controls and evaluated associations overall, by subtype (ALL, acute myeloid leukemia (AML)), and by presence (+) or absence (−) of *MLL* rearrangements.

Results—Homozygosity for a variant *IKZF1* allele (rs11978267) increased risk of infant AML (Odds Ratio (OR)=3.9, 95% Confidence Interval (CI)=1.8–8.4); the increased risk was similar for AML/*MLL*+ and *MLL*− cases. In contrast, risk of ALL/*MLL*− was increased in infants homozygous for the *IKZF1* variant (OR=5.1, 95%CI=1.8–14.5) but the variant did not modify risk of ALL/*MLL*+. For *ARID5B* (rs10821936), homozygosity for the variant allele increased risk for the ALL/*MLL*− subgroup only (OR=7.2, 95%CI=2.5–20.6). There was little evidence of an association with the *CEBP* variant (rs2239633).

Conclusion—*IKZF1* is expressed in early hematopoiesis, including precursor myeloid cells. Our data provide the first evidence that *IKZF1* modifies susceptibility to infant AML, irrespective of *MLL* rearrangements, and could provide important new etiologic insights into this rare and heterogeneous hematopoietic malignancy.

Keywords

leukemia; genetic susceptibility; infants

INTRODUCTION

Leukemias in infancy (<12 months) are extremely rare; ~40 cases per million infants are diagnosed each year in the US. Infant leukemias (IL) are biologically distinct from

leukemias in later childhood and adolescence. In older children, acute lymphoblastic leukemia (ALL) is almost five times more common than acute myeloid leukemia (AML); in infants the ratio is closer to 1.3 [1]. Most infant cases (~60–80% ALL, 30–50% AML) present with mixed lineage leukemia (*MLL*) gene rearrangements in their leukemia cells [2–7], whereas *MLL* rearrangements occur in only 6% and 14% of childhood ALL and AML, respectively [8]. Evidence from molecular studies of twins suggests that ILs arise *in utero*, possibly due to transplacental carcinogen exposure [9]. However, given the rarity of IL, it is extremely difficult to identify contributing factors. Like childhood leukemia, it is feasible that IL arises from a combination of events, perhaps on the background of underlying genetic susceptibility.

Very few genetic susceptibility studies have specifically focused on IL. Candidate gene studies suggest a potential role of single nucleotide polymorphisms (SNPs) in methylenetetrahydrofolate (*MTHFR*), NAD(P)H:quinone oxidoreductase (*NQO1*), and N-acetyltransferase 2 (*NAT2*); some report significant associations only with cases harboring *MLL* gene translocations [10–12]. However, these studies each had fewer than 50 IL cases, emphasizing challenges with interpretation and replication.

Genome wide association (GWA) studies of childhood ALL have identified candidate susceptibility loci at *IKZF1*, *ARID5B*, and *CEBPE* [13,14]. No studies have explored these loci in IL. We genotyped these SNPs in 171 non-Hispanic white IL cases and 384 non-Hispanic white controls to determine associations by leukemia subtype and presence of a *MLL* gene rearrangement.

MATERIALS AND METHODS

Study Samples

IL cases were enrolled in Children's Oncology Group (COG) Protocol AE24 [15, 16]. Infants (<12 months) with a confirmed diagnosis of ALL or AML (ICD-9 codes: 9801, 9803, 9821, 9823, 9861, 9863, 9910, 9913) during 1996–2006 and treated at U.S. and Canadian COG institutions were eligible. Down syndrome cases were excluded. Case mothers submitted buccal cell samples or released existing biological specimens from their child for genetic analysis. DNA was extracted from cytobrushes via a Purgene kit protocol (Gentra Systems, Minneapolis, MN). DNA was extracted from archived biological samples (e.g., blood smears, bone marrow samples, cerebrospinal fluid) using a phenol-chloroform extraction protocol [17]. All isolated DNA was stored at –80°C prior to genotyping. Mothers also released their child's diagnostic information, including results of Southern blot, RT-PCR, fluorescent *in situ* hybridization, or other cytogenetics testing to permit central review. Three independent reviewers (S.M.D., N.A.H., and J.M.H.) evaluated submitted materials to confirm diagnosis and determine if there was evidence of *MLL* gene rearrangement (*MLL*+), no rearrangement (*MLL*–), or insufficient evidence to classify. The current analysis was restricted to infants who provided DNA specimens and who were categorized as non-Hispanic white based on maternal report (n=189) to avoid potential spurious associations due to confounding by population admixture [18]. Anonymized plasma samples from 384 healthy, non-Hispanic white blood donors were used as controls. DNA was isolated from the plasma via a DNA blood midi column protocol (Qiagen, Inc., Valencia, CA) and was stored at –20°C prior to genotyping. Institutional Review Boards at the University of Minnesota and participating COG institutions approved the study. Mothers of IL cases provided informed consent for the use of the biospecimens in genetic analyses.

Genotyping

The selected SNPs (*IKZF1* rs11978267, *ARID5B* rs10821936 and rs10994982, *CEBPE* rs2239633) were genotyped by fluorogenic PCR-based allelic discrimination (Taqman) assays using the ABI Prism 7900HT RT-PCR System (Applied Biosystems, Inc., Carlsbad, CA). Primers and probes were designed by ABI (Taqman SNP assays C_199413_10, C_26140184_10, C_30824850_10, and C_335486_1). PCR conditions are available on request. Genotype calls were made manually upon visual inspection of allelic discrimination plots by identifying clusters around reference controls for each allele. Among the case samples, there were 18 samples that could not be genotyped across all SNPs. Of the remaining 171 cases (including 102 ALL, 67 AML, and 2 biphenotypic), genotypes for the *IKZF1* and *ARID5B* SNPs could not be called for 3–7% and 19% for the *CEBPE* SNP. Of these 171 cases, 94 were *MLL*+, 54 *MLL*– and 23 indeterminate. For the control samples, the call rate was 95% for each SNP. As a quality control measure, 5–8% of samples were selected for duplication for each SNP, and there was 100% concordance between the replicates. Control genotypes for all SNPs were in Hardy Weinberg Equilibrium ($p > 0.05$) and closely followed the genotype distributions reported in similar populations [13, 16].

Statistical Analysis

Case genotypes were compared to those of controls at each locus of interest via unconditional logistic regression (SAS version 9.2, SAS Institute Inc., Cary, NC). Genomic odds ratios (ORs), as well as corresponding 95% confidence intervals (CIs), were calculated to compare individual genotypes (AA vs. BB, AB vs. BB), and allelic ORs and p-values for trend were calculated from ordinal variables indicating the number of high risk alleles (0, 1, 2) to assess potential dose response. An *ARID5B* risk score was summed for each subject indicating the number of risk alleles across the two *ARID5B* SNPs and an overall genetic risk score was likewise compiled representing the total number of risk alleles across the four SNPs examined. Individuals with missing genotypes were excluded from the risk scores. Case-control comparisons were performed for all cases combined and for subgroups by *MLL* gene status (*MLL*+, *MLL*–), leukemic subtype (ALL, AML), and combinations of these categories versus the entire control group.

RESULTS

There was a significantly increased risk of IL overall associated with two copies of the *IKZF1* variant (OR=2.3, 95%CI=1.3–4.2) (Table 1). This association was seen in *MLL*– and AML cases but not in ALL cases overall, and was only apparent for cases homozygous (OR=4.0, 95%CI=1.8–8.6; OR=3.9, 95%CI=1.8–8.4, respectively) for the variant allele. Upon further stratification by subtype and *MLL* status (Table 2), strong associations were observed among AML cases with two copies of the variant allele regardless of *MLL* involvement. For ALL, there was a strong association for *MLL*– cases with two copies of the variant allele (OR=5.1, 95%CI=1.8–14.5), but no association in ALL/*MLL*+ cases (OR=0.7, 95%CI=0.2–2.2).

There were no significant associations observed overall for either of the variant *ARID5B* alleles (Table 1). However, when stratified by leukemia subtype or *MLL* status, there was an increased risk associated with two copies of the variant rs10821936 allele among *MLL*– cases only (OR=2.8, 95%CI=1.3–6.2). Moreover, there was an inverse association among *MLL*+ cases heterozygous for rs10994982 (OR=0.5, 95%CI=0.3–0.8). Further stratification revealed a significant risk with rs10821936 among ALL/*MLL*– cases only; homozygosity for the variant allele was associated with a 7.2-fold increased risk (95%CI=2.5–20.6) (Table 2). An inverse effect of rs10994982 was only apparent among AML/*MLL*+ cases, although this observation is based on a small number of cases. Finally, for *CEBPE* (rs2239633) there

was a significant inverse association among heterozygous *AML/MLL*– cases (OR=0.3, 95% CI=0.1–0.9), but no reduction in risk in infants with the homozygous variant genotype, raising concern that this may be a spurious observation. There were no associations of *CEBPE* (rs2239633) with any of the other case groups. Across all SNPs, we found little evidence of an increasing risk associated with increasing number of variant alleles, suggesting each locus imparts an independent role.

DISCUSSION

Our results provide evidence for a distinct genetic etiology for infant ALL cases with and without *MLL* gene rearrangements, but similarity for infants with AML with or without *MLL* rearrangement. Clinical observations support these observations. Five-year survival rates for infant ALL vary markedly by *MLL* status. Eighty percent of infants with ALL and no *MLL* rearrangement survive at five years, while survival rates for infant *ALL/MLL*+ cases are less than 50% [19, 20]. In contrast, *MLL* status has little influence on survival for infant AML [21]. Further, infant *ALL/MLL*+ cases are more often diagnosed prior to six months of age, whereas infant *ALL/MLL*– cases are more frequently diagnosed in older infants; age differences by *MLL* status are less distinct among AML cases [22]. Gene expression studies demonstrate clustering by selected cell phenotypes (including pre-B cell versus infant ALL, pediatric ALL versus AML) as well as by *MLL* status (rearranged versus germline) [23–26]. However, we are not aware of a specific gene expression study that directly compares *infant ALL and AML* cases by *MLL* status. Taken together, the biological distinctions between these subgroups of leukemia may reflect origins in precursors arising at different stages of hematopoietic development, and modifiers of susceptibility may be expected to vary also.

Ikaros is a zinc finger transcription factor that plays a major role in B cell development [27]; it is highly expressed in liver, lymph node, thymus, bone marrow, and placenta [28]. Ikaros drives differentiation of hematopoietic stem cells and multi-potent progenitors into the B-cell lineage, and suppresses differentiation into the myeloid lineage. The functional significance of the *IKZF1* polymorphism is not fully elucidated. mRNA expression was evaluated in transformed Epstein Barr Virus lymphocytes and significantly lower dose-dependent expression was found with each copy of the variant *IKZF1* allele [13]. Our results, as well as those in B-lineage leukemia in older children suggest that reduced expression of Ikaros increases risk of leukemogenesis, and it is possible that low levels of Ikaros delay progression through B-cell maturation and increase the number of precursors at risk of leukemogenesis. Data suggest that *MLL* rearrangements associated with fusion to *AF4* (most common in infant ALL) occur very early in embryological development, perhaps in a population of mesodermal prehematopoietic precursors, still capable of differentiation into multiple cell lineages, including mesenchymal stem cells [29]. It is possible that such an early precursor would be less influenced by Ikaros, as our data show, in contrast to leukemia arising later in the pathway of lymphoid differentiation.

Involvement of Ikaros in AML has not been reported previously, and is perhaps more surprising. While Ikaros appears to play a less significant role in myelopoiesis, the protein is highly expressed in early myeloid precursor cells, and it is clear that suppression of myeloid differentiation is as important a function as the promotion of lymphoid development [27]. Thus, it is conceivable that reduced expression of Ikaros during these early stages contributes to susceptibility to infant AML overall. The *IKZF1* genotype has not been studied in older children with AML. In an *ad hoc* analysis, we genotyped 450 AML cases (ages 0–19 years) enrolled on COG studies 2941 and 2961 and found no overall associations with the variant allele, suggesting that this susceptibility is specific to infants and is not a feature of AML generally.

ARID5B encodes the AT-rich interactive domain 5B, a member of the ARID family of proteins, which are highly conserved and play a key role in development. *ARID5B* homozygous null mice show transient immune abnormalities including a reduction in early B-cell progenitors [30]. Expression of *ARID5B* also correlated with *RAG1* expression in bone marrow [31], which supports a role for this gene in early B-cell development. Our data point to a role of *ARID5B* only in *ALL/MLL*– cases, in agreement with observations in childhood pre-B cell ALL [13, 28]. Fewer studies suggest a role for *CEBPE*, a regulator of myelopoiesis in childhood ALL [22]; our data do not support an important role in IL.

In conclusion, this study suggests a role for *IKZF1* in early myeloid leukemia. While there were a relatively small number of IL cases in our study, the ORs are quite strong, and higher than those typically reported in genetic susceptibility studies. While the data would be strengthened by replication in independent datasets, this is challenging due to the low frequency of IL, and the need for cases to be carefully phenotyped for lineage (*ALL* vs *AML*) and *MLL* involvement. Additional functional studies are also needed to understand the role of these genes in early hematopoiesis.

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Table 1

Associations between genetic variants, *MLL* status, and leukemia subtype

	Controls			Overall cases			<i>MLL</i> + cases			<i>MLL</i> - cases			ALL cases			AML cases		
	N	N	OR (95%CI)	N	N	OR (95%CI)	N	N	OR (95%CI)	N	N	OR (95%CI)	N	N	OR (95%CI)	N	N	OR (95%CI)
<i>IKZF1</i>																		
rs11978267																		
AA	204	81	1	49	1		24	1		53	1		26	1				
AG	152	53	0.9 (0.6–1.3)	28	0.8 (0.5–1.3)		14	0.8 (0.4–1.6)		31	0.8 (0.5–1.3)		22	1.1 (0.6–2.1)				
GG	28	26	2.3 (1.3–4.2)**	10	1.5 (0.7–3.3)		13	4.0 (1.8–8.6)		12	1.7 (0.8–3.5)		14	3.9 (1.8–8.4)				
Per G allele			1.3 (1.0–1.7)		1.0 (0.7–1.5)			1.7 (1.1–2.6)			1.1 (0.8–1.5)			1.8 (1.2–2.6)				
p, trend*			0.07		0.89			0.01			0.67			0.003				
<i>ARID5B</i>																		
rs10821936																		
TT	169	72	1	44	1		18	1		39	1		32	1				
TC	171	67	0.9 (0.6–1.4)	35	0.8 (0.5–1.3)		22	1.2 (0.6–2.3)		42	1.1 (0.7–1.7)		25	0.8 (0.4–1.4)				
CC	43	27	1.5 (0.9–2.6)	11	1.0 (0.5–2.1)		13	2.8 (1.3–6.2)		18	1.8 (1.0–3.5)		8	1.0 (0.4–2.3)				
Per C allele			1.1 (0.9–1.5)		0.9 (0.7–1.3)			1.6 (1.1–2.4)			1.3 (0.9–1.8)			0.9 (0.6–1.4)				
p, trend*			0.36		0.63			0.02			0.13			0.65				
rs10994982																		
GG	101	51	1	36	1		8	1		27	1		23	1				
GA	187	68	0.7 (0.5–1.1)	31	0.5 (0.3–0.8)		28	1.9 (0.8–4.3)		42	0.8 (0.5–1.4)		26	0.6 (0.3–1.1)				
AA	95	40	0.8 (0.5–1.4)	21	0.6 (0.3–1.1)		13	1.7 (0.7–4.4)		25	1.0 (0.5–1.8)		14	0.7 (0.3–1.3)				
Per A allele			0.9 (0.7–1.2)		0.8 (0.5–1.0)			1.3 (0.8–1.9)			1.0 (0.7–1.4)			0.8 (0.5–1.1)				
p, trend*			0.44		0.07			0.27			0.95			0.20				
<i>CEBPB</i>																		
rs2239633																		
AA	90	32	1	15	1		14	1		19	1		13	1				
AG	165	64	1.1 (0.7–1.8)	39	1.4 (0.7–2.7)		15	0.6 (0.3–1.3)		43	1.2 (0.7–2.3)		21	0.9 (0.4–1.8)				
GG	108	42	1.1 (0.6–1.9)	23	1.3 (0.6–2.6)		12	0.7 (0.3–1.6)		23	1.0 (0.5–2.0)		18	1.2 (0.5–2.5)				
Per G allele			1.0 (0.8–1.4)		1.1 (0.8–1.6)			0.8 (0.5–1.3)			1.0 (0.7–1.4)			1.1 (0.7–1.6)				
p, trend*			0.76		0.55			0.42			0.98			0.67				

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; *MLL*⁺, evidence of an *MLL* gene rearrangement; *MLL*⁻, no evidence of an *MLL* gene rearrangement; OR, odds ratio.

* P-value for trend calculated via Cochran-Armitage trend test.

** Values in bold are statistically significant at $P < 0.05$ level.

Table II

Genetic variant associations stratified by *MLL* status and leukemia subtype

Controls		ALL/ <i>MLL</i> + cases		ALL/ <i>MLL</i> − cases		ALL/ <i>MLL</i> + cases		ALL/ <i>MLL</i> − cases	
		N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)
<i>IKZF1</i>									
rs11978267									
AA	204	40	1	10	1	8	1	13	1
AG	152	19	0.6 (0.4–1.2)	9	1.2 (0.5–3.1)	9	1.5 (0.6–4.0)	5	0.5 (0.2–1.5)
GG	28	4	0.7 (0.2–2.2)	7	5.1 (1.8–14.5)**	6	5.5 (1.8–16.9)	6	3.4 (1.2–9.6)
Per G allele			0.7 (0.5–1.2)		2.1 (1.2–3.7)		2.2 (1.2–4.1)		1.5 (0.8–2.7)
p, trend*			0.18		0.009		0.007		0.22
<i>ARID5B</i>									
rs10821936									
TT	169	29	1	6	1	15	1	11	1
TC	171	29	1.0 (0.6–1.7)	10	1.7 (0.6–4.6)	6	0.4 (0.2–1.0)	12	1.1 (0.5–2.5)
CC	43	6	0.8 (0.3–2.1)	11	7.2 (2.5–20.6)	4	1.1 (0.3–3.3)	2	0.7 (0.2–3.4)
Per C allele			0.9 (0.6–1.4)		2.8 (1.6–5.0)		0.8 (0.4–1.5)		0.9 (0.5–1.7)
p, trend*			0.73		0.0002		0.42		0.82
rs10994982									
GG	101	22	1	2	1	14	1	5	1
GA	187	25	0.6 (0.3–1.1)	15	4.1 (0.9–18.1)	6	0.2 (0.1–0.6)	13	1.4 (0.5–4.1)
AA	95	15	0.7 (0.4–1.5)	8	4.3 (0.9–20.5)	5	0.4 (0.1–1.1)	5	1.1 (0.3–3.8)
Per A allele			0.8 (0.6–1.2)		1.7 (0.9–3.0)		0.5 (0.3–0.9)		1.0 (0.6–1.9)
p, trend*			0.33		0.08		0.02		0.92
<i>CEBPE</i>									
rs2239633									
AA	90	12	1	5	1	3	1	9	1
AG	165	29	1.3 (0.6–2.7)	10	1.1 (0.4–3.3)	10	1.8 (0.5–6.8)	5	0.3 (0.1–0.9)
GG	108	16	1.1 (0.5–2.5)	6	1.0 (0.3–3.4)	7	1.9 (0.5–7.7)	5	0.5 (0.2–1.4)
Per G allele			1.0 (0.7–1.5)		1.0 (0.6–1.8)		1.3 (0.7–2.5)		0.6 (0.3–1.2)
p, trend*			0.84		0.99		0.37		0.14

ALL/*MLL*⁺, acute lymphoblastic leukemia with evidence of an *MLL* gene rearrangement; ALL/*MLL*⁻, acute lymphoblastic leukemia with no evidence of an *MLL* gene rearrangement; AML/*MLL*⁺, acute myeloid leukemia with evidence of an *MLL* gene rearrangement; AML/*MLL*⁻, acute myeloid leukemia with no evidence of an *MLL* gene rearrangement; CI, confidence interval; OR, odds ratio.

* P-value for trend calculated via Cochran-Armitage trend test.

** Values in bold are statistically significant at P < 0.05 level.