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***MLL* gene rearrangements in infant leukemia vary with age at diagnosis and selected demographic factors: A Children's Oncology Group (COG) study**

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Abstract

Background—Infant leukemias have a high frequency of mixed lineage leukemia (*MLL*) gene rearrangements.

Procedure—Using data from a large etiologic study, we evaluated the distribution of selected demographic factors among 374 infant leukemia cases by leukemic subtype, *MLL* status and diagnosis age.

Results—Overall, 228 cases were *MLL*+. Compared to white infants, black infants were significantly less likely to have *MLL*+ leukemia. Further, there was a statistically significantly higher age at diagnosis for infants with t(9;11) translocations compared to all other translocation partners in both acute lymphoblastic leukemia and acute myeloid leukemia cases.

Conclusion—These patterns may provide important etiological insight into the biology of infant leukemia.

Keywords

epidemiology; infants; leukemia; *MLL*

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

INTRODUCTION

Infant leukemias (<12 months) are rare (40/1,000,000 person-years)[1] and distinct from leukemias in older children. Using current treatment protocols, 5-year survival has increased to >80% for leukemias diagnosed at 1–9 years, while the infant leukemia rate is 50%[2]. Other important presenting characteristics include hyperleukocytosis, absence of CD10+ expression on blasts, and central nervous system involvement [reviewed in [3]]. Further, there is a high proportion of chromosomal aberrations involving the mixed lineage leukemia (*MLL*) gene on 11q23. The commonest *MLL* aberrations involve translocation of the N-terminus with various partners. To date, >60 translocation partners have been identified[4]. *MLL* rearrangements occur in 6% and 14% of childhood acute lymphoid leukemias (ALL) and acute myeloid leukemias (AML), respectively[5]. The infant frequency is much higher, with 61–79% of ALL and 31–45% of AML cases affected[6–11].

Differences in leukemias in infants versus older children suggest divergent underlying causes. Here, we aimed to characterize a large infant leukemia case series by leukemic subtype, *MLL* status, and translocation partner chromosome, as subgroup differences may inform hypotheses in future etiologic investigations.

MATERIALS AND METHODS

Patients enrolled on Children's Oncology Group (COG) Protocol AE24 comprised the case series. Detailed methods have been published previously[12]. Briefly, acute leukemia cases diagnosed at <12 months between 1/1/96–10/13/02 (Phase 1) and 1/1/03–12/31/06 (Phase 2) at U.S. and Canadian COG institutions were eligible if their biological mothers were available for telephone interview and spoke English or Spanish (Phase 2 only). Individuals with Down syndrome were excluded. Interview response rates were 69% (Phase 1) and 59% (Phase 2). Mothers provided demographic data via telephone interview and released diagnostic information to permit central review (by N.A.H., J.M.H., S.M.D.).

Cases were classified by leukemia type, *MLL* rearrangement status, and *MLL* translocation partner chromosome. Cases were categorized as *MLL*+ (n=228) if there was evidence of *MLL* rearrangement or balanced translocation from molecular methods (Southern blot, PCR), conventional cytogenetics, and/or fluorescence *in situ* hybridization. Cases with no evidence of *MLL* rearrangement were considered *MLL*– (n=146). Those with insufficient information (i.e., poor karyotype, deletion) were regarded as indeterminate (n=69).

Partner chromosomes were determined for 194/228 (85%) *MLL*+ cases, including chromosomes 1 (n=14), 4 (n=78), 9 (n=33), 10 (n=23), 19 (n=31), or less common partners (n=15), while partners were not determined for 34 cases. t(11;19) included 2 cases of t(11;19)*ELL*, 18 cases of t(11;19)*ENL*, and 11 cases without identified breakpoints. Although t(11;19)*ELL* and t(11;19)*ENL* are biologically different[13], small numbers prohibited separate examination.

University of Minnesota and COG institutions' Institutional Review Boards approved the protocol. Case mothers provided informed consent prior to participation.

Statistical Methods

Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated via unconditional logistic regression for Table I characteristics. Kaplan-Meier analysis was performed to identify significant differences between age of diagnosis (continuous) and leukemic subtypes (ALL versus AML, *MLL*+ versus *MLL*–) and translocation partner

(t(9;11), t(4;11), others). Differences in distribution functions were evaluated via Wilcoxon test. Statistical analyses were performed in SAS v9.2 (Cary, NC).

RESULTS

This series included 264 ALL, 172 AML and 7 AUL/biphenotypic infant cases (Table I). Of 152 AML cases with known morphologic subgroup, 42% were M5, 20% M4, 17% M7, 8% M0, and 5% M2; few cases comprised remaining subgroups. Overall, 228 cases were classified as *MLL*+, 146 *MLL*−, and 69 undetermined. Most ALL cases were *MLL*− (60%), while proportions of *MLL*− (40%) and *MLL*− (38%) AML cases were comparable. The most common translocation in infant ALL was t(4;11) (48%). In AML, t(10;11) was most common (26%), followed by t(9;11) (25%).

Females were 35% more likely than males to have *MLL* rearrangements (95% CI:0.89–2.05)(Table II). For leukemia overall and ALL, infants of other races/ethnicities were less likely to be *MLL*− compared to whites, but the OR was only statistically significant for black infants (OR_{Overall}=0.27, 95% CI:0.11–0.70; OR_{ALL}=0.24, 95% CI:0.07–0.84).

MLL− cases had a greater median age of diagnosis (7.1 months, n=146) than *MLL*− cases (5.2 months, n=228). Further, the diagnosis age distribution was significantly different across the two groups ($p_{\text{Wilcoxon}}=0.004$), which was driven by ALL cases (ALL *MLL*−: 4.8 months, n=157; ALL *MLL*−: 9.0 months, n=77; $p_{\text{Wilcoxon}}<0.0001$). In contrast, AML *MLL*− cases had a slightly older diagnosis age (7.0 months, n=68) compared to AML *MLL*− cases (5.1 months, n=66)($p_{\text{Wilcoxon}}=0.04$). In examining 3-month age groups, infants diagnosed at 10–12 months had reduced odds of *MLL*− ALL compared to diagnosis at 3 months (OR=0.16, 95% CI:0.07–0.36). Conversely, infants diagnosed with AML at 10–12 months had the highest odds of *MLL* rearrangements (OR=2.36, 95% CI:0.77–7.21).

ALL cases with t(9;11) were diagnosed at significantly greater ages than those with either t(4;11) or other translocations($p_{\text{Wilcoxon}}=0.01$) (Figure 1a). Ages of the latter two groups were not significantly different($p_{\text{Wilcoxon}}=0.58$). Because there were only 3 t(4;11) AML cases, we combined them with other translocations and compared that group to t(9;11) AML cases (Figure 1b). As with ALL, t(9;11) cases were significantly older than all other translocations combined($p_{\text{Wilcoxon}}=0.04$).

DISCUSSION

In this large series, 60% of infant ALL and 40% of infant AML cases exhibited *MLL* rearrangements, which is consistent with prior reports[6–10]. The observation that females were more likely to have *MLL* rearrangements in their leukemia cells was also consistent with earlier studies[14]. To our knowledge, the finding that black infants had significantly lower odds of *MLL*− leukemia has not been previously reported, although this estimate is based on a small number of black cases. No associations were observed between Hispanic ethnicity and *MLL*− ALL either here or in a prior report[15].

Observed differences may provide important clues to the underlying biology of *MLL*− infant leukemia. Our results indicate that female sex may be a risk factor and black race may be protective for *MLL*− leukemia. These findings could be leveraged in designing targeted, testable hypotheses for future studies, where investigations are focused on what might be biologically different about female versus male infants or black versus white infants. For example, hormonal differences between sexes may influence *MLL*− leukemia development; one *in vitro* study showed that estrogen induced *MLL* translocations in human lymphoblastoid cells[16]. Or, male fetuses with *MLL* rearrangements may be less

viable[14]. Reduced *MLL*+ leukemia odds among black infants may result from differences in genetic susceptibility and/or environmental exposures.

Selection bias may also play a role, however, since not all eligible cases were ultimately enrolled. We compared our case-control data to U.S. cancer registry data [17]. Similar infant leukemia risks were found for females versus males ($OR_{AE24}=1.05$, incidence rate ratio(IRR) $_{SEER}=0.93$) and non-Hispanic whites versus blacks ($OR_{AE24}=0.76$, $IRR_{SEER}=0.80$). These comparable results diminish selection bias concerns.

In our study, *MLL*+ cases were diagnosed earlier than *MLL*- cases for ALL but not AML. Previous studies have shown that *MLL*+ infant leukemias are diagnosed earlier (<6 months) than *MLL*- infant leukemias[9,10,18]. The observation that specific ALL translocations differed by diagnosis age has also been described previously [7,10].

One possible explanation for an age-translocation association is that specific translocations may arise systematically in different cells of origin, resulting in varying leukemia latency periods. Some support comes from Menendez et al[19], where the *MLL-AF4* fusion gene was identified in bone marrow mesenchymal cells from 8 infants with *MLL*+ leukemia, while neither *MLL-AF10* nor *MLL-ENL* was observed. Animal models suggesting different translocations may result in varying latency to disease[20,21] provide additional support. However, due to differences in strains, malignancy induction, and disease outcome, generalizability to human studies is limited.

Alternatively, fusion products may affect different downstream targets. Microarray studies have shown differential gene expression of *MLL*+ versus *MLL*- infant leukemias[22]. Furthermore, t(4;11), t(9;11), and t(11;19)*ENL* cases clustered separately[22], arguing for possible unique downstream targets of particular fusion products. Partner-specific alterations may alter susceptibility for secondary genetic/epigenetic events and ultimately affect timing to overt leukemia.

Study strengths include the large number of cases with known *MLL* status and translocation partner chromosome. In addition, we recruited from a nearly population-based source of U.S. and Canadian cases, since COG institutions see nearly all leukemia patients ages 0–4 years[23].

Limitations include possible selection bias (discussed above), unknown *MLL* status for 16% of cases, and undetermined translocation partner for 14% of *MLL*+ cases. To evaluate the robustness of our results, we reclassified cases with undetermined *MLL* status as *MLL*+ or *MLL*-, respectively, and found no marked deviations from the results reported above. Similar results were obtained after assigning *MLL*+ cases with unknown translocation partners to t(4;11) and t(9;11).

In this large descriptive study of infant leukemia, we observed that blacks have lower *MLL*+ leukemia risk than whites, and that *MLL-AF9* leukemias (ALL and AML) are diagnosed later than other *MLL* fusion gene leukemias, which may provide clues into etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Kaplan-Meier graph comparing 75 t(4;11), 16 t(9;11) and 40 infant ALL cases with less common translocations. Median age of diagnosis was 4.44 months for t(4;11) cases, 7.57 months for t(9;11) cases and 4.06 months for others ($p_{\text{Wilcoxon}}=0.01$). Diagnostic ages of the t(4;11) and other translocation groups were not significantly different ($p_{\text{Wilcoxon}}=0.58$) (A). Kaplan-Meier graph comparing 17 t(9;11) and 43 infant AML cases with other translocation partners including t(4;11). Median age of diagnosis was 8.02 months for t(9;11) cases and 5.46 months for all others ($p_{\text{Wilcoxon}}=0.04$). (Grouping t(4;11) cases with other translocations did not significantly change the median age of diagnosis.) (B)

Table I

Demographic distribution of cases by leukemia subtype

	Total N=443 N (%)	ALL N=264 N (%)	AML N=172 N (%)	Biphenotypic/AUL N=7 N (%)
Sex				
Female	225 (50.8)	131 (49.6)	88 (51.2)	6 (85.7)
Male	218 (49.2)	133 (50.4)	84 (48.8)	1 (14.3)
Race/ethnicity				
White	310 (70.0)	189 (71.6)	118 (68.6)	3 (42.9)
Black	24 (5.4)	13 (4.9)	11 (6.4)	0
Hispanic	63 (14.2)	33 (12.5)	26 (15.1)	4 (57.1)
Native American/Alaskan Native	8 (1.8)	3 (1.1)	5 (2.9)	0
Asian/Pacific Islander	24 (5.4)	17 (6.4)	7 (4.1)	0
Other	13 (2.9)	8 (3.0)	5 (2.9)	0
Missing	1 (0.2)	1 (0.4)	0	0
Age of diagnosis				
0–3 months	114 (25.7)	63 (23.9)	47 (27.3)	4 (57.1)
4–6 months	123 (27.8)	74 (28.0)	47 (27.3)	2 (28.6)
7–9 months	111 (25.1)	61 (23.1)	50 (29.1)	0
10–12 months	95 (21.4)	66 (25.0)	28 (16.3)	1 (14.3)
<i>MLL</i> status				
<i>MLL</i> +	228 (51.5)	157 (59.5)	68 (39.5)	3 (42.9)
<i>MLL</i> –	146 (33.0)	77 (29.2)	66 (38.4)	3 (42.9)
Indeterminate	69 (15.6)	30 (11.4)	38 (22.1)	1 (14.3)
Translocation partners *				
t(1;11)	14 (6.1)	8 (5.1)	6 (8.8)	0
t(4;11)	78 (34.2)	75 (47.8)	3 (4.4)	0
t(9;11)	33 (14.5)	16 (10.2)	17 (25.0)	0
t(10;11)	23 (10.1)	3 (1.9)	18 (26.5)	2 (66.7)
t(11;19) **	11 (4.8)	7 (4.5)	4 (5.9)	0
t(11;19) <i>ELL</i>	2 (0.9)	0	2 (2.9)	0
t(11;19) <i>ENL</i>	18 (7.9)	16 (10.2)	1 (1.5)	1 (33.3)
Other	15 (6.6)	6 (3.8)	9 (13.2)	0
Undetermined	34 (14.9)	26 (16.6)	8 (11.8)	0

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; AUL = acute undifferentiated leukemia; *MLL* + = evidence of an *MLL* gene rearrangement; *MLL* – = no evidence of an *MLL* gene rearrangement

* Percentages reflect proportion of *MLL* + cases

** Breakpoint not determined

Table II

Associations between *MLL* status and sex, race/ethnicity and age of diagnosis

	Leukemias				ALL				AML			
	<i>MLL</i> −	<i>MLL</i> +	OR	95% CI	<i>MLL</i> −	<i>MLL</i> +	OR	95% CI	<i>MLL</i> −	<i>MLL</i> +	OR	95% CI
Sex												
Male	77 (52.7)	103 (45.2)	1.00		44 (57.1)	72 (45.9)	1.00		33 (50.0)	31 (45.6)	1.00	
Female	69 (47.3)	125 (54.8)	1.35	0.89 – 2.05	33 (42.9)	85 (54.1)	1.57	0.91 – 2.73	33 (50.0)	37 (54.4)	1.19	0.61 – 2.35
Race/ethnicity*												
White	93 (63.7)	171 (75.3)	1.00		50 (64.9)	121 (77.6)	1.00		42 (63.6)	48 (70.6)	1.00	
Black	14 (9.6)	7 (3.1)	0.27	0.11 – 0.70	7 (9.1)	4 (2.6)	0.24	0.07 – 0.84	7 (10.6)	3 (4.4)	0.38	0.09 – 1.54
Hispanic	21 (14.4)	31 (13.7)	0.80	0.44 – 1.48	11 (14.3)	17 (10.9)	0.64	0.28 – 1.46	8 (12.1)	13 (19.1)	1.42	0.54 – 3.76
Native American/Alaskan Native	4 (2.7)	1 (0.4)	0.14	0.02 – 1.23	1 (1.3)	1 (0.6)	0.41	0.03 – 6.74	3 (4.5)	0 (0.0)	--	
Asian/Pacific Islander	8 (5.5)	12 (5.3)	0.82	0.32 – 2.07	5 (6.5)	10 (6.4)	0.83	0.27 – 2.54	3 (4.5)	2 (2.9)	0.58	0.09 – 3.66
Other	6 (4.1)	5 (2.2)	0.45	0.14 – 1.53	3 (3.9)	3 (1.9)	0.41	0.08 – 2.12	3 (4.5)	2 (2.9)	0.58	0.09 – 3.66
Age of diagnosis												
3 months	35 (24.0)	64 (28.1)	1.00		13 (16.9)	47 (29.9)	1.00		22 (33.3)	14 (20.6)	1.00	
4–6 months	31 (21.2)	75 (32.9)	1.32	0.74 – 2.38	11 (14.3)	56 (35.7)	1.41	0.58 – 3.44	18 (27.3)	19 (27.9)	1.66	0.66 – 4.20
7–9 months	37 (25.3)	58 (25.4)	0.86	0.48 – 1.54	19 (24.7)	35 (22.3)	0.51	0.22 – 1.17	18 (27.3)	23 (33.8)	2.01	0.81 – 4.99
10–12 months	43 (29.5)	31 (13.6)	0.39	0.21 – 0.73	34 (44.2)	19 (12.1)	0.16	0.07 – 0.36	8 (12.1)	12 (17.6)	2.36	0.77 – 7.21

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

* Does not include 1 case with missing race/ethnicity.