Leukemia (2005) 19, 415–419

www.nature.com/leu

RAS mutation is associated with hyperdiploidy and parental characteristics in pediatric acute lymphoblastic leukemia

JL Wiemels[1], Y Zhang[1], J Chang[2], S Zheng[3], C Metayer[2], L Zhang[2], MT Smith[2], X Ma[4], S Selvin[2], PA Buffler[2] and JK Wiencke[3]

[1]*Laboratory for Molecular Epidemiology, Department of Epidemiology and Biostatistics, and UCSF Comprehensive Cancer Center, University of California, San Francisco, CA, USA;* [2]*School of Public Health, University of California, Berkeley, CA, USA;* [3]*Department of Neurological Surgery, University of California San Francisco, CA, USA; and* [4]*Department of Epidemiology and Public Health, Yale University, New Haven, CT, USA*

We explored the relationship of *RAS* gene mutations with epidemiologic and cytogenetic factors in a case series of children with leukemia. Diagnostic bone marrow samples from 191 incident leukemia cases from the Northern California Childhood Leukemia Study were typed for *NRAS* and *KRAS* codon 12 and 13 mutations. A total of 38 cases (20%) harbored *RAS* mutations. Among the 142 B-cell acute lymphoblastic leukemia (ALL) cases, *RAS* mutations were more common among Hispanic children ($P=0.11$) or children born to mothers <30 years ($P=0.007$). Those with hyperdiploidy at diagnosis (>50 chromosomes) had the highest rates of *RAS* mutation ($P=0.02$). A multivariable model confirmed the significant associations between *RAS* mutation and both maternal age and hyperdiploidy. Interestingly, smoking of the father in the 3 months prior to pregnancy was reported less frequently among hyperdiploid leukemia patients than among those without hyperdiploidy ($P=0.02$). The data suggest that *RAS* and high hyperdiploidy may be cooperative genetic events to produce the leukemia subtype; and furthermore, that maternal age and paternal preconception smoking or other factors associated with these parameters are critical in the etiology of subtypes of childhood leukemia.

Leukemia (2005) **19,** 415–419. doi:10.1038/sj.leu.2403641
Published online 27 January 2005
Keywords: childhood leukemia; *RAS*; hyperdiploidy; smoking; maternal age

Introduction

The etiology of childhood leukemia is uncertain but may be different for distinct molecular subtypes of leukemia. A dominant-acting oncogene, *RAS*, is mutated in a large percentage of human tumors, in particular those with putative chemical causes such as lung and colon cancers,[1] and in chemically induced rodent tumors.[2] In leukemia, *RAS* mutations are historically correlated to pediatric and adult myeloid and less so to lymphoblastic subtypes.[3] *RAS* is not associated with prognostic outcome of leukemia in studies of childhood AML[4] and ALL,[5] but has been associated in etiology studies with occupational chemical exposures in adult AML.[6,7]

The *RAS* genes are part of the small GTPase family and consist of three separate genes, *NRAS*, *KRAS2*, and *HRAS*. *HRAS* is rarely mutated in hematologic tumors and is expressed at a low level compared to the other two isoforms in leukemia and the hematopoietic cells from which they derive,[8] and hence is not further considered here. The three *RAS* genes code for proteins that are nearly identical except for the C-terminus, and recent work has shown that these differences lead to discrete subcellular locations of RAS proteins, and distinct interacting proteins including nucleotide exchange and GTPase-activating proteins (reviewed in Hingorani and Tuveson[9] and Ehrhardt *et al*[10]). The RAS proteins activate several downstream pathways to promote proliferation, differentiation, survival, and apoptosis depending on cellular conditions.

We assessed *RAS* mutation status in cases derived from the Northern California Childhood Leukemia Study (NCCLS) with the hypothesis that the *RAS*-mutation positive subgroup would be associated with exposures to chemicals, specifically carcinogens from parental cigarette smoke. We also considered the relationship of *RAS* mutation with our most prevalent cytogenetic subgroup – high hyperdiploid leukemia (those with >50 chromosomes) – and patient demographic characteristics. *RAS* mutations were unexpectedly linked to hyperdiploidy; and among hyperdiploid patients, a negative association with paternal smoking at the time of pregnancy was apparent.

Materials and methods

Study population

Subjects were derived from the Northern California Childhood Leukemia Study (NCCLS). Included in this study were 191 incident cases of childhood leukemia (0–14 years), who were enrolled in from 1995 to 2000 and had cryopreserved pretreatment bone marrow aspirates obtained from the clinical center that first diagnosed the case. A detailed description of this approximately population-based study design can be found elsewhere,[11] as well as the subset used for this analysis.[12] Parental demographic characteristics and smoking information was provided by the case mother (97.5%) or father (2.5%) through in-person interviews in the home of the parents.

Cytogenetics

Patient diagnostic cytogenetics were subjected to a standardized review, supported by Fluorescence *In Situ* Hybridization (FISH) to help categorize *TEL-AML1* translocations and cryptic high hyperdiploidy (hereafter referred to as 'hyperdiploidy'). Hyperdiploidy is defined here as the presence of 50 or more chromosomes in diagnostic karyotypes (overt hyperdiploidy) or the concurrent present of more than two chromosomes 21 and X (identified by centromeric FISH) among cases where the karyotype failed or was unavailable (cryptic hyperdiploidy). The presence of extra chromosomes 21 and X distinguish 97% of hyperdiploid leukemia[13] and has been used elsewhere to indicate hyperdiploidy.[14]

Correspondence: Dr J Wiemels, Laboratory for Molecular Epidemiology, University of California San Francisco, 500 Parnassus Ave, MU-420W, Box 0560, San Francisco, CA 94143, USA;
Fax: +1 415 476-6014; E-mail: wiemels@itsa.ucsf.edu
Received 28 September 2004; accepted 29 November 2004; Published online 27 January 2005

RAS characterization

Screening for *RAS* mutations was performed by laboratory personnel on coded samples. Laboratory personnel at UC San Francisco did not have access to epidemiologic variables or cytogenetic status, information which was kept at UC Berkeley. DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen) from 5 μl bone marrow sample.

A Restriction Endonuclease-Mediated Selective (REMS)-PCR Screen was first used to identify *RAS* mutations, essentially as described.[15] REMS-PCR is a two-step nest PCR, the second PCR being subjected to a selective restriction endonuclease digestion of the normal wild-type sequence to allow for detection of the mutant. Primers and specific methods are available from the authors by request.

In order to confirm the results obtained by REMS-PCR, oligonucleotides were synthesized specific for each mutation, nonradioactively labeled, and used on dot blots of the first round PCR reaction essentially as previously described.[16] Finally, all mutations were confirmed by Sanger DNA sequencing (ABI 377, Foster City, CA, USA).

Statistical analysis

Pearson's χ^2 tests or Fisher's exact tests (when 25% of the cells had expected counts <5) were used to compare the distribution of *RAS* mutation by childhood leukemia subtypes, child's demographics, and parental characteristics. Multivariable analysis was performed using log-linear regression to assess the associations between seven variables (presence of *RAS* mutation, presence of hyperdiploidy, paternal smoking 3 months before pregnancy, maternal smoking during pregnancy, maternal age, child's race/ethnicity, and income) (see Table 2). The variable 'income' was included in this model since it has been found to be associated with leukemia risk in previous analyses.[11,17] Although log-linear model and logistic model are generally similar, log-linear model is more appropriate for this analysis because it does not require an outcome variable. Log-linear model was chosen for the analysis because it is not clear whether the hyperdiploidy is an antecedent event to the *RAS* mutation. Application of log-linear model is an extension of the Pearson's χ^2-test used to assess association in a two-dimensional (row by column) contingency table and it allows evaluation of associations in a multidimensional contingency table. A log-linear model consists of two parts, the additive part with the main effect terms and the part with the measures of association terms (interaction terms). The statistical significance of each interaction term was assessed by a *P*-value generated from the log-likelihood ratio test comparing the sub-model without the measure of association (interactive) term to the full model including the measures of association. This process identifies the degree of pairwise associations between the *RAS* mutation and hyperdiploidy and five other variables that make up the analysis (Table 2).

Results

A total of 157 cases were diagnosed with acute lymphoblastic leukemia (ALL), 32 with acute myeloid leukemia (AML), and two with chronic myeloid leukemia (CML). The mean and median ages of the cases were 6.1 and 5.0 years, respectively (range: 0.2–14.9 years). In all, 49% of cases were non-Hispanic (NH) White, 33% were Hispanic, and the remaining belonged to other racial/ethnic groups (Table 1). In our case series, 36% of fathers smoked in 3 months prior to pregnancy, and 12% of mothers smoked during pregnancy.

RAS mutations were present in 20% (38/191) of all leukemias including 17 *KRAS*, 18 *NRAS,* and three with both *K* and *NRAS*, and were similarly prevalent in AML and ALL (21 *vs* 16%, $P=0.49$). The presence of *RAS* was similar in B- and T-lineage leukemias (20 and 29%, respectively). Subsequent analyses were confined to the B-cell leukemia subgroup since it was the largest homogeneous group. A higher proportion of *RAS* mutant bone marrows were observed among the hyperdiploid B-cell ALL group compared to other B-cell subtypes ($P=0.02$, Table 1). Maternal age was inversely associated with *RAS* mutation ($P=0.007$, Table 1).

In order to detect associations between variables while adjusting for other covariates, a multivariable log-linear analysis was performed with ALL cases (Table 2). This model includes the seven variables of interest as main effects as well as in interaction with each other, in categorization of 157 ALL cases. In the multivariable model, *RAS* mutation remained significantly associated with maternal age ($P=0.01$) and hyperdiploidy ($P=0.03$). In addition, paternal smoking 3 months prior to pregnancy was significantly *inversely* associated with hyperdiploidy among ALL cases (7/42, or 17% among hyperdiploids *vs* 29/63, or 46% among nonhyperdiploids, $P=0.002$ in univariable analysis, and $P=0.02$ in the multivariable model log-linear model, Table 2).

Discussion

This is the first study to consider *RAS* mutation along with cytogenetic subtypes of pediatric ALL and epidemiologically derived variables in a series of leukemias. We report the association of mutations within the *RAS* oncogene (*N* and *K* loci) with mother's age at time of the child's birth, and hyperdiploidy (Table 1). We also report an unexpected inverse association between paternal smoking and hyperdiploidy. These associations are not likely to be explained by bias, since patient families were not aware of their child's *RAS* mutation status. Likewise, laboratory personnel were blinded to the subjects' cytogenetic status and epidemiologic information. Lastly, all subjects were cases, and all subjects would share any interview or response bias introduced by case status.

Childhood leukemia like other cancers is thought to be a multistep process in which two or more mutations occur at different periods in development of the child as well as the ontological development of the blood cell.[18–20] Hyperdiploidy is the gain of a number of extra chromosomes (5–22 more than the diploid 46) which is thought to occur in a single catastrophic mitosis,[21] and in some cases appears to be a prenatal event.[22,23] The association of *RAS* mutations with maternal age suggests that *RAS* mutation may also be a prenatal event, and indicates that an examination of archived neonatal blood samples for *RAS* mutations in children with *RAS*-mutation positive leukemia could be informative. A recent animal model suggests that *RAS* may operate as an initiating or a second event in a two-hit disease;[24,25] our data suggest that hyperdiploidy may represent a complementary genetic event in leukemias with *RAS* mutation. Recent evidence that *FLT3* mutations may also be such a complementary event in hyperdiploid leukemia[26,27] is compatible with the current results, as *FLT3* signals in part through the *RAS* pathway. Furthermore, mutations in another *RAS*-pathway gene, *PTPN11*, are genetically restricted to leukemias that do not have *RAS* mutations, and additionally are found in the *TEL*-

Table 1 *RAS* mutations in pediatric B-cell acute lymphoblastic leukemia, and relationships to patient and parental characteristics in the NCCLS study

	RAS mutation		*P-value*[a]
	Yes (%)	*No (%)*	
Childhood leukemia ($n=191$)[b]	38 (20)	153 (80)	—
Childhood leukemia phenotypes[c]			
ALL ($n=157$)	33 (21)	124 (79)	0.49
AML ($n=32$)	5 (16)	27 (84)	
ALL subtypes			
B lineage ($n=142$)	29 (20)	113 (80)	0.48
T lineage ($n=14$)	4 (29)	10 (71)	
B-lineage ALL cytogenetic subtypes[d]			
Nonhyperdiploid B cell ($n=86$)	12 (14)	74 (86)	0.02
Hyperdiploid B cell ($n=56$)	17 (30)	39 (70)	
Gender[d]			
Female ($n=69$)	10 (14)	59 (86)	0.09
Male ($n=73$)	19 (26)	54 (74)	
Race/ethnicity[d]			
Hispanic ($n=48$)	13 (27)	35 (73)	0.11
Non-Hispanic White ($n=65$)	8 (12)	57 (88)	
Other ($n=23$)	6 (26)	17 (74)	
Father's age[d]			
<30 ($n=58$)	15 (26)	43 (74)	0.16[e]
$\geqslant 30$ ($n=75$)	12 (16)	63 (84)	
Mother's age[d]			
<30 ($n=73$)	21 (29)	52 (71)	0.007[e]
$\geqslant 30$ ($n=61$)	6 (10)	55 (90)	
Income[d]			
$<\$30\,000$ ($n=43$)	11 (26)	32 (74)	0.46[f]
\$30 000–\$75 000 ($n=56$)	11 (20)	45 (80)	
$>\$75\,000$ ($n=35$)	5 (14)	30 (86)	
Father smoked 3 months prior to pregnancy[d]			
Yes	4 (12)	29 (88)	0.13
No	16 (25)	47 (75)	
Mother smoked 3 months prior to pregnancy[d]			
Yes	4 (19)	17 (81)	0.89
No	23 (20)	90 (80)	
Mother smoked during pregnancy[d]			
Yes	2 (15)	11 (85)	0.65
No	25 (21)	96 (79)	

[a]*P*-value derived from χ^2-test.
[b]Three cases had both *KRAS* and *NRAS* mutations.
[c]The two CML cases included in the study did not have *RAS* mutations.
[d]These included 152 ALL cases for whom the diagnosing hospital or the UC Berkeley performed FISH screening for t(12;21) and hyperdiploidy AND for whom the t(12;21) and hyperdiploidy information was captured from the hospital clinical cytogenetics report.
[e]*P*-value for trend when father's age treated as a continuous variable = 0.40; *P*-value for trend for mother's age = 0.02.
[f]*P*-value for trend when treated as a six-level ordinal variable = 0.20.

AML1-negative common ALL subgroup, particularly those with hyerpdiploidy,[28] the same subgroup we have found an excess of *RAS* mutations. Like *PTPN11*, *FLT3* mutations were shown to be genetically restricted to pediatric (myeloid) leukemias without *RAS* mutations.[29] Future work will need to determine whether mutations in *RAS* and *FLT3* are exclusive to each other in childhood ALL.

We did not detect a higher prevalence of parental smoking among *RAS*-mutation positive cases compared to *RAS*-mutation negative cases or case subtypes (Table 1), perhaps due to limited power. Previous studies suggest that *RAS* mutations may be associated with chemical exposures. Two epidemiologic studies have linked *RAS* mutation in adult myeloid leukemia with 'high risk' occupations for leukemogenesis.[6,7] Another case–case pediatric leukemia study (like the current one) suggested a role for parental hydrocarbon exposures including some specific for the father for leukemias with *RAS* mutations compared to those without.[30] In addition, mutagenic chemicals from maternal smoking cross the placenta enhancing the plausibility of an effect of parental smoking on pediatric leukemia risk.[31] Our analysis showed a significant association between paternal smoking and hyperdiploid leukemia, in the *inverse* direction, when compared to other leukemia subtypes (see Results). Because parental smoking was not significantly associated with *RAS* mutation-positive leukemia overall (Table 1), this suggests that another molecular subtype of leukemia may be *positively* associated with paternal smoking. Future studies should strive to assess the role of parental smoking and other hydrocarbons in pediatric leukemia among the various key tumor genetics subtypes (eg *TEL-AML1, MLL,* hyperdiploidy, and *RAS*). It should be noted that the current study did not include population-based controls and therefore population risks were not assessed.

The unexpected significant inverse association with preconception paternal smoking and hyperdiploid leukemia is unprecedented. This relationship was, however, not seen for maternal smoking, possibly since the prevalence of maternal smoking at the time of pregnancy was far lower (37% paternal, 12% maternal). No risk factors have been associated with hyperdiploidy apart from age of the child in past studies. The apparent lower prevalence of preconception paternal smoking among hyperdiploid cases needs to be further assessed by examining leukemia risk in a case–control analysis. We cannot exclude the possibility that smoking associated mutagens may be toxic to hyperdiploid clones, which have been shown to be especially sensitive to therapeutic chemical agents albeit those associated with poisoning the folate metabolic pathway.[32,33]

RAS mutation was more than twice as frequent among Hispanics (28%) compared to non-Hispanic whites (13%). Also, maternal age was clearly associated with incidence of *RAS* mutation (Tables 1 and 2). However, when both of these factors were included in the same model, ethnicity was not a significant factor but maternal age remained significant (Table 2), suggesting that ethnic identity may be confounded with maternal age which is the true associated factor. Maternal age at child's birth is not an established risk factor in leukemia, but a slightly increased risk in children born from older mothers has been observed.[34] The association with maternal age in the current study, that is, higher prevalence of *RAS*-mutant positive leukemias in children born of younger mothers, may be a reflection of an increased prevalence of other genetic subtypes in children born of older mothers. Furthermore, maternal age may be confounded with another causal variable, which should be considered in future studies.

The current study raises many questions and highlights the potential interactive role of *RAS* mutation, maternal age, ethnicity, cytogenetics, and parental smoking. The significant associations found here should guide the design of future etiology studies, which have at their heart a goal of teasing out the causal pathway to childhood leukemia, and emphasize the

Table 2 Multivariable log-linear model and selective associations: B-cell acute lymphoblastic leukemia cases in the NCCLS ($n = 152$)

Variables included in the model:[a]

$$\text{Var1} = \text{FSMpre};\ \text{Var2} = \text{MSMpreg};\ \text{Var3} = \text{MomAge} < 30;\ \text{Var4} = \text{Hyperdip};\ \text{Var5} = \text{RAS};\ \text{Var6} = \text{Race};\ \text{Var7} = \text{Income}$$

Full log-linear model:[a]

$$\begin{aligned}\text{Log(count)} = {} & a + b_1(\text{Var1}) + \ldots + b7(\text{Var7}) + c_1(\text{Var1} * \text{Var2}) + c_2(\text{Var1} * \text{Var3}) + \ldots + c_6(\text{Var1} * \text{Var7}) \\ & + d_1(\text{Var2} * \text{Var3}) + d_2(\text{Var2} * \text{Var4}) + \ldots + d_5(\text{Var2} * \text{Var7}) + e_1(\text{Var3} * \text{Var4}) + e_2(\text{Var3} * \text{Var5}) + \ldots + e_4(\text{Var3} * \text{Var7}) \\ & + f_1(\text{Var4} * \text{Var5}) + f_2(\text{Var4} * \text{Var6}) + f_3(\text{Var4} * \text{Var7}) + g_1(\text{Var5} * \text{Var6}) + g_2(\text{Var5} * \text{Var7}) + h_1(\text{Var6} * \text{Var7})\end{aligned}$$

Measure of association terms	*DF*[b]	χ^2-*value*[c]	*P-value*[d]
FSMpre*RAS	1	0.35	0.55
MSMpreg*RAS	1	0.10	0.75
FSMpre*Hyperdip	1	5.89	0.02
MSMpreg*Hyperdip	1	0.52	0.47
MomAge < 30*RAS	1	4.47	0.03
Hyperdip*RAS	1	6.43	0.01
RAS*Race	2	0.39	0.82

[a]Details: FSMpre = paternal smoking during preconception (two categories, one parameter); MSMpreg = maternal smoking during pregnancy (two categories, one parameter); MomAge < 30 = maternal age at birth < 30 years (two categories, one parameter); Hyperdip = hyperdiploidy (two categories, one parameter); Ras = any Ras mutation (two categories, one parameter); Race = child's race/ethnicity (three categories, two parameters); Income = annual household income (three categories, two parameters).
[b]DF = degrees of freedom.
[c]χ^2 value derived from log-likelihood ratio test comparing the submodel without the measure of the association term with the full model including the measure of association term.
[d]*P*-value associated with the χ^2 value derived from log-likelihood ratio test.

critical role of tumor genetic subgroups in epidemiologic study of pediatric leukemia.

Acknowledgements

This work was supported by grants form the National Cancer Institute (RO1 CA89032) and National Institute of Environmental Health Sciences (PS42 ES04705 and RO1 ES09137). JLW is a scholar of the Leukemia and Lymphoma Society of America.

References

1 Bos JL. RAS oncogenes in human cancer: a review. *Cancer Res* 1989; **49**: 4682–4689.
2 Sills RC, Boorman GA, Neal JE, Hong HL, Devereux TR. Mutations in RAS genes in experimental tumours of rodents. *IARC Science Publication*. Lyon: IARC Press, 1999, pp 55–86.
3 Parry TE. The non-random distribution of point mutations in leukaemia and myelodysplasia – a possible pointer to their aetiology. *Leuk Res* 1997; **21**: 559–574.
4 Vogelstein B, Civin CI, Preisinger AC, Krischer JP, Steuber P, Ravindranath Y *et al*. RAS gene mutations in childhood acute myeloid leukemia: a Pediatric Oncology Group study. *Genes Chromosomes Cancer* 1990; **2**: 159–162.
5 Perentesis JP, Bhatia S, Boyle E, Shao Y, Shu XO, Steinbuch M *et al*. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. *Leukemia* 2004; **18**: 685–692.
6 Barletta E, Gorini G, Vineis P, Miligi L, Davico L, Mugnai G *et al*. Ras gene mutations in patients with acute myeloid leukaemia and exposure to chemical agents. *Carcinogenesis* 2004; **25**: 749–755.
7 Taylor JA, Sandler DP, Bloomfield CD, Shore DL, Ball ED, Neubauer A *et al*. RAS oncogene activation and occupational exposures in acute myeloid leukemia. *J Natl Cancer Inst* 1992; **84**: 1626–1632.
8 Shen WP, Aldrich TH, Venta-Perez G, Franza Jr BR, Furth ME. Expression of normal and mutant ras proteins in human acute leukemia. *Oncogene* 1987; **1**: 157–165.
9 Hingorani SR, Tuveson DA. Ras redux: rethinking how and where Ras acts. *Curr Opin Genet Dev* 2003; **13**: 6–13.
10 Ehrhardt A, Ehrhardt GR, Guo X, Schrader JW. Ras and relatives – job sharing and networking keep an old family together. *Exp Hematol* 2002; **30**: 1089–1106.
11 Ma X, Buffler PA, Selvin S, Matthay KK, Wiencke JK, Wiemels JL *et al*. Daycare attendance and risk of childhood acute lymphoblastic leukaemia. *Br J Cancer* 2002; **86**: 1419–1424.
12 Zheng S, Ma X, Zhang L, Gunn L, Smith MT, Wiemels JL *et al*. Hypermethylation of the 5′ CpG island of the FHIT gene is associated with hyperdiploid and translocation negative subtypes of pediatric leukemia. *Cancer Res* 2004; **64**: 2000–2006.
13 Raimondi SC, Pui CH, Hancock ML, Behm FG, Filatov L, Rivera GK. Heterogeneity of hyperdiploid (51–67) childhood acute lymphoblastic leukemia. *Leukemia* 1996; **10**: 213–224.
14 Investigators UCCS. The United Kingdom Childhood Cancer Study: objectives, materials and methods. *Br J Cancer* 2000; **82**: 1073–1102.
15 Roberts NJ, Impey HL, Applegate TL, Fuery CJ, Ward RL *et al*. Rapid, sensitive detection of mutant alleles in codon 12 of KRAS by REMS-PCR. *Biotechniques* 1999; **27**: 418–420, 422.
16 Verlaan-de Vries M, Bogaard ME, van den Elst H, van Boom JH, van der Eb AJ, Bos JL. A dot-blot screening procedure for mutated ras oncogenes using synthetic oligodeoxynucleotides. *Gene* 1986; **50**: 313–320.
17 Ma X, Buffler PA, Gunier RB, Dahl G, Smith MT, Reinier K *et al*. Critical windows of exposure to household pesticides and risk of childhood leukemia. *Environ Health Perspect* 2002; **110**: 955–960.
18 Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer* 2003; **3**: 639–649.
19 Wiemels JL, Leonard BC, Wang Y, Segal MR, Hunger SP, Smith MT *et al*. Site-specific translocation and evidence of postnatal origin of the t(1;19) E2A-PBX1 fusion in childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 2002; **99**: 15101–15106.
20 Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G *et al*. Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet* 1999; **354**: 1499–1503.

21 Onodera N, McCabe NR, Rubin CM. Formation of a hyperdiploid karyotype in childhood acute lymphoblastic leukemia. *Blood* 1992; **80**: 203–208.
22 Panzer-Grumayer ER, Fasching K, Panzer S, Hettinger K, Schmitt K, Stockler-Ipsiroglu S *et al.* Nondisjunction of chromosomes leading to hyperdiploid childhood B-cell precursor acute lymphoblastic leukemia is an early event during leukemogenesis. *Blood* 2002; **100**: 347–349.
23 Maia AT, Tussiwand R, Cazzaniga G, Rebulla P, Colman S, Biondi A *et al.* Identification of preleukemic precursors of hyperdiploid acute lymphoblastic leukemia in cord blood. *Genes Chromosomes Cancer* 2004; **40**: 38–43.
24 Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J *et al.* Somatic activation of oncogenic KRAS in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc Natl Acad Sci USA* 2004; **101**: 597–602.
25 Chan IT, Kutok JL, Williams IR, Cohen S, Kelly L, Shigematsu H *et al.* Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. *J Clin Invest* 2004; **113**: 528–538.
26 Armstrong SA, Mabon ME, Silverman LB, Li A, Gribben JG, Fox EA *et al.* FLT3 mutations in childhood acute lymphoblastic leukemia. *Blood* 2004; **103**: 3544–3546.
27 Taketani T, Taki T, Sugita K, Furuichi Y, Ishii E, Hanada R *et al.* FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. *Blood* 2004; **103**: 1085–1088.
28 Tartaglia M, Martinelli S, Cazzaniga G, Cordeddu V, Iavarone I, Spinelli M *et al.* Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. *Blood* 2004; **104**: 307–313.
29 Meshinchi S, Stirewalt DL, Alonzo TA, Zhang Q, Sweetser DA, Woods WG *et al.* Activating mutations of RTK/ras signal transduction pathway in pediatric acute myeloid leukemia. *Blood* 2003; **102**: 1474–1479.
30 Shu XO, Perentesis JP, Wen W, Buckley JD, Boyle E, Ross JA *et al.* Parental exposure to medications and hydrocarbons and ras mutations in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1230–1235.
31 Milunsky A, Carmella SG, Ye M, Hecht SS. A tobacco-specific carcinogen in the fetus. *Prenat Diagn* 2000; **20**: 307–310.
32 Whitehead VM, Vuchich MJ, Lauer SJ, Mahoney D, Carroll AJ, Shuster JJ *et al.* Accumulation of high levels of methotrexate polyglutamates in lymphoblasts from children with hyperdiploid (greater than 50 chromosomes) B-lineage acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood* 1992; **80**: 1316–1323.
33 Belkov VM, Krynetski EY, Schuetz JD, Yanishevski Y, Masson E, Mathew S *et al.* Reduced folate carrier expression in acute lymphoblastic leukemia: a mechanism for ploidy but not lineage differences in methotrexate accumulation. *Blood* 1999; **93**: 1643–1650.
34 Reynolds P, Von Behren J, Elkin EP. Birth characteristics and leukemia in young children. *Am J Epidemiol* 2002; **155**: 603–613.