[CANCER RESEARCH 61, 3432–3438, April 15, 2001]

Gestational Age and Gender-specific *In Utero* V(D)J Recombinase-mediated Deletions[1]

Makoto Yoshioka, J. Patrick O'Neill, Pamela M. Vacek, and Barry A. Finette[2]

Department of Pediatrics [M. Y., B. A. F.], Genetics Laboratory [P. O.], Vermont Cancer Center [P. O., B. A. F.], Departments of Medical Biostatistics [P. M. V.] and Microbiology and Molecular Genetics [B. A. F.], University of Vermont, Burlington, Vermont 05405

ABSTRACT

Recent studies have brought to the forefront the importance of somatic mutations during human fetal development and malignant transformation in children, specifically leukemia. Therefore, a better understanding of the frequency and mutational spectrum of spontaneous *in utero* mutations is essential for understanding the genetic mechanisms associated with pediatric malignancies. Previously we reported that the frequency of somatic mutations during the late stages of fetal development was dependent on both gestational age and gender. Here we present the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) reporter gene mutational spectra analysis for 60 T-cell mutant isolates from the umbilical cord blood of preterm newborns to gain insight into background mutational events during the late stages of fetal development. Logistic regression analyses showed a significant increase in *HPRT* deletions mediated by V(D)J recombinase in preterm newborns compared with full-term newborns ($P = 0.009$). A comparative analysis of deletion mutations also revealed that V(D)J recombinase-mediated *HPRT* deletions increased with decreasing gestational age ($P = 0.012$) and were significantly higher in females than males of the same developmental status ($P = 0.031$). Developmental and gender-specific differences in *HPRT* deletions mediated by V(D)J recombinase provide insight into the gender-specific differences seen in infant leukemia.

INTRODUCTION

Significant progress has been made toward understanding the molecular basis and clinical relevance of germinal mutations and inherited human diseases. In contrast, only recently has there been significant evidence demonstrating that somatic mutational events during fetal development also have direct clinical consequences for both pediatric and adult multifactorial diseases, especially cancer.

Recent studies have linked for the first time specific *in utero* genetic events and the development of cancer in children. Specifically, *in utero* somatic mutational events involving the *MLL/AF4* (1, 2) and *TEL-AML1* (3, 4) gene fusions in T cells and immunoglobulin heavy chain and T-cell receptor rearrangements in B cells (5) have been correlated with the subsequent development of infant and childhood leukemia.

Previously, we reported developmental and gender-specific differences in the *in utero* frequency of somatic mutations (Mf) at the *HPRT*[3] reporter gene (6). Specifically, the Mf of preterm newborns was higher compared with full-term newborns, with the mean Mf of female preterm newborns being inversely related to gestational age and significantly higher than that found for male preterm newborns (6).

We report here a comparative analysis of the mutational spectra at the *HPRT* reporter gene mutations in T cells from this cohort. Statistical analyses revealed a significant increase of V(D)J recombinase-mediated *HPRT* deletions in preterm newborns compared with full-term infants. In addition, there was a significant increase in V(D)J recombinase-mediated deletions in both preterm and full-term female newborns compared with preterm and full-term male newborns. This gender-specific difference in V(D)J recombinase-mediated events may be relevant to understanding the higher incidence of infant leukemia observed among females.

MATERIALS AND METHODS

Study Population. Heparinized umbilical cord blood samples from 20 preterm newborns (gestation, <36 weeks; Ref. 6) and 33 full-term newborns (gestation, ≥36 weeks; Ref. 7) were acquired from the labor and delivery unit of Fletcher Allen Hospital of the University of Vermont College of Medicine. Informed consents were obtained after the procedure was approved by the Committee on Human Research at the University of Vermont.

***HPRT* T-Cell Cloning Assay.** Determination of *HPRT* Mf and the isolation of mutant clones were described previously (6, 7). *HPRT* mutant isolates were expanded and stored at −80°C at either 1×10^4 cells for reverse transcription-PCR or 5×10^4 cells for genomic multiplex PCR before molecular analysis.

Molecular Analysis of *HPRT* Mutant Isolates. The *HPRT* locus, located at Xq26, contains 9 exons, is 43 Kb in size, and has been completely sequenced. The coding sequence is 657-bp long. Molecular analyses of mutant cells both at the genomic and cDNA level have been well described (7–9). *HPRT* mutations observed previously include: (*a*) base substitutions at more that 270 sites in all nine exons; (*b*) small deletions and insertions; (*c*) large structural alterations; (*d*) splice site changes in introns; and (*e*) specialized genetic events such as V(D)J recombinase-mediated deletions (9–11).

Because the *HPRT* gene is located on the X-chromosome, molecular analysis at the DNA/RNA level is performed in different ways for mutant isolates from males and females (7). Mutant isolates from males first were analyzed by multiplex genomic *HPRT* PCR to determine the presence or absence of the nine *HPRT* exons (8). Mutant isolates from males showing no genomic alterations were characterized by reverse transcriptase-mediated production of *HPRT* cDNA, nested PCR amplification, and DNA sequencing of the amplified products (7). The multiplex PCR primer pairs for exons 1–9 also permitted sequence analyses of both intron and exon segments involved in most splice-sequence mutations, reflected as exon exclusions or intron inclusions in cDNA. For mutant isolates from females, multiplex genomic PCR analysis was not performed because the inactive X chromosome precludes deletion determination. Therefore, *HPRT* mutant isolates from females first were analyzed with specific primers to screen for V(D)J recombinase-mediated exon 2–3 deletion mutants (11). Then, those mutant isolates which showed no V(D)J recombinase-mediated deletions were analyzed by reverse transcription-PCR and DNA sequencing.

Statistical Analysis. Logistic regression was used to assess the effects of gender and development on the proportions of the different types of mutations. Models with interaction terms were fitted to test whether the effects of gender and development were independent. *In utero* exposure to tobacco smoke was included in some models to determine whether the effects of development and gender were attributable to differences in transplacental exposure to tobacco smoke. Detailed information about maternal smoking and passive smoke exposure was unavailable for many preterm infants, so smoke exposure was represented as dichotomous variables (exposed or not exposed). All models

Received 11/15/00; accepted 2/13/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Research was supported by Child Health and Human Development (NICHD) Grants 1K11HD01010 and 1R29HD35309, National Cancer Institute Grant 1KO1CA77737, and National Cancer Institute Grant P30CA22435 to the University of Vermont Cancer Center DNA Analysis Facility.

[2] To whom requests for reprints should be addressed, at Department of Pediatrics, Medical Alumni Building, Burlington VT 05405. Phone: (802) 656-2296; Fax: (802) 656-2077; E-mail: finette@salus.med.uvm.edu.

[3] The abbreviations used are: HPRT, hypoxanthine-guanine phosphoribosyltransferase; Mf, mutation frequency; RSS, recombination signal sequences; OR, odds ratio; N nucleotides, nontemplated nucleotides; DSB, double-strand break.

included a random effect to account for the correlation between multiple mutations from the same newborn.

RESULTS

A summary of mutations at the *HPRT* locus for each mutant isolate, as well as sex and gestational age of the subjects, is shown in Table 1. A total of 66 mutant isolates representing 60 independent mutations from 20 preterm newborns and 85 mutant isolates representing 78 independent mutations from 33 full-term newborns were characterized. Independent mutations were defined as single *HPRT* mutational events corrected for *in vivo* clonal expansion of mutant isolates. Clonal expansion was evident in subjects PS21 (deletion exons 1–9); PS30 ($C^{508}\rightarrow T$); PS29 (deletion exon 2); PS5 (exclusion exons 2–6); MFS89 (deletion exons 1–9 and deletion exons 2–9); MFS36 (deletion exons 7–9); and MFS65 ($G^{3}\rightarrow T$). Mutational spectrum data for full-term infants was reported previously, except for those indicated in Table 1 (7).

Distribution Analyses of *HPRT* Mutations. The distribution analysis for *HPRT* mutations during the late stages of fetal development is summarized in Table 2 and shown graphically in Fig. 1.

Mutations were first designated as small alterations, large alterations, and uncharacterized, as defined previously (7). A comparison between all mutations revealed a greater proportion of large alterations compared with small alterations for both preterm and full-term infants. This is consistent with previously reported *HPRT* mutational spectra in children from birth through 5 years of age (11). Logistic regression analysis demonstrated that the proportions of small and large alterations were not significantly related to gestational age or gender.

Distribution analysis of small alterations revealed a higher proportion of transition mutations compared with transversion mutations in both preterm and full-term newborns. Logistic regression analysis of the proportions of transitions and transversions did not demonstrate a relationship with either development or gender.

There were two predominant large alterations observed: *HPRT* deletions of exons 2 and 3 mediated by V(D)J recombinase (V(D)J deletions) as defined previously (9, 11), and exon deletions not mediated by V(D)J recombinase (non-V(D)J deletions). Distribution analysis revealed a higher proportion of V(D)J deletions compared with non-V(D)J deletions among all preterm infants and term females, but not among term males. Notably, logistic regression analysis revealed that the proportion of V(D)J deletions to non-V(D)J deletions was significantly related to both development and gender. Specifically, the proportion of V(D)J deletions were significantly higher compared with non-V(D)J deletions in preterm infants than full-term infants of the same gender (OR, 3.5; $P = 0.012$) as well as higher in females compared with males of the same developmental status (OR, 4.1; $P = 0.043$). Similar results were obtained when development was represented as gestational age rather than being classified as preterm or full-term, with ORs indicating that the proportion of V(D)J deletions increased ~13% with each week of decreasing gestational age from birth ($P = 0.038$).

Previously, we have reported that transplacental exposure to tobacco smoke results in a significant increase in V(D)J-mediated *HPRT* deletions in healthy full-term infants (7). In this study, an analysis of transplacental tobacco exposure was not related to any type of alteration in those preterm infants for whom exposure status was available.

Analysis of V(D)J Recombinase-mediated Breakpoints. Characteristic V(D)J recombinase-mediated sequence signature markings for *HPRT* V(D)J deletions are summarized in Fig. 2. A total of 10 V(D)J recombinase-mediated mutants from female preterm newborns and 21 V(D)J recombinase-mediated mutant isolates from male preterm newborns were characterized. All but two isolates from male subjects were Class I V(D)J deletion mutants, with the remaining mutant isolates being Class III V(D)J deletion mutants (9). There were five mutant isolates from male subjects and two mutant isolates from female subjects that lacked the N nucleotides additions. The percentage of the breakpoint sequences lacking the N nucleotide in the V(D)J mutant isolates from preterm newborns (22.6%, 7 of 31) was higher than the percentage observed previously for full-term newborns (5.6%; 1 of 18; Ref. 7). In addition, two atypical V(D)J recombinase events were observed. The V(D)J deletion breakpoint sequence from PS7M11 contained a 14-base N nucleotide insertion with an unusually long 26-base nibbling at the 3′ side of the breakpoint, whereas another mutation from subject PS7M20 contained a tandem direct repeat of a motif 5′-CACATCCCTTTCATG-3′, which is separated by four bases upstream of the 5′ breakpoint.

DISCUSSION

In this study we observed a unique mutational spectra for large alterations during the late stages of fetal development that was not seen for small alterations (including single-bp transversions and transitions). The most significant finding is that the proportion of V(D)J recombinase-mediated *HPRT* deletions are significantly higher during fetal development compared with after birth, and that this genetic event is related to gestational age and gender. Specifically, 63% (54 of 86) of all large alterations found in preterm and full-term newborns are V(D)J-mediated *HPRT* deletions, with a significantly higher distribution of V(D)J deletions in females (84%) compared with males (57%). To the best of our knowledge, this is the first demonstration of a gender-specific mutational spectra in humans, especially one with potential clinical consequences. We hypothesize that the high frequency of V(D)J recombinase-mediated *HPRT* deletions observed are the consequence of aberrant V(D)J genomic recombination events occurring at a time of high V(D)J recombinase activity required for *in utero* T-cell maturation. The reason for the predominance of these V(D)J recombinase rearrangements in females is unclear. Yet our observations may still be relevant, because an increase in these aberrant V(D)J-mediated genomic rearrangements may be responsible for the higher incidence of infant leukemia among females (12).

V(D)J recombinase-mediated rearrangements have been observed with cytogenetic alterations associated with T- and B-cell leukemia (13–16). In these studies, sequence analysis of translocation and deletion breakpoint sites in malignant clones demonstrated hallmark V(D)J recombinase signature markings that include palindromic bases (P nucleotides) and nucleotide nibbling and/or insertion of nontemplated bases (N nucleotides) at heptamer (CAC/TGTG)/nonamer (GGTTTTTGT) RSSs containing 12- and 23-bp spacers. Recently, chromosomal rearrangements involving the *MLL* (*ALL-1*, *HRX-AF-9*) gene at chromosome band 11q23 and the *TEL-AML1* gene fusion in children with infant leukemia (<12 months of age) have been shown to have occurred *in utero* (3, 17, 18). In addition, molecular analysis of Guthrie blood spots in monozygotic twins demonstrated clonal *MLL* fusion rearrangements that were subsequently identified in leukemic cells from these children, providing additional support to the hypothesis that *in utero* somatic mutational events are associated with the development of pediatric leukemia (1, 19, 20). These and other studies in *MLL-AF9* knockin mice (21) also demonstrate that other genetic events and environmental influences likely affect the length of the latent period for tumor development. For example, the expression of mutant isoforms of a transcription factor Ikaros has also been

Table 1 *Molecular analysis of in utero HPRT mutations*

Mutant isolate	Sex	GA[a]	*HPRT* Mutation		Transversion/Transition
			cDNA	Genomic DNA	
Preterm infants					
PS21M1	M	26		Deletion exons 1–9[b]	
PS21M4				Deletion exons 1–9[b]	
PS21M3				V(D)J class I	
PS21M5				V(D)J class I	
PS21M6			$G_{143}\rightarrow A$		Transition (CpG)
PS21M2			$T_{595}\rightarrow G$		Transversion
PS8M2	F	27		V(D)J class I	
PS8M3			$G_{606}\rightarrow C$		Transversion
PS30M1	M	29	$C_{508}\rightarrow T$[b]		Transition (CpG)
PS30M6			$C_{508}\rightarrow T$[b]		Transition (CpG)
PS30M10			$C_{508}\rightarrow T$[b]		Transition (CpG)
PS30M5			$C_{454}\rightarrow T$		Transition
PS30M2				V(D)J class I	
PS30M3				V(D)J class I	
PS30M7				V(D)J class I	
PS9M2	F	29		V(D)J class I	
PS23M2	M	31		Deletion exons 2–9	
PS26BM2	M	31	Exclusion exons 2–3	V(D)J class I	
PS26BM3				Deletion exons 1–9	
PS26BM4			Exclusion exons 2–6	Insertion GCTGCCG @IVS5+1	
PS29M1	M	31		Deletion exons 1–9	
PS29M2				V(D)J class I	
PS29M5				V(D)J class I	
PS29M4				Deletion exon 2[b]	
PS29M6				Deletion exon 2[b]	
PS26aM1	F	31		V(D)J class I	
PS26aM3			Exclusion exons 2–3	V(D)J class I	
PS26aM6			Exclusion exons 2–3	V(D)J class I	
PS26aM12			Exclusion exons 2–3	V(D)J class I	
PS26aM9			Exclusion exon 4	IVS4+1 G→A	Transition
PS26aM4			Exclusion exons 3–7	$A_{307}\rightarrow T$	Transversion
PS26aM5			$T_{81}\rightarrow C$		Transition
PS4M1	M	31		V(D)J class I	
PS4M3				V(D)J class I	
PS4M4				V(D)J class I	
PS4M5				V(D)J class I	
PS4M7				V(D)J class I	
PS4M2				Deletion exons 7–8	
PS4M8			Deletion A_{381}		
PS4M9			Exclusion exon 8	NC[c]	
PS18M1	M	32		V(D)J class I	
PS18M2				Deletion 537–564	
PS19M6	M	32	Exclusion 429–475	NC[c]	
PS19M7			$C_{508}\rightarrow T$		Transition (CpG)
PS6M1	M	33		V(D)J class I	
PS16M2	M	34		V(D)J class 3	
PS16M9			$C_{508}\rightarrow T$		Transition (CpG)
PS20M1	M	34		V(D)J class I	
PS20M2				$C_{69}\rightarrow A$	Transversion
PS12BM1	F	34		V(D)J class I	
PS12BM2			$C_{508}\rightarrow T$		Transition (CpG)
PS13M1	M	35	$G_{207}\rightarrow T$		Transversion
PS15M2	M	35		V(D)J class I	
PS15M5				V(D)J class 3	
PS15M4			$C_{145}\rightarrow T$		Transition
PS15M6				Deletion exon 1	
PS7M11	M	35		V(D)J class I	
PS7M20				V(D)J class I	
PS7M13			Exclusion exon 6	IVS5-1 G→A	Transition
PS14M2	F	35		V(D)J class I	
PS14M4				V(D)J class I	
PS5M1	F	35	$C_{508}\rightarrow T$		Transition (CpG)
PS5M8				V(D)J class I	
PS5M4			Exclusion exons 2–6[b]	NC[c]	
PS5M6			Exclusion exons 2–6[b]	NC[c]	
PS5M9			Exclusion exons 2–6[b]	NC[c]	
Term infants[d]					
MFS61aM2	F	36	$G_{606}\rightarrow T$		Transversion
MFS89M2	M	36	$C_{454}\rightarrow T$		Transition
MFS89M3				Deletion exons 1–9[b]	
MFS89M18				Deletion exons 1–9[b]	
MFS89M10			$C_{508}\rightarrow T$		Transition (CpG)
MFS89M12				Deletion exons 2–9[b]	
MFS89M6				Deletion exons 2–9[b]	
MFS89M4				V(D)J class I	
MFS89M14				V(D)J class I	
MFS89M15				V(D)J class I	
MFS101M1	M	36	$T_{536}\rightarrow A$[b]		Transversion
MFS101M2			$T_{536}\rightarrow A$[b]		Transversion
MFS101M3				Deletion exons 6–9	

Table 1 *Continued*

Mutant isolate	Sex	GA[a]	*HPRT* Mutation		Transversion/Transition
			cDNA	Genomic DNA	
MFS7M1	M	36		V(D)J class I	
MFS7M3				V(D)J class I	
MFS7M4				Deletion exon 1	
MFS36M1	M	37		Deletion exons 7–9[b]	
MFS36M2				Deletion exons 7–9[b]	
MFS36M5				Deletion exons 7–9[b]	
MFS36M4				Deletion exon 6	
MFS12M4	F	38	Exclusion exons 2–3	V(D)J class I	
MFS12M37				V(D)J class I	
MFS12M34			$C_{508}\rightarrow T$		Transition (CpG)
MFS12M40			$A_{401}\rightarrow G$		Transition
MFS65M3	M	38	$G_3\rightarrow T$[b]		Transversion
MFS65M19			$G_3\rightarrow T$[b]		Transversion
MFS65M4			$G_3\rightarrow T$[b]		Transversion
MFS65M5				Deletion exons 7–9	
MFS65M9			Exclusion 8	IVS8+2 T→C	Transition
MFS65M10				V(D)J class I	
MFS65M11			$G_{190}\rightarrow C$		Transversion
MFS65M15			$C_{151}\rightarrow T$		Transition (CpG)
MFS65M17				V(D)J class I	
MFS65M18			$T_{82}\rightarrow G$		Transversion
MFS94M3[e]	F	38	Exclusion exons 2–3	NC[c]	
MFS94M1			$Deletion_{429–475}$		
MFS3M1	M	39	$A_{484}\rightarrow C$		Transversion
MFS3M2				V(D)J class I	
MFS85M3	F	39	Exclusion exons 2–3	NC[c]	
MFS83M3	M	39		Deletion exons 1–9	
MFS87M1	M	39		Deletion exons 1–9	
MFS87M2				V(D)J class I	
MFS1M2	M	39		Deletion exons 1–9	
MFS1M4				V(D)J class I	
MFS43M4	M	39	$ins_{108}AT$		
MFS59M2	M	39		Deletion exons 7–8	
MFS5M2	M	39		V(D)J class I	
MFS58M1	M	39	$G_{538}\rightarrow A$		Transition
MFS58M3			$G_{580}\rightarrow C$		Transversion
MFS58M4				Deletion exons 1–3	
MFS72M3	M	39		V(D)J class I	
MFS72M8				Deletion exons 2–4	
MFS37M1	M	39	$G_{134}\rightarrow A$		Transition
MFS37M2				Deletion exons 2–9	
MFS37M4			$Del_{230–234}$ ACCTG		
MFS13M4	F	39	$G_{628}\rightarrow A$		Transition
MFS8M2	F	40	$Del_{218–221}$ AATT		
MFS8M3			Inclusion of first 49 bp of intron 1	IVS1+1G→A	Transition
MFS25M4	M	40	Exclusion exon 8	IVS8+5G→A	Transition
MFS60M1	M	40	$G_{606}\rightarrow T$		Transversion
MFS60M2				Deletion exon 4	
MFS38M4	F	40	Exclusion exon 7	IVS6-1G→A	Transition
MFS53M1	F	40		V(D)J class I	
MFS53M2				V(D)J class I	
MFS84M3	M	40		Deletion exon 1	
MFS84M4				Deletion exons 2–9	
MFS2M1	M	40		V(D)J class I	
MFS2M2[f]			Exclusion exon 6	NC[c]	
MFS2M4				V(D)J class I	
MFS2M12			Exclusion exon 6	IVS6+1G→T	Transversion
MFS2M13				V(D)J class I	
MFS79M2	F	40		V(D)J class I	
MFS6M1	F	40	Exclusion exons 2–3	V(D)J class I	
MFS6M2			TCR α insert intron 1		
MFS57M2	M	41		Deletion exon 1	
MFS57M3			$C_{151}\rightarrow T$		Transition (CpG)
MFS57M5				V(D)J class I	
MFS68M1	M	41	$G_{197}\rightarrow A$		Transition
MFS68M2			$C_{508}\rightarrow T$		Transition (CpG)
MFS68M3			$C_{151}\rightarrow T$		Transition (CpG)
MFS68M5			Inclusion of first 49-bp of intron 1	IVS1+5G→A	Transition
MFS14M1	M	42	Ins_{108}.AT		
MFS88M1	M	42	$C_{508}\rightarrow T$		Transition (CpG)
MFS88M2				V(D)J class I	
MFS88M5				Deletion exons 1–9	

[a] GA, gestational age in weeks.

[b] These mutants are considered to represent a single independent *HPRT* mutational event in this subject with subsequent *in vivo* clonal expansion.

[c] NC, not characterized.

[d] Mutational spectra data for term infants has been previously reported (7) except for the following subjects: MFS101; MFS85; MFS5; MFS58; MFS72; MFS37; MFS13; MFS38; MFS53; and MFS6.

[e] MFS94M3 showed the exclusion of exons 2 and 3 from cDNA, but is not a V(D)J recombinase-mediated deletion. It is probably a splice alteration mutation, but this could not be confirmed by genomic DNA analysis because the mutant arose in a female.

[f] MFS2M2 showed the exclusion of exon 6 from cDNA, but no mutations could be found in the genomic region containing exon 6, therefore it is considered an uncharacterized splicing alteration.

Table 2 *Distribution analysis of in utero HPRT mutations*[a]

	Preterm				Term				Combined			
	Male		Female		Male		Female		Male		Female	
	n	%[b]	*n*	%[b]	*n*	%[b]	*n*	%[b]	*n*	%[b]	*n*	%[b]
No. of subjects	14	27	6	11	23	43	10	19	37	70	16	30
No. of mutants	47	31	19	13	68	45	17	11	115	76	36	24
Independent mutations[c]	43	31	17	12.5	61	44	17	12.5	104	75	34	25
All mutations	43	100	17	100	61	100	17	100	104	100	34	100
Small alterations	11	25	6	35	23	37	6	35	34	33	12	35
Transitions	7	16	3	17.5	13	21	5	29	20	19	8	23
Transversions	3	7	3	17.5	8	13	1	6	11	11	4	12
≤2-bp insertions	0	0	0	0	2	3	0	0	2	2	0	0
≤2-bp deletions	1	2	0	0	0	0	0	0	1	1	0	0
Large alterations	30	70	10	59	37	61	9	53	67	64	19	56
V(D)J deletions	21	49	10	59	17	28	6	35	38	36	16	47
Non-V(D)J deletions[d]	8	19	0	0	20	33	2	12	28	27	2	6
>2-bp insertions	1	2	0	0	0	0	1	6	1	1	1	3
Uncharacterized	2	5	1	6	1	2	2	12	3	3	3	9
Small alterations	11	100	6	100	23	100	6	100	34	100	12	100
Transitions	7	64	3	50	13	56	5	83	20	59	8	67
Transversions	3	27	3	50	8	35	1	17	11	32	4	33
≤2-bp insertions	0	0	0	0	2	9	0	0	2	6	0	0
≤2-bp deletions	1	9	0	0	0	0	0	0	1	3	0	0
Large alterations	30	100	10	100	37	100	9	100	67	100	19	100
V(D)J deletions	21	70	10	100	17	46	6	67	38	57	16	84
Non-V(D)J deletions[d]	8	27	0	0	20	54	2	22	28	42	2	11
>2-bp insertions	1	3	0	0	0	0	1	11	1	1	1	5

[a] Distribution analysis was confined to a comparative analysis between independent characterized *HPRT* mutation events.
[b] Percentage was calculated as the ratio of the number of each specific mutation (*n*) over the number of independent mutations for each group.
[c] Represents single *HPRT* mutational events by correcting for *in vivo* clonal expansion.
[d] Represent deletions >2 bp that are not mediated by V(D)J recombinase.

correlated with infant leukemia (22). The etiology of a number of *MLL* rearrangements observed is not clear. Cell lines established from leukemia patients with t(4;11)(q21;q23) *MLL* translocations have showed the hallmarks of V(D)J recombinase at the chromosomal breakpoints, which include cryptic RSSs and random base insertions at chromosomal breakpoints (23).

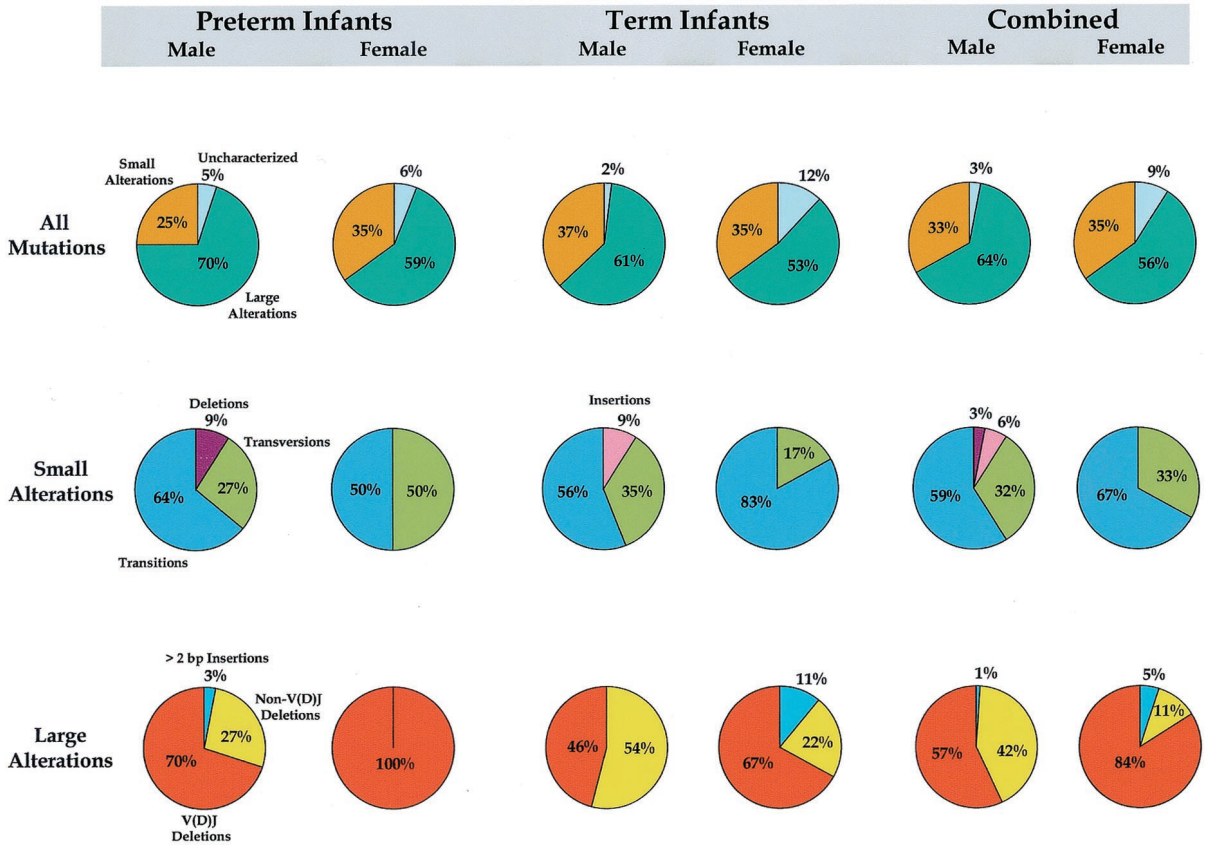

Fig. 1. Distribution analysis of *HPRT* mutations in T cells from preterm and term infants.

MALE	GA	N - Insertions	Class I
5′Intron 1		GTTTTGCACA***CACTGTA***	
PS21M3	26	GTTTTGCA-- *AAA*	-GTCTCAAAAGGTAT
PS21M5		GTTTTGCACA	--TCTCAAAAGGTATCAAAAGAGAATAGC
PS30M2	29	GTTTTGCAC- *TC*	------AAAAGGTATCAAAAGAGAATAGC
PS30M3		GTTTT----- *TACCTT*	------AAAAGGTATCAAAAGAGAATAGC
PS30M7		GTTTTGCAC-	----TCAAAAGGTATCAAAAGAGAATAGC
PS26bM2	31	GTTTTGC--- *CCC*	--TCTCAAAAGGTATCAAAAGAGAATAGC
PS29M2		GTTTTGCACA *TTGATG*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS29M5		GTTTTGCACA *C*	---CTCAAAAGGTATCAAAAGAGAATAGC
PS4M1	31	GTTTTGC--- *CATC*	-----CAAAAGGTATCAAAAGAGAATAGC
PS4M3		GTTTTGCAC-	---CTCAAAAGGTATCAAAAGAGAATAGC
PS4M4		GTTTTGC--- *CT*	--TCTCAAAAGGTATCAAAAGAGAATAGC
PS4M5		GTTTTGCACA *TACGA*	-----CAAAAGGTATCAAAAGAGAATAGC
PS4M7		GTTTTGCAC-	---CTCAAAAGGTATCAAAAGAGAAT
PS18M1	32	GTTTTGCACA	TGTCTCAAAAGGTATCAAAAGAGAATAGC
PS6M1	33	GTTTTGCAC- *TTTA*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS20M1	34	GTTTTGC--- *CCTACTCGG*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS7M11	35	GTTTTGC--- *TCAAAGTACTTTTG*	----------AGC
PS7M20		GTTTTGCACA *TCCCTTTCATGACTCCACATCCCTTTCA*	TGTCTCAAAAGGTATCAAAAGAGAATAGC
PS15M2	35	GTTTTGCACA *TAATAGCA*	-----CAAAAGGTATCAAAAGAGAATAGC
		3′Intron 3 ***GTGTGTG***TGTCTCAAAAGGTATCAAAAGAGAATAGC	

			Class III
5′Intron 1		GTTTTGCACA***CACTGTA***	
PS16M2	34	GTTTTGCA-- *GGGGAG*	AATCCCAAATACAGCACAATTTT
PS15M5	35	GTTTTGC--- *CCTCGG*	----------GCACAATTTT
		3′Intron 3 ***TAGTGTG***AATCCCAAATACAGCACAATTTT	

Female	GA	N - Insertions	Class I
5′Intron 1		GTTTTGCACA***CACTGTA***	
PS8M2	27	GTTTTGC--- *CCTG*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS9M2	29	GTTT------ *CCCAACCCC*	---------AGGTATCAAAAGAGAATAGC
PS26aM1	31	GTTTTGCAC- *CCG*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS26aM3		GTTTTGC--- *CATCTGGG*	---CTCAAAAGGTATCAAAAGAGAATAGC
PS26aM6		GTTTTGC--- *CGAGG*	-GTCTCAAAAGGTATCAAAAGAGAATAGC
PS26aM12		GTTTTGCAC- *GT*	--TCTCAAAAGGTATCAAAAGAGAATAGC
PS12bM1	34	GTTTTGCACA	------AAAAGGTATCAAAAGAGAATAGC
PS14M2	35	GTTTTGC---	----TCAAAAGGTATCAAAAGAGAATAGC
PS14M4		GTTTTGCA-- *GGGGATCCCA*	TGTCTCAAAAGGTATCAAAAGAGAATAGC
PS5M8	35	GTTTTGCACA *TGGAGA*	----------GTATCAAAAGAGAATAGC
		3′Intron 3 ***GTGTGTG***TGTCTCAAAAGGTATCAAAAGAGAATAGC	

Fig. 2. Breakpoint sequence analysis of *HPRT* V(D)J recombinase-mediated deletions in preterm infants. Intron 1 and 3 breakpoint regions for both Class I and III regions are shown. *Bold* nucleotides represent conserved genomic V(D)J RSSs associated with 12- or 23-bp spacer sequences. DNA sequences for Class I and III *HPRT* mutant clones show the hallmark signature markings of V(D)J recombinase-mediated events at breakpoint sites, including nibbling back, the presence of templated P nucleotides (*underline*), and the insertion of nongermline templated bases, N nucleotides (*italics*).

MLL rearrangements have also been associated with chemotherapy using topoisomerase II inhibitors (24, 25). These chemotherapeutic agents have been shown to increase the frequency of V(D)J recombinase-mediated *HPRT* deletions in the CCRF-CEM lymphoid cell line that constitutively expresses RAG1 and RAG2 (26), therefore suggesting a link between DNA DSB repair and V(D)J recombinase-mediated rearrangements. In addition, some components of the DSB repair system are shared by V(D)J recombinase, including the catalytic subunit of DNA-dependent protein kinase (27), Ku70 (28), and Ku80 (29, 30). Breakpoints associated with some t(4;11) translocations involving *MLL* genes have recently been reported to display short tandem repeats, inversions, and short homologous sequences at the chromosomal breakpoints, suggesting a DNA repair mechanism (31, 32). Of interest, P nucleotide sequences were observed at some of these breakpoints, which may indicate that V(D)J recombinase activity may have also participated in these translocations.

In this report, 22.6% of V(D)J breakpoints in preterm newborns did not contain N nucleotide insertion compared with 5.6% in full-term newborns. A decrease in the insertion of N nucleotides has been associated with the early stages of murine B-cell (33) and γδ T-cell development (34) as well as in human T-cell development attributable to decreased *in utero* expression of terminal deoxytransferase. The lower frequency of *in utero* N nucleotides we observed is in agreement with these previous reports. In addition, V(D)J mutant, PS7M20, contained a tandem repeat (5′-CACATCCCTTTCATG-3′). Such tandem repeats were also observed at *MLL* breakpoint sites (31, 32).

Therefore, during *in utero* lymphoid development, a synergistic relationship may exist between components of the V(D)J recombinase and DSB repair systems that increases the frequency of aberrant genomic deletions and chromosome translocations that are responsible for the development of leukemia in infants and children.

ACKNOWLEDGMENTS

We thank Holly Pasackow for obtaining preterm cord blood samples.

REFERENCES

1. Gale, K. B., Ford, A. M., Repp, R., Borkhardt, A., Keller, C., Eden, O. B., and Greaves, M. F. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. Proc. Natl. Acad. Sci. USA, *94:* 13950–13954, 1997.
2. Fasching, K., Panzer, S., Haas, O. A., Marschalek, R., Gadner, H., and Panzer-Grumayer, E. R. Presence of clone-specific antigen receptor gene rearrangements at birth indicates an in utero origin of diverse types of early childhood acute lymphoblastic leukemia. Blood, *95:* 2722–2724, 2000.

3. Wiemels, J. L., Cazzaniga, G., Daniotti, M., Eden, O. B., Addison, G. M., Masera, G., Saha, V., Biondi, A., and Greaves, M. F. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet, *354:* 1499–1503, 1999.
4. Wiemels, J. L., Ford, A. M., Wering, E. R. V., Postma, A., and Greaves, M. Protracted and variable latency of acute lymphoblastic leukemia after *TEL-AML1* gene fusion in utero. Blood, *94:* 1057–1062, 1999.
5. Yagi, T., Hibi, S., Tabata, Y., Kuriyama, K., Teramura, T., Hashida, T., Shimizu, Y., Takimoto, T., Todo, S., Sawada, T., and Imashuku, S. Detection of clonotypic IGH and TCR rearrangements in the neonatal blood spots of infants and children with B-cell precursor acute lymphoblastic leukemia. Blood, *96:* 264–268, 2000.
6. Yoshioka, M., Vacek, P. M., Poseno, T., Silver, R., and Finette, B. A. Gender-specific frequency of background somatic mutations at the hypoxanthine phosphoribosyltransferase locus in cord blood T lymphocytes from preterm newborns. Proc. Natl. Acad. Sci. USA, *96:* 586–591, 1999.
7. Finette, B. A., O'Neill, J. P., Vacek, P. M., and Albertini, R. J. Gene mutations with characteristic deletions in cord blood T lymphocytes associated with passive maternal exposure to tobacco smoke. Nat. Med., *4:* 1144–1151, 1998.
8. Gibbs, R. A., Nguyen, P-N., Edwards, A., Civitello, A. B., and Caskey, C. T. Multiplex DNA deletion detection and exon sequencing of the *hypoxanthine phosphoribosyltransferase* gene in Lesch-Nyhan families. Genomics, *7:* 235–244, 1990.
9. Fuscoe, J. C., Zimmerman, L. J., Lippert, M. J., Nicklas, J. A., O'Neill, J. P., and Albertini, R. J. V(D)J Recombinase-like activity mediates *hprt* gene deletion in human fetal T-lymphocytes. Cancer Res., *51:* 6001–6005, 1991.
10. Cariello, N. F., Douglas, G. R., Gorelick, N. J., Hart, D. W., Wilson, J. D., and Soussi, T. Database and software for the analysis of mutations in the human *p53* gene, human *hprt* gene, and both the *lacI* and *lacz* gene in transgenic rodents. Nucl. Acids Res., *26:* 198–199, 1998.
11. Finette, B. A., Poseno, T., and Albertini, R. J. V(D)J recombinase-mediated *HPRT* mutations in peripheral blood lymphocytes of normal children. Cancer Res., *56:* 1405–1412, 1996.
12. Ross, J. A., and Robison, L. L. MLL rearrangements in infant leukemia: is there a higher frequency in females? Leuk. Res., *21:* 793–795, 1997.
13. Haluska, F. G., Finver, S., Tsujimoto, Y., and Croce, C. M. The t(8;14) chromosomal translocation occurring in B-cell malignancies results from mistakes in V-D-J joining. Nature (Lond.), *324:* 158–161, 1986.
14. Breit, T. M., Mol, E. J., Wovers-Tettero, I. L. M., Ludwig, W-D., and Wering, E. R. Site-specific deletions involving the *tal*-1 and *sil* genes are restricted to cells of the T-cell receptor α/β lineage: T-cell receptor δ gene deletion mechanism affects multiple genes. J. Exp. Med., *177:* 965–977, 1993.
15. Bash, R. O., Crist, W. M., Shuster, J. J., Link, M. P., Amylon, M., Pullen, J., Carroll, A. J., Buchanan, G. R., Smith, R. G., and Baer, R. Clinical features and outcome of T-cell acute lymphoblastic leukemia in childhood with respect to alterations at the TAL1 locus: a Pediatric Oncology Group study. Blood, *81:* 2110–2117, 1993.
16. Duro, D., Bernard, O., Valle, V. D., Leblanc, T., Berger, R., and Larsen, C-J. Inactivation of the $P16^{INK4}$/*MTS1* gene by a chromosome translocation t(9;14)(p21–22; q11) in an acute lymphoblastic leukemia of B-cell type. Cancer Res., *56:* 848–854, 1996.
17. Felix, C. A., and Lange, B. J. Leukemia in infants. Oncologist, *4:* 225–240, 1999.
18. Sandler, D. P., and Ross, J. A. Epidemiology of acute leukemia in children and adults. Semin. Oncol., *24:* 3–16, 1997.
19. Ford, A. M., Ridge, S. A., Cabrera, M. E., Mahmoud, H., Steel, C. M., Chan, L. C., and Greaves, M. *In utero* rearrangements in the trithorax-related oncogene in infant leukaemias. Nature (Lond.), *363:* 358–360, 1993.
20. Gill-Super, H. J., Rothberg, P. G., Kobayashi, H., Freeman, A. I., Diaz, M. O., and Rowley, J. D. Clonal, nonconstitutional rearrrangements of the *MLL* gene in infant twins with acute lymphoblastic leukemia: *in utero* chromosome rearrangement of 11q23. Blood, *83:* 641–644, 1994.
21. Corral, J., Lavenir, I., Impey, H., Warren, A. J., Forster, A., Larson, T. A., Bell, S., McKenzie, A. N. J., King, G., and Rabbits, T. H. An MII-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell, *85:* 853–861, 1996.
22. Sun, L., Heerema, N., Crotty, L., Wu, X., Navara, C., Vassilev, A., Sensel, M., Reaman, G. H., and Uckun, F. M. Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. USA, *96:* 680–685, 1999.
23. Gu, Y., Cimino, G., Alder, H., Nakamura, T., Prasad, R., Canaani, O., Moir, D. T., Jones, C., Nowell, P. C., Croce, C. M., and Canaani, E. The (4;11)(q21;q23) chromosome translocations in acute leukemias involve the VDJ recombinase. Proc. Natl. Acad. Sci. USA, *89:* 10464–10468, 1992.
24. Pedersen-Bjergaard, J., Pedersen, M., Roulston, D., and Philip, P. Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. Blood, *86:* 3542–3552, 1995.
25. Pui, C-H. Childhood leukemias. N. Engl. J. Med., *332:* 1618–1630, 1995.
26. Chen, C-L., Fuscoe, J. C., Liu, Q., and Relling, M. V. Etoposide causes illegitimate V(D)J recombination in human lymphoid leukemic cells. Blood, *88:* 2210–2218, 1996.
27. Blunt, T., Finnie, N. J., Taccioli, G. E., Smith, G. C. M., Demengeot, J., Gottlieb, T. M., Mizuta, R., Varghese, A. J., Alt, F. W., Jeggo, P. A., and Jackson, S. P. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. Cell, *80:* 813–823, 1995.
28. Gu, Y., Jin, S., Gao, Y., Weaver, D. T., and Alt, F. W. Ku70-deficient embryonic stem cells have increased ionizing radiosensitivity, defective DNA end-binding activity, and inability to support V(D)J recombination. Proc. Nat. Acad. Sci. USA, *94:* 8076–8081, 1997.
29. Taccioli, G. E., Gottieb, T. M., Blunt, T., Priestly, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P., and Jeggo, P. A. Ku80: product of the *XRCC5* gene and its role in DNA repair and V(D)J recombination. Science (Washington DC), *265:* 1442–1445, 1994.
30. Smider, V., Rathmell, W. K., Lieber, M. R., and Chu, G. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. Science, *266:* 288–291, 1994.
31. Reichel, M., Gillert, E., Nilson, I., Siegler, G., Greil, J., Fey, G. H., and Marschalek, R. Fine structure of translocation breakpoints in leukemic blasts with chromosomal translocation t(4;11): the DNA damage-repair model of translocation. Oncogene, *17:* 3035–3044, 1998.
32. Gillert, E., Leis, T., Repp, R., Reichel, M., Hosch, A., Breitenlohner, I., Angermuller, S., Borkhardt, A., Harbott, J., Lampert, F., Griesinger, F., Greil, J., Fey, G. H., and Marschalek, R. A DNA damage repair mechanism is involved in the origin of chromosomal translocations t(4;11) in primary Leukemic cells. Oncogene, *18:* 4663–4671, 1999.
33. Gu, H., Forster, I., and Rajewsky, K. Sequence homologies, N sequence insertion, and J_H gene utilization in V_HDJ_H joining: implications for the joining mechanism and the ontogentic timing of LY1 B cell and B-CLL progenitor generation. EMBO J., *9:* 2133–2140, 1990.
34. Lafaille, J. J., DeCloux, A., Bonneville, M., Takagaki, Y., and Tonegawa, S. Junctional sequences of T-cell receptor $\gamma\delta$ genes: implications for $\gamma\delta$ T-cell lineages and for a novel intermediate of V-(D)-J joining. Cell, *59:* 859–870, 1989.