multicenter studies investigating effective chemotherapy regimens for infant ALL (Interfant 99 and POG/COG9407) have been completed in Europe and in the United States, and these results will be important in designing future protocols for this age group, as will studies to develop innovative targeted therapies for infants with *MLL*-rearranged ALL, now underway.[29,30]

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

No *nucleophosmin* mutations in pediatric acute myeloid leukemia with normal karyotype: a study of the Japanese Childhood AML Cooperative Study Group

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Acute myeloid leukemia (AML) with normal karyotype had a heterogenous prognosis. In this subgroup, *FLT3*-internal tandem duplication (ITD) was strongly associated with a poor prognosis.[1–3] Recently, it was reported that mutations of *nucleophosmin* (*NPM*) gene occur in 50–60% of adult AML with normal karyotype and were frequently associated with *FLT3*-ITD. In the AML patients with normal karyotype and *FLT3*-ITD, patients with *NPM* gene mutations showed a better prognosis than those without *NPM* gene mutations.[4–6] However, the frequency and clinical impact of *NPM* gene mutations in pediatric AML patients with normal karyotype remained uncertain because there were a few number of reports.[7,8]

We searched for *NPM* gene mutations in 33 (20.9%) of 158 patients with normal karyotype who were treated on Japanese Childhood AML Cooperative protocol, AML 99 (0–15 years old, median 8 years old).[9] We amplified exon 12 of *NPM* gene using the primers; *NPM* cDNA Fow, 5′-AAAGGTGGTTCTCTTCCC AAA-3′ and *NPM* cDNA Rev, 5′-GCATTATAAAAAGGACAGCC AGA-3′ and directly sequenced on a DNA sequencer (ABI 310; Applied Biosystems, Foster City, CA, USA) using a BigDye terminator cycle sequencing kit (Applied Biosystems).[9] We could not find any *NPM* gene mutations in this study.

It was reported that the frequency of *NPM* gene mutations in children (<18 years old) was very low (children 1 out of 47 (2.1%) versus adults 32 out of 126 (25.4%), $P<0.001$).[8] Furthermore, they suggested that *NPM* gene mutations were also rarely detected in patients younger than 40 years old (3 (3.5%) out of 85). On the other hand, it was reported that *NPM* mutations were found in seven (27.1%) of 26 pediatric AML patients with normal karyotype from Italy.[7] These seven patients ranged from 5.0–17.9 years old, 10 years old ($n=2$), 11 years old ($n=2$) and 5, 8, 17 years old ($n=1$). Notably, two AML patients with *NPM* gene mutations have been reported in adult Japanese AML study (15 and 16 years old).[5]

We also analyzed *FLT3*-ITD and *RAS* gene alterations in these patients and found *FLT3*-ITD in nine (27.3%), *NRAS* mutation in two (6.1%) and *KRAS* mutation in three (9.1%). The frequencies of these gene alterations were compatible with those of previous reports.[2,3]

We considered that *NPM* gene mutations may be infrequent in Asian pediatric AML patients with normal karyotype, especially less than 15 years old. Frequency of *NPM* gene mutations depends on age and may depend on races. Further larger studies of *NPM* gene analysis are needed to clarify this item.

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Letter to the Editor

Low Frequency of *KIT* Gene Mutation in Pediatric Acute Myeloid Leukemia with inv(16)(p13q22): A Study of the Japanese Childhood AML Cooperative Study Group

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Acute myeloid leukemia (AML) patients with t(8;21)(q22;q22) or inv(16)(p13q22) are known to have a good prognosis. Recently, mutations of the *KIT* gene have been found in 12.7% to 48.1% of adult AML patients with t(8;21) or inv(16) and in approximately 20% of pediatric AML patients with t(8;21) [1-5]. *KIT* gene mutations in adult and pediatric AML patients with t(8;21) and in adult AML patients with inv(16) have been associated with a poorer prognosis than in those without *KIT* gene mutations [1-5]. However, the frequency and clinical impact of *KIT* gene mutations in pediatric AML patients with inv(16) remain unknown. Pediatric AML patients with inv(16) have been reported to represent 3.4% to 6% of the total number of pediatric AML patients. Thus, the number of patients in this subgroup is very small [6,7].

Three hundred eighteen patients were enrolled in the Japanese Childhood AML Cooperative Study Group Protocol AML 99 from January 2000 to December 2002, and 12 (3.8%) of these AML patients comprised 11 patients with inv(16) and 1 patient with t(16;16)(p13;q22) [5,8]. The 5-year overall survival rate was 100%, and the event-free survival rate was 90.9%. Of these 12 AML patients with inv(16) or t(16;16), 7 patients were available for molecular analysis (age range, 11 months to 14 years; median, 10 years) (Table 1). The 5-year overall survival rate for these 7 patients was 100%, and the event-free survival rate was 85.7% (Table 1). We used the reverse transcriptase–polymerase chain reaction method in a mutational analysis of the extracellular domain (exons 8 and 9), the transmembrane domain (exon 10), the juxtamembrane domain (exon 11), and the second intracellular kinase domain (exons 17 and 18) of the *KIT* gene and then carried out a sequencing analysis [5]. Sequencing was performed directly or, if necessary, after subcloning.

KIT mutation (deletion of D419 in exon 8) was found in an 11-month-old male patient (1 [14.3%] of 7 patients). The initial white blood cell count for this patient (no. 7) was 199,000/μL, and a karyotype analysis revealed 46,XY,inv(16)(p13q22). This patient received a total of 6 consecutive chemotherapies; however, he relapsed 16 months after the initial diagnosis. He then underwent unrelated allogeneic stem cell transplantation during the second complete remission and has been alive for 40 months from the diagnosis. The remaining 6 AML patients with inv(16) have maintained a complete remission without relapse for more than 41 months.

As for *FLT3* and *RAS* gene alterations, we found 2 *FLT3* D835 mutations (28.6%) and 2 *NRAS* mutations (28.6%) in these 7 AML patients with inv(16) (Table 1). No patient had an *FLT3* internal tandem duplication or a *KRAS* gene mutation. The majority of these patients (5 [71.4%] of 7) had one of the chimeric *CBFβ-MYH11* transcripts, which have most frequently been found in AML cases with inv(16) (*CBFβ* at nucleotide 495 fused to

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Table 1.
Correlations of Clinical Features with *KIT*, *FLT3*, and *RAS* Gene Mutations in 7 Acute Myeloid Leukemia Patients with inv(16) or t(16;16)*

Patient No.	Age	Sex	FAB Classification	Karyotype	*CBFβ-MYH11*	*KIT* Mt	*FLT3* D835 Mt	*NRAS* Mt	EFS Time, mo
1	14 y	M	M4Eo	46,XY,inv(16)(p13q22), add(7)(q32)	†	–	–	+‡	63+
2	10 y	M	M4Eo	46,XY,inv(16)(p13q22)	1	–	–	–	67+
3	7 y	M	M1	46,XY,inv(16)(p13q22)	1	–	–	–	63+
4	13 y	F	M1	46,XX,inv(16)(p13q22)	1	–	+	–	59+
5	3 y	F	M5a	47,XX,+8,t(16;16)(p13;q22)	2	–	–	+‡	43+
6	14 y	F	M5b	46,XX,inv(16)(p13q22)	1	–	+	–	41+
7	11 mo	M	M4Eo	46,XY,inv(16)(p13q22)	1	+§	–	–	16

**CBFβ-MYH11* transcripts were detected by reverse transcriptase–polymerase chain reaction analysis and direct sequencing (1, nucleotide 495 of *CBFβ* fused to nucleotide 1921 of *MYH11*; 2, nucleotide 399 of *CBFβ* fused to nucleotide 1201 of *MYH11*). *KRAS* mutations were not found in any of the 7 patients. FAB, French-American-British; Mt, mutation; EFS, event-free survival.

†Chimeric transcripts were not detected.

‡Codon 12.

§Deletion D419 in exon 8.

MYH11 at nucleotide 1921, Table 1) [9,10]. The *FLT3* D835 mutation, *NRAS* mutation, and subtypes of *CBFβ-MYH11* transcripts were not associated with the clinical outcome.

We also looked for *KIT* mutations in 11 pediatric AML patients with inv(16) who were treated with the previous protocol in Japan (age range, 8 months to 15 years; median, 3 years), but we did not identify *KIT* mutations in any of the patients. Interestingly, 3 of the 11 AML patients with inv(16) were infants, and 2 of them died, although all 3 exhibited no mutations in *KIT*, *FLT3*, or *RAS*. These data together with those described in our previous report [11] suggest that infant AML patients with inv(16) have a poor prognosis, regardless of the status of these genes.

A few reports have suggested that adult AML patients who have inv(16) with *KIT* mutations were associated with a poorer prognosis than those without KIT mutations [2,3]. A recent study by the Berlin-Frankfurt-Münster Study Group revealed that 6 (54.5%) of 11 pediatric AML patients with inv(16) had *KIT* mutations but that the clinical impact was limited [12]. The Acute Leukemia French Association (ALFA) and the Leucémies Aiguës Myéloblastiques de l'Enfant (LAME) cooperative study groups also suggested that *KIT* gene mutations were not associated with a poor prognosis in pediatric and adult AML patients with inv(16) [4]. We considered the frequency of *KIT* gene mutations (1 [5.6%] of 18) among the pediatric AML patients with inv(16) in this study to be lower than that of adult AML patients with inv(16) [2-4]. We must await the results of a larger study regarding the correlation between *KIT* gene mutations and prognosis in pediatric AML patients with inv(16).

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Tandem Duplications of *MLL* and *FLT3* Are Correlated With Poor Prognoses in Pediatric Acute Myeloid Leukemia: A Study of the Japanese Childhood AML Cooperative Study Group

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Background. *Mixed-lineage leukemia* (*MLL*)-partial tandem duplication (PTD) is associated with poor prognosis in adult acute myeloid leukemia (AML), but its relationship to pediatric AML is unknown. ***Procedure.*** One hundred fifty-eight newly diagnosed AML patients, including 13 FAB-M3 and 10 Down syndrome (DS) patients, who were treated on the Japanese Childhood AML Cooperative Treatment Protocol AML 99 were analyzed for *MLL*-PTD, as well as internal tandem duplication (ITD) and the kinase domain mutation (D835Mt) in the *FLT3* gene. ***Results.*** We found *MLL*-PTD in 21 (13.3%) of 158 AML patients, but not in FAB-M3 or DS patients. The differences between patients with and without *MLL*-PTD were significant for 3-year overall survival (OS) (56.3% vs. 83.2%, $P=0.018$), disease-free survival (DFS) (41.7% vs. 69.6%, $P=0.010$), and relapse rate (RR) (54.3% vs. 27.6%, $P=0.0085$) of 135 AML patients excluding the FAB-M3 and DS patients. Furthermore, ITD and D835Mt in the *FLT3* gene were found in 17 (12.6%) and 8 (5.9%) of these 135 patients, respectively. The differences between patients with *FLT3*-ITD and the wild-type allele were significant for 3-year OS (35.3% and 84.3%, $P<0.0000001$), DFS (40.0% and 66.9%, $P<0.003$), and RR (52.4% and 30.3%, $P<0.005$). Coduplication of both genes was found in only 3 (1.9%) patients. ***Conclusion.*** AML patients with *FLT3*-ITD, but not D835Mt, showed a poor prognosis. AML patients with *MLL*-PTD were also correlated with poor prognosis in this study. Pediatr Blood Cancer 2008;50:264–269. © 2007 Wiley-Liss, Inc.

Key words: AML; childhood; cytogenetics; *FLT3*; *MLL*; *tandem duplication*

INTRODUCTION

Risk classification of acute myeloid leukemia (AML) patients based on cytogenetic abnormalities has been widely accepted in adult and pediatric AML studies [1–4]. AML patients with t(8;21), inv(16), and t(15;17) have been classified into a low risk (LR) group, those with monosomy 5 and monosomy 7 into a high risk (HR) group, and others into an intermediate risk (IR) group [2–4]. Patients with normal karyotype were classified into the IR group and showed various prognoses. Classification by gene alterations other than karyotypic abnormalities would be preferable for improving the treatment outcome of pediatric AML patients.

Chromosome 11q23 abnormalities involving the *mixed-lineage leukemia* (*MLL*) gene are found in about 5% of adult AML patients and in ~50% of infants with AML [5–7]. *MLL*-partial tandem duplication (PTD) is reported in ~10% of adult AML patients, but in 20–50% of adult AML patients with a normal karyotype and trisomy 11 [8–13]. *MLL*-PTD is associated with a poor prognosis in adult AML patients and a high relapse rate (RR) [10–13]. On the other hand, the prevalence and prognosis of *MLL*-PTD in pediatric AML patients remains obscure, although a relatively high prevalence of *MLL*-PTD has been reported in a few articles [14,15].

Fms-related tyrosine kinase 3 (*FLT3*) is one of the class III receptor tyrosine kinases that is normally expressed in hematopoietic stem cells and early progenitor cells [16,17]. Internal tandem duplication (ITD) of the juxtamembrane domain (JM) of the *FLT3* gene occurs in approximately 30% of adult AML patients [18–20] and in ~20% of pediatric AML patients [21–23]. *FLT3*-ITD is strongly associated with poor prognosis, especially in patients with a normal karyotype [18–23]. Furthermore, ~10% of adult AML patients have an activating loop mutation in the kinase domain specifically, a point mutation in aspartic acid residue at codon 835 (D835Mt). These patients show a poor prognosis [19,20,24]. The prevalence and prognostic significance of *FLT3*-D835Mt in pediatric AML patients are controversial [21,23].

We have previously reported the existence of the coduplication of *MLL* and *FLT3* in pediatric AML patients who had poor prognoses [25]. These results were confirmed in adult patients with a normal karyotype and trisomy 11 [12,13,26,27]. We here performed mutation analysis of both *MLL* and *FLT3* genes in 158 unselected pediatric AML patients treated on the Japanese pediatric AML collaborative treatment protocol AML99. These data suggest that *FLT3*-ITD and *MLL*-PTD are both important markers of poor prognosis in pediatric AML patients.

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PATIENTS AND METHODS

Patients

The diagnosis of AML was made according to the French–American–British (FAB) classification. Cytogenetic analysis was performed using the G-banding method. Among 318 newly diagnosed de novo AML patients enrolled from January 2000 to December 2002, 158 samples were available for molecular analysis (Table I). Among the 158 patients, there were 13 patients with FAB-M3 and 10 patients with Down syndrome (DS) who were treated with different treatment protocols [28–30]. There were no significant differences between the 135 analyzed patients without FAB-M3 and DS and the 105 non-analyzed patients in terms of age (median 6 years (range: 0–15 years) vs. 6 years (range: 0–15 years)) and initial WBC count (median 24.8×10^9/L (range: 1.65–621.0×10^9/L) vs. 13.8×10^9/L (range: 1.0–489.0×10^9/L, $P = 0.0764$)). Patients who were younger than 2 years old or had an initial WBC count <100,000/μl were treated with the Induction A regimen (etoposide (VP16), cytarabine (CA) and mitoxantrone (MIT), (ECM)). Patients who were older than 2 years old and had an initial WBC count >100,000/μl were treated with the Induction B regimen (VP16, CA and idarubicin (IDA), (ECI)). If patients achieved complete remission (CR), the patients were classified into three risk groups (62 in low, 57 in intermediate and 10 in high) according to the results of cytogenetic analyses or the achievement of CR after initial 2 courses of chemotherapy [28–30] (Supplemental Fig. 1 which has been reported in Blood [30], http://bloodjournal.hematologylibrary.org/cgi/data/2005-08-3408/DC1/2). AML patients with t(8;21) (except for those with WBC counts >50,000/μl) or inv(16)(p11q22) were classified into the LR group. Patients with monosomy 7, 5q-, t(16;21), or Ph1 were classified into the HR group. Patients were treated with additional chemotherapy or allogeneic stem cell transplantation (allo-SCT) in each risk group (Supplemental Fig. 1).

Informed consent was obtained from the patients or patients' parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Center approved this project.

Detection of *MLL*-PTD

Total RNA (4 μg) extracted from the bone marrow or peripheral blood samples at diagnosis was reverse transcribed to cDNA with a cDNA Synthesis Kit (Amersham Bioscience, Tokyo, Japan). *MLL*-PTD was examined by simple first round reverse transcriptase-polymerase chain reaction (RT-PCR) with 35 cycles using the primer pair 6.1 (located on exon 9) and E3AS (located on exon 4), according to the conditions previously reported [10,25,31]. We did not use the nested RT-PCR method because a previous report suggested that the *MLL*-PTD transcripts were highly detected in the healthy controls [31]. We used the CTS cell line as a positive control for *MLL*-PTD and water as a negative control for RT-PCR analysis

TABLE I. Clinical Characteristics of Patients With *MLL* or *FLT3* Gene Alterations

	All patients	*MLL*-PTD	*FLT3*-ITD	*FLT3*-D835Mt
Age, median (year)	6 (0–15)	10 (2–15)	9 (2–15)	11 (2–14)
WBC count, median ($\times 10^9$/L)	20.7 (1.0–620.0)	31.4 (3.6–343.4)	33.2 (3.0–620.0)	45.0 (3.3–440.0)
Male/female	89/69	12/9	8/12	7/4
FAB classification				
M0	6	1	1	0
M1	24	7(2[a])	4(2[a])	2
M2	46	5	4	2
M3	13	0	3	3
M4	22	4(1[a])	1(1[a])	1
M5	25	3	5	3
M6	1	0	0	0
M7	19	1	1	0
Unclassified	2	0	1	0
Karyotypic abnormalities				
Normal	33	8(2[a])	9(2[a])	2
t(8;21)	46	4	2	1
11q23 abnormalities	20	5	0	1
t(15;17)[b]	13	0	3	3
inv(16)	7	0	0	2
DS[b]	10	0	0	0
Others[c]	27	4(1[a])	5(1[a])	2
Unknown	2	0	1	0
Total	158	21	20	11
Risk group				
Low	62	4	2	3
Intermediate	57	13(2[a])	8(2[a])	4
High	10	3	2	0
Non-CR	6	1(1[a])	5(1[a])	1
Total	135	21	17	8

[a]Cases who showed *MLL*-PTD and *FLT3*-ITD simultaneously; [b]DS—Down syndrome, patients with FAB-M3 or DS were treated with the different protocol; [c]others contain −7, +8 or complex karyotypes.

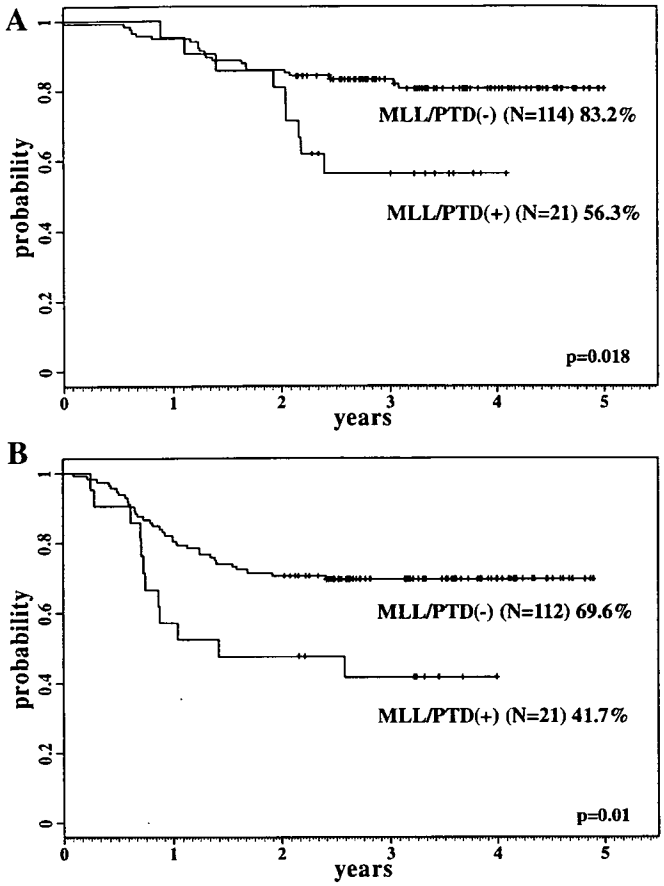

Fig. 1. Probabilities of 3-year OS (**A**) and 3-year DFS (**B**) in 135 AML patients excluding those with FAB-M3 and Down syndrome. Kaplan–Meier method estimates for patients with and without *MLL*-PTD are shown. The difference in patient numbers between OS and DFS resulted from the death of two patients during induction therapy.

[32,33]. Furthermore, we analyzed *MLL*-PTD in 10 normal bone marrow samples. Five microliter of the PCR products were electrophoresed in a 3% agarose gel. The amplified products were purified and directly sequenced.

Detection of *FLT3*-ITD and D835Mt

Using 1 μl of the cDNA, PCR amplification was performed for the JM and tyrosine kinase domain of the *FLT3* gene. The PCR procedure has been reported previously using primer pairs R5, R6, and 17F, TKR [30,34,35]. If a longer size product was found, the product was cut from the gel, purified with a QIAquick gel extraction kit (Qiagen, Chatsworth, CA), and directly sequenced on a DNA sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, CA) using a BigDye terminator cycle sequencing kit (Applied Biosystems). D835Mt was confirmed using *Eco*RV digestion and followed by direct sequencing as previously reported [24,30,34,35].

Statistical Analysis

Estimation of the survival distributions was performed using the Kaplan–Meier method and the differences were compared using the log-rank test. Disease-free survival (DFS) was defined as the time from diagnosis until the date of relapse. Overall survival (OS) was defined as the time from diagnosis until death owing to any cause or the last follow-up. Statistical difference analysis was performed using the χ^2 test. The prognostic significance of the clinical variables was assessed by using Cox proportional hazards model. These statistical analyses were performed with statistical software R. For all analyses, the *P*-values were two-tailed, and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

MLL-PTD

MLL-PTD was found in 21 (13.3%) of 158 pediatric AML patients (Table I). One type of fusion transcript (exon 9 and exon 3) was found in 10 patients, and the other type (exon 10 and exon 3) was found in 11 patients. Only one patient showed both fusion transcripts corresponding to alternatively spliced exons 10 and 11 to exon 3 (Supplemental Fig. 2). Furthermore, 10 normal bone marrow samples did not show *MLL*-PTD transcripts. *MLL*-PTD was frequently found in FAB-M1, M4 and patients with normal karyotype or 11q23 abnormalities (Table I). *MLL*-PTD was not found in FAB-M3 and DS patients. Patients with trisomy 11 were not found in this study. Remarkably, more than half of the patients with *MLL*-PTD were classified into the IR group (13 of 21 (61.9%)). The median age of patients with *MLL*-PTD was 10 years old (2–15) and no patients with *MLL*-PTD under 2 years old were found. Excluding the FAB-M3 and DS patients, the statistical differences in the clinical outcome between patients with and without *MLL*-PTD were significant for 3-year OS (56.3% vs. 83.2%, $P = 0.018$), DFS (41.7% vs. 69.6%, $P = 0.01$), and RR (54.3% vs. 27.6%, $P = 0.0085$) (Fig. 1). Allo-SCT was performed in 18 (85.7%) of 21 *MLL*-PTD patients, and 9 (50.0%) of them have been alive for a median of 42.0 months. The three patients without allo-SCT are all alive. Notably, six of the eight patients who received allo-SCT in the 1st CR and three of four patients who received allo-SCT in the 2nd CR are still alive.

FLT3-ITD and D835Mt

FLT3-ITD was found in 20 (12.7%) of 158 patients (Table I). All patients except for one showed both *FLT3*-ITD and *FLT3*-WT transcripts by RT-PCR. Half of the *FLT3*-ITD consisted of an in-frame tandem repeat of exon 11 (12–147 bp). The other half of *FLT3*-ITD showed insertions of 1–15 bp between the duplicated regions. *FLT3*-D835Mt was found in 11 (7.0%) of 158 patients. D835Mt consisted of D835Y (seven patients), D835V (two patients) and D835H (two patients). Differences in the median age of patients with *FLT3*-ITD, D835Mt, and the wild-type gene (WT) were not statistically significant (9, 11, and 5 years old, respectively). All patients with *FLT3*-ITD or D835Mt were older than 2 years old. The difference in the median initial WBC count between patients with *FLT3*-ITD and WT was significant ($P = 0.014$). Excluding FAB-M3 and DS patients, the differences between AML patients with *FLT3*-ITD, D835Mt, and WT were significant for the 3-year OS (35.3%, 100% and 84.3%, $P < 0.0000001$), DFS (40.0%, 87.5%, and 66.9%, $P < 0.003$), and RR (52.4%, 11.8% and 30.3%, $P < 0.005$) (Fig. 2). *FLT3*-ITD was found in five (83.3%) of six patients who did not

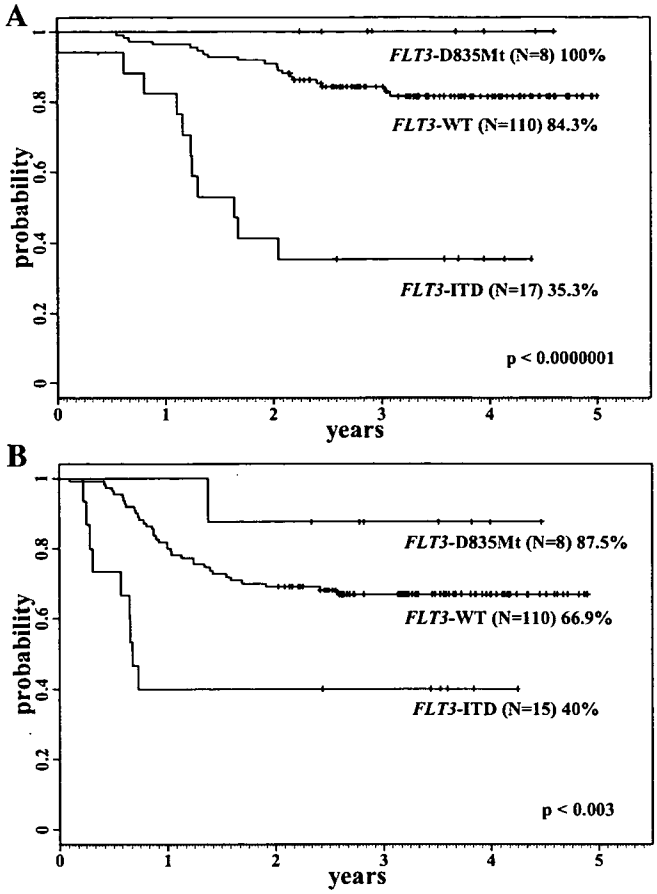

Fig. 2. Probabilities of 3-year OS (**A**) and 3-year DFS (**B**) in 135 AML patients, excluding those with FAB-M3 and Down syndrome. The Kaplan–Meier method for patients with *FLT3*-ITD, D835Mt, and WT is shown. The difference in patient numbers between OS and DFS resulted from the death of two patients during induction therapy.

attain CR. Allo-SCT was performed in 12 (70.6%) of 17 *FLT3*-ITD patients; of whom 4 (33.3%) were still alive for a median 43.5 months. The remaining eight patients died. Furthermore, four of seven patients who received allo-SCT in the 1st CR are still alive. Three of five patients without allo-SCT are also alive.

FLT3-ITD and D835Mt were found in 3 (23.1%) of 13 patients with FAB-M3. Both alterations of the *FLT3* gene did not influence the clinical outcome of FAB-M3 patients. Furthermore, these *FLT3* alterations were not found in DS patients.

Coduplication of the *MLL* and *FLT3* Genes

Coduplication of the *MLL* and *FLT3* genes were found in only 3 (1.9%) of 158 patients (Table I). Two patients had normal karyotype and one patient had +8. All three patients received allo-SCT, and two of them died because of disease progression.

Multivariate Analysis of Clinical Outcome

Multivariate analysis of *FLT3*-ITD, *MLL*-PTD, M1 marrow after induction therapy and initial high WBC count (more than 100×10^9/L) was carried out for 3-year OS and DFS data from 135 AML patients. Only *FLT3*-ITD was significant for 3-year OS (hazard ratio 8.4, 95% confidence interval (95% CI) 3.2–21.8, $P < 0.0001$). For 3-year DFS, *FLT3*-ITD, and M1 marrow after induction therapy were significant (hazard ratio 3.6 and 3.1, 95% CI 1.7–7.6 and 1.4–7.0, $P < 0.001$ and 0.007). Moreover, multivariate analysis was performed for 108 patients excluding those who received allo-SCT in 1st CR. Only *FLT3*-ITD was significant for 3-year OS (hazard ratio 16.0, 95% CI 4.7–54.7, $P < 0.00001$) (Table II). On the other hand, *MLL*-PTD was significant for 3-year DFS (hazard ratio 3.2, 95% CI 1.3–7.7, $P < 0.01$) (Table III).

DISCUSSION

In this study, *MLL*-PTD was found in 21 (15.6%) of 135 pediatric AML patients excluding those with FAB-M3 and DS. We used the simple first round RT-PCR method and not the nested RT-PCR method to minimize the possibility of detecting false positive *MLL*-PTD transcripts. *MLL*-PTD in pediatric AML has been reported at a relatively high frequency in a small number of patients: 2 (20%) of 10 patients [14] and 5 (9.4%) of 53 patients [15]. These data are compatible with our results. However, Shih et al. [36] have recently reported that *MLL*-PTD was rarely found in pediatric AML patients (one of 123, 0.8%). The difference of these frequencies in pediatric AML remains unknown but it may be partially due to the patient's age; although the median age of 16 patients with *MLL* rearrangements, including one *MLL*-PTD, is 1.3 years (1 day to 5.5 years) in the paper by Shih et al. [36], that of 21 patients with *MLL*-PTD is 10 years (2–15 years), and 17 of 21 patients with *MLL*-PTD is more than 6 years old in our study.

Patients with *MLL*-PTD showed a poor prognosis, a short duration of remission, and a high RR, as previously reported for adult AML patients [10–14,26]. Multivariate analysis suggested that *MLL*-PTD was a marker of poor prognosis for 3-year DFS, but not for 3-year OS, in AML patients excluding those who received allo-SCT in 1st CR in this study. This result may be explained by the effectiveness of allo-SCT in 2nd CR for patients with *MLL*-PTD. Indeed, four patients received allo-SCT in 2nd CR, and three of these patients are still alive.

Regarding karyotypic abnormalities, our results also confirmed that *MLL*-PTD was frequently found in AML patients with a normal karyotype as reported for adult patients [10–14]. Interestingly, *MLL*-PTD was found in AML patients with 11q23 translocations in this study. Moreover, *MLL*-PTD was also found in AML patients with

TABLE II. Prognostic Factors for 3-year Overall Survival in 108 AML Patients Treated on AML99 Protocol, Excluding Those Who Received Allo-SCT in 1st CR

Variable	*P*-values	Hazard ratio	95% CI
FLT3-ITD	<0.00001	16.0	4.7–54.7
MLL-PTD	0.25	2.1	0.6–7.4
M1 marrow after induction therapy	0.092	5.3	0.8–37.3
WBC > 100×10^9/L	0.14	0.19	0.02–1.7

TABLE III. Prognostic Factors for 3 Year Disease-Free Survival in 108 AML Patients Treated on AML99 Protocol, Excluding Those Who Received Allo-SCT in 1st CR

Variable	*P*-values	Hazard ratio	95% CI
FLT3-ITD	<0.0001	7.7	2.9–20.6
MLL-PTD	0.0099	3.2	1.3–7.7
M1 marrow after induction therapy	0.028	9.3	2.1–40.1
WBC > 100×10^9/L	0.013	3.1	1.3–7.5

t(8;21), which has not previously been reported in adult AML [10–14,26]. Unfortunately, we could not analyze DNA because genomic samples were not available. Two of the 4 t(8;21)-AML patients with *MLL*-PTD were also found to have *KIT* mutations in our previous study [30], suggesting that some patients possibly had genetic instability. We must await further studies to clarify these issues.

As for *FLT3* gene, multivariate analysis also strongly suggested that *FLT3*-ITD was an independent marker of poor prognosis in pediatric AML as previously reported [18,20,22]. D835Mt did not represent a poor prognosis in this study, confirming a previous report of pediatric AML [21], although D835Mt has been reported to be associated with poor prognosis in adult AML [18–20,24]. The difference between adult and pediatric AML remains unknown.

The coduplication of both genes was found in 3 (1.9%) of 158 patients in this study, which is compatible with previous reports (4 (1.6%) of 250 and 16 (1.7%) of 956 adult AML patients) [12,26]. The mechanism of formation of *MLL*-PTD and *FLT3*-ITD remains unknown. *MLL* and *FLT3* loci demonstrate similar susceptibilities to agents that modify chromatin configuration, including topoisomerase II inhibitors [27]. We conclude that the coduplication of *MLL* and *FLT3* genes is rare in pediatric AML as well as adult AML.

There was no definitive result as to the effectiveness of allo-SCT for the pediatric patients with *MLL*-PTD or *FLT3*-ITD. In this study, the majority of patients received allo-SCT due to the protocol agreement or relapse (18 (85.7%) of 21 *MLL*-PTD and 12 (70.6%) of 17 *FLT3*-ITD). Eight *MLL*-PTD patients and seven *FLT3*-ITD patients received allo-SCT in the 1st CR. Although similar results for 3-year DFS were found in patients with *MLL*-PTD (41.7%) and *FLT3*-ITD (40.0%), there was a difference in the 3-year OS between *MLL*-PTD (56.3%) and *FLT3*-ITD (35.3%) ($P = 0.024$). This difference was possibly due to the effectiveness of allo-SCT for the patients with *MLL*-PTD rather than those with *FLT3*-ITD as a lack of effectiveness of allo-SCT has been recently reported for patients with *FLT3*-ITD [37].

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Genetic abnormalities involved in t(12;21) *TEL–AML1* acute lymphoblastic leukemia: Analysis by means of array-based comparative genomic hybridization

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The *TEL (ETV6)–AML1 (RUNX1)* chimeric gene fusion is the most common genetic abnormality in childhood acute lymphoblastic leukemias. Evidence suggests that this chimeric gene fusion constitutes an initiating mutation that is necessary but insufficient for the development of leukemia. In a search for additional genetic events that could be linked to the development of leukemia, we applied a genome-wide array-comparative genomic hybridization technique to 24 *TEL–AML1* leukemia samples and two cell lines. It was found that at least two chromosomal imbalances were involved in all samples. Recurrent regions of chromosomal imbalance (>10% of cases) and representative involved genes were gain of chromosomes 10 (17%) and 21q (25%; *RUNX1*) and loss of 12p13.2 (87%; *TEL*), 9p21.3 (29%; *p16INK4a/ARF*), 9p13.2 (25%; *PAX5*), 12q21.3 (25%; *BTG1*), 3p21 (21%; *LIMD1*), 6q21 (17%; *AIM1* and *BLIMP1*), 4q31.23 (17%; *NR3C2*), 11q22-q23 (13%; *ATM*) and 19q13.11-q13.12 (13%; *PDCD5*). Enforced expression of *TEL* and to a lesser extent *BTG1*, both single genes known to be located in their respective minimum common region of loss, inhibited proliferation of the *TEL–AML1* cell line Reh. Together, these findings suggest that some of the genes identified as lost by array-comparative genomic hybridization may partly account for the development of leukemia. (*Cancer Sci* 2007; 98: 698–706)

TEL (ETV6)–AML1 (RUNX1), generated by the chromosomal translocation t(12;21) (p13;q22), is the most prevalent fusion gene in pediatric cancer, occurring in some 25% of childhood acute lymphoblastic leukemias (ALL).[1,2] Twin studies and retrospective analysis of archived neonatal blood spots of patients with ALL indicate that the chimeric gene arises predominantly during fetal hemopoiesis at a rate that considerably exceeds that of overt clinical ALL, and that *TEL–AML1*-harboring fetal clones expand and can persist in a clinically covert state for more than a decade.[1,2] These observations indicate that additional 'secondary' and postnatal genetic events are required for overt clinical leukemia. Findings obtained with animal models of the *TEL–AML1* translocation are consistent with this notion.[3–5]

Fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) studies have all been used in efforts to identify the genetic events required for *TEL–AML1*-harboring clones to become overtly leukemic.[6–14] Genomic loss of untranslocated *TEL* and gain of the *AML1* gene have often been found; however, the methods used were not broadly applied to the genome at large, or the analyses met with limited resolution.

In the study presented here, we used the array-CGH technique, which covers genome-wide chromosomal imbalances (gains/losses) with high resolution, to analyze a total of 24 *TEL–AML1* leukemia clinical samples and two cell lines. The results obtained revealed that all the samples, in addition to the *TEL–AML1* translocation, harbored at least two chromosomal imbalances, including previously unidentified ones.

Materials and Methods

Patient material and cell lines. Leukemia cell samples were collected from the National Nagoya Hospital in Japan (16 cases), and the Japanese Red Cross Nagoya First Hospital (eight cases). Informed consent was obtained from parents or guardians of all patients to have their samples used for banking and molecular analysis. Leukemia cells accounted for at least 70% of all clinical samples. All samples featured the *TEL–AML1* chimeric transcript as detected by reverse transcription–polymerase chain reaction (PCR) analysis. The ALL cell lines harboring the *TEL–AML1* translocation, Reh and KOPN41, were obtained from the American Type Culture Collection (Manassas, VA, USA), Dr K. Sugita and Dr S. Nakazawa (University of Yamanashi, Nakakoma, Japan), respectively. Cells were maintained in RPMI-1640 medium (Gibco-BRL, New York, NY, USA), supplemented with 10% fetal calf serum, at 37°C under 5% CO_2/95% air.

Comparative genomic hybridization. CGH analysis was carried out as described previously,[15] on DNA obtained from bone marrow cells of 24 patients and the various cell lines. At least 10 metaphases were visualized using a BX-60-RF microscope (Olympus, Tokyo, Japan) equipped with IP Laboratory Scientific Imaging Software (Scanalytics, Fairfax, VA, USA). The threshold sets corresponded to a mean hybridization ratio between tumor and normal of >1.2:1 for gain and <0.80:1 for loss.[15] Overrepresentation was interpreted as high-level amplification when the ratio exceeded 2.0. Heterochromatic regions in the centromeric and paracentromeric parts of chromosomes, the short arm of acrocentric chromosomes, and all regions adjacent to telomeres were not included in the evaluation. Aberrations of chromosomes 19 and 22 were not evaluated as they have been reported to show false-positive results in negative controls.[16] The cut-off value for the X chromosome of female patients was less than two copies (Table 1).

Array-CGH. A genome-wide scanning array with 2304 bacteria artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) clones, covering the whole human genome at a resolution of roughly 1.3 Mb, was used in this study,[17] whereas in some experiments a contig BAC array for chromosome 12p13.2 encompassing the *TEL* gene was used. The contig array contained

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Table 1. Summary of patient characteristics and chromosomal imbalances detected by a conventional comparative genomic hybridization (CGH) method

Case/cell line	Age (years)	Sex	WBC counts at Dx	NCI risk	CR	Relapse	Survival	Losses by CGH	Gains by CGH
1	2	M	14 700	SR	Y	Y	N	6q21-q22.1	10, 162
2	4	M	3 600	SR	Y	Y	Y	None	None
3	2	M	11 000	SR	Y	N	Y	12p11	None
4	5	F	7 400	SR	Y	N	Y	None	None
5	3	M	34 200	SR	Y	Y	Y	None	10
6	5	F	3 900	SR	Y	N	Y	12p11.2-p13.2	10, 21
7	5	F	77 800	HR	Y	N	Y	None	None
8	13	F	700	HR	Y	N	Y	12p11.2-pter	None
9	4	M	14 800	SR	Y	Y	Y	None	21
10	2	M	86 000	HR	Y	N	Y	None	None
11	3	M	14 000	SR	Y	U	N	6q22	21
12	3	F	39 200	SR	Y	Y	Y	11q13.3-qter, 12p, 6q26-qter	None
13	2	F	159 000	HR	N	NE	N	12p	Xp
14	7	M	8 400	SR	Y	N	Y	7q32-qter	10
15	6	F	37 500	HR	Y	N	Y	None	Xq26-qter
16	2	M	5 100	SR	Y	N	Y	None	None
17	8	M	11 700	HR	Y	N	Y	11q21-qter	21q21-qter, Xq21.3-qter
18	4	F	36 400	HR	Y	N	Y	12p	None
19	8	F	10 100	HR	Y	N	Y	6q21-q23	21
20	3	M	10 800	HR	Y	N	Y	None	None
21	4	F	8 100	SR	Y	N	Y	12p11-p12	None
22	6	M	51 800	HR	Y	N	Y	None	21q22-qter
23	2	F	12 900	HR	Y	N	Y	1p34-pter, 9p, 11q12, 12p12.1-per, 16p, 17p	None
24	3	F	16 700	HR	Y	N	Y	12p13	None
Reh	NA	NA	NA	NA	NA	NA	NA	3p21.1-p21.3	21q21-qter
KOPN41	NA	NA	NA	NA	NA	NA	NA	12p13.2-pter	21q21-qter

All cases were confirmed to have TEL–AML1 transcripts by reverse transcription–polymerase chain reaction. CR, complete remission; Dx, diagnosis; HR, high risk; NA, not applicable; NCI, National Cancer Institute; NE, not evaluable; SR, standard risk; U, unknown; WBC, white blood cell count.

19 BAC clones that included four BAC for TEL and its adjacent genes covering 2 Mb. The location of all clones used for the array-CGH were confirmed by standard FISH analyses. BAC/PAC clones were amplified by degenerate oligonucleotide primer (DOP)-PCR and spotted on glass slides, as described previously.[17,18] DNA preparation from cells, labeling, hybridization and scanning analyses were carried out according to the protocol previously described,[19] with minor modifications.[20] The data obtained were processed as described elsewhere to detect chromosomal imbalances.[21]

Oligonucleotide array-CGH analyses of some DNA samples were conducted according to the manufacturer's instructions. Briefly, 3 μg of genomic DNA from the reference (46, XY male) and the corresponding experimental sample were digested with *Alu*I and *Rsa*I, and then filtered with the aid of QIAprep spin columns (Qiagen, Hilden, Germany). A genomic DNA labeling kit (Agilent Technologies, Santa Clara, CA, USA) was used to label the resultant DNA with Cy3-dUTP or Cy5-dUTP, and Microcon YM-30 filter units (Millipore, Billeria, MA, USA) were used for filtering. Experimental and reference DNAs thus labeled were then mixed and hybridized with Human Genomic CGH 244K Microarrays (Agilent) in the presence of *Cot-1* DNA at 65°C for 40 h. After washing, the microarray slides were scanned with an Agilent Microarray Scanner, and the data were processed with Future Extraction software (Agilent).

Retroviral transduction and cell proliferation assays. cDNA for *INK4a* and *ARF* were kindly provided by Dr Scott Lowe (Cold Spring Harbor Laboratory, Cold Springs Harbor, NY, USA). cDNAs for *TEL* and *BTG1* were cloned by the PCR method using primer sets harboring restriction sites that allowed for subsequent subcloning; TTTAGATCTAAA*GAA-TTC*TCTGGGTTGGGGAGAGGAA/TTT*GTCGAC*TTCCCGGGTCTCTTCCTTTA for *TEL*, and TTTT*CTCGAGAATTC* TCCCCTAGAACCAGTAGCC/TTTT*CTCGAG*ACCTGATAC-AGTCATCATATTG for *BTG1*. The resulting products were, respectively, digested with *Bgl*II/*Sal*I and *Xho*I, and then inerted into the *BamH*I/*Xho*I or *Xho*I site of the pcDNA-Flag vector (Invitrogen, Carlsbad, CA, USA). Respective cDNAs tagged with Flag at the carboxyl terminus were then isolated with *EcoR*I and inserted into the *EcoR*I site upstream of the ires-green fluorescent protein (GFP) cassette of the pMXs-iresGFP retroviral vector (a kind gift from Dr T. Kitamura, University of Tokyo). Retrovirus was produced by cotransfection into 293T cells of the respective retroviral vector and the packaging plasmid with VSV-G envelope, pCGCGP (a kind gift from Dr A. Abe, Nagoya University). Reh cells were infected with the virus on retronectin-coated dishes (Takara, Otsu, Japan), according to the manufacturer's instruction.

Retrovirally infected cells were monitored for GFP expression by flow-cytometry on a FACS Caliber (BD Bioscience, San Jose, CA, USA). In some experiments, infected cells were stained with PKH-26 dye (Sigma, St Louis, MI, USA) and monitored for red fluorescence, along with GFP to analyze cell division.

Results

CGH analyses of *TEL–AML1* clinical samples. We first applied a conventional CGH technique to 24 *TEL–AML1* clinical samples to identify chromosomal imbalances. The results are summarized in Table 1. Eighteen cases showed chromosomal imbalances, including loss of 12p11-p13 (nine cases) and 6q22 (three cases),

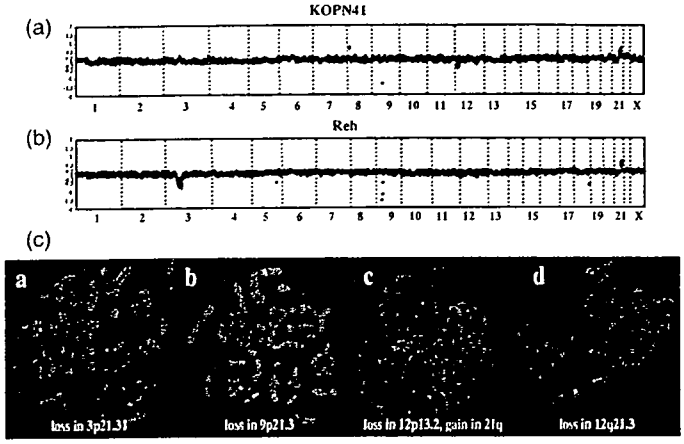

Fig. 1. Array-comparative genomic hybridization (CGH) profiles and fluorescent *in situ* hybridization (FISH) analysis of *TEL–AML1* cell lines. Log_2 ratios of signals of cell lines/sample versus normal control are plotted for all clones based on their chromosomal position, with chromosomes separated by vertical lines. Normal range of the log_2 ratios are within ±0.2. Clones are arranged from chromosome 1–22 and X within each chromosome on the basis of the Sanger Center Mapping Position, July 2004 version. (a) In the KOPN41 cell line, chromosomal imbalances detected were loss of 9p21.3, 12p12.3-pter and 12q21.3, and gain of 8p23.2 and 21q22.12-qter. (b) In the Reh cell line, loss of 3p14.3-p22.3, 5q23.2, 9p21.3, 12p13.2, 12q21.3 and 18q23, and gain of 21q22.12-qter were detected. (c) FISH analyses of Reh cells for the indicated chromosomal imbalances. BAC clones used were (a) RP11-509I21 (green) and RP11-335I9 (red), (b) RP11-149I2 (green) and RP11-30O14 (red), (c) RP11-71L1 (green) and RP11-525I3 (red), and (d) RP11-887P2 (green) and RP11-796E2 (green). Loss of signals by RP11-509I21 (3p21.31) in (a), RP11-149I2 (9p21.3) in (b), RP11-525I3 (12p13.2) in (c), a diminished signal by RP11-796E2 (12q21.3) in (d), and an extra signal by RP11-71L1 in (c) were clearly detected. Note that BAC RP11-525I3 (12p13.2) used in (c) is not included in the array-CGH analysis shown in (b), but is included in the contig-array in Fig. 3, where the corresponding region of loss is presented.

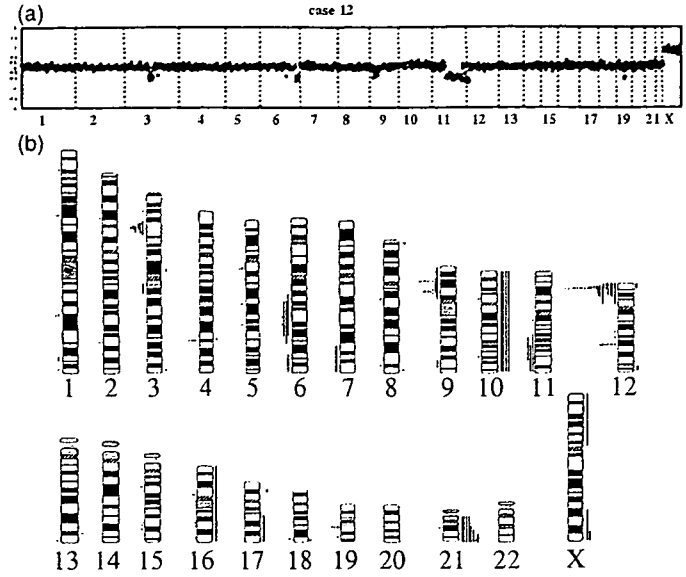

Fig. 2. Summary of chromosomal imbalances. (a) A representative array-comparative genomic hybridization (CGH) profile of patient samples (case 12) is presented. Detected were loss of 3q11.2-q13.13, 6q22.1, 6q25-qter, 9p13.3, 9p21.3, 9p23, 11q14.1-qter, 12p11.22-p13.2, 12q21.33 and 19q13.11-q13.12. (b) Chromosomal imbalances detected in all patient samples (red lines) and two cell lines (black lines) are presented. Regions of loss and gain are represented by vertical lines, on the left (loss) and right (gain) side of each ideogram.

and gain of chromosome 10 (four cases), chromosome 21 (four cases) and 21q22-qter (three cases), whereas six cases showed no alterations. These frequently altered genomic regions have been reported previously.[6,7,22,23]

Genomic profile of patient samples and *TEL–AML1* cell lines. To analyze genomic alterations in more detail, we next used the array-CGH technique. Our initial analysis was conducted on cell lines with the *TEL–AML1* translocation. Array-CGH profiles of KOPN41 (Fig. 1a) and Reh (Fig. 1b) cells revealed several common regions of chromosomal imbalance, including gain of regions on chromosome 21q22-qter and loss of regions on 9p21.1, 12p13 and 12q21. Among these, gain of 21q22-qter and loss of regions on 3p21.31, 9p21.3, 12p13.2 and 12q21.3 were subsequently confirmed in Reh cells by FISH analysis (Fig. 1c). Of particular note, loss of the region on 12q21.3 (the region where the *BTG1* gene resides) was marginally detected by array-CGH (the log2 ratio was −0.27, whereas the normal limit was within ±0.2), but the loss was subsequently confirmed by FISH analysis as a partial deletion of BAC, thus demonstrating the high-resolution capacity of the array-CGH method used.

We then analyzed the 24 *TEL–AML1* clinical samples using array-CGH and found that all cases exhibited at least two chromosomal imbalances. A representative array-CGH profile of a patient (case no. 12) is shown in Fig. 2a. Loss of regions on chromosomes 3q11.2-q13.13, 6q22.1, 6q25-qter, 9p13.3, 9p21.3, 9p23, 11q14.1-qter, 12p11.22-p13.2, 12q21.33 and 19q13.11-q13.12 were clearly detected. Among these, loss of 3q11.2-q13.13, 6q22.1, 9p13.3, 9p21.3, 9p23, 12q21.33 and 19q13.11-q13.12 were not detected using conventional CGH (Table 1).

A summary of chromosomal gains and losses identified with the array-CGH in the 24 *TEL–AML1* cases and two cell lines is presented in Fig. 2. Recurrent chromosomal imbalances found in no less than three patient samples included a gain of 21q (six cases), 10 (four cases), and loss of 12p13.2 (21 cases), 9p21.3 (seven cases), 9p13.2 (six cases), 12q21.3 (six cases), 3p21 (five case), 6q21 (four cases), 4q31.23 (four cases), 11q22-q23 (three cases) and 19q13.11-q13.12 (three cases). These findings suggest that a limited number of non-random genomic alterations could be causally related to the development of *TEL–AML1* leukemia.

Array analysis of the 12p13.2 chromosome region in *TEL–AML1* cases. We next sought to identify minimal common regions within clustered regions representing genomic deletions. Because deletion at 12p13 is known to be the most prevalent, occurring in some 60–80% of *TEL–AML1* cases,[1] we initially focused on this region with special attention to the *TEL* gene. Our initial arrays did not contain BAC for *TEL*, but 10 out of 24 cases (case 3, 6, 7, 8, 10, 13, 18, 21, 23 and 24) showed deletion of BAC encompassing a minimum of 25 Mb (BAC RP11-277P12, RP11-267J23 and RP11-180-M15). This suggests the occurrence of large-scale deletions including *TEL* and its neighboring genes at both the telomeric and centromeric sides (Fig. 3). To analyze cases that did not show such large deletion areas surrounding the *TEL* gene (14 out of 24 cases: cases 1, 2, 4, 5, 9, 11, 12, 14, 15, 16, 17, 19, 20 and 22, and the Reh and KOPN41 cell lines), we constructed a new array that included two BAC for the *TEL* gene and one BAC each directly adjacent to the *TEL* gene at both the telomeric and centromeric sides. Results of the array analysis of the deleted regions in each of the patient samples are summarized in Fig. 3. In all, 21 out of 24 cases (87.5%), as well as the Reh and KOPN41 cell lines, showed deletion at 12p13.2. Case 19 showed gain of *TEL* and its telomeric regions. It is known that chromosomal translocations such as *TEL–AML1* fusion often accompany deletion of the chromosomal regions adjacent to the break point on the allele involved in the translocation. This type of deletion therefore conceivably coincides with the *TEL–AML1* translocation that represents the 'first hit' event associated with the development of

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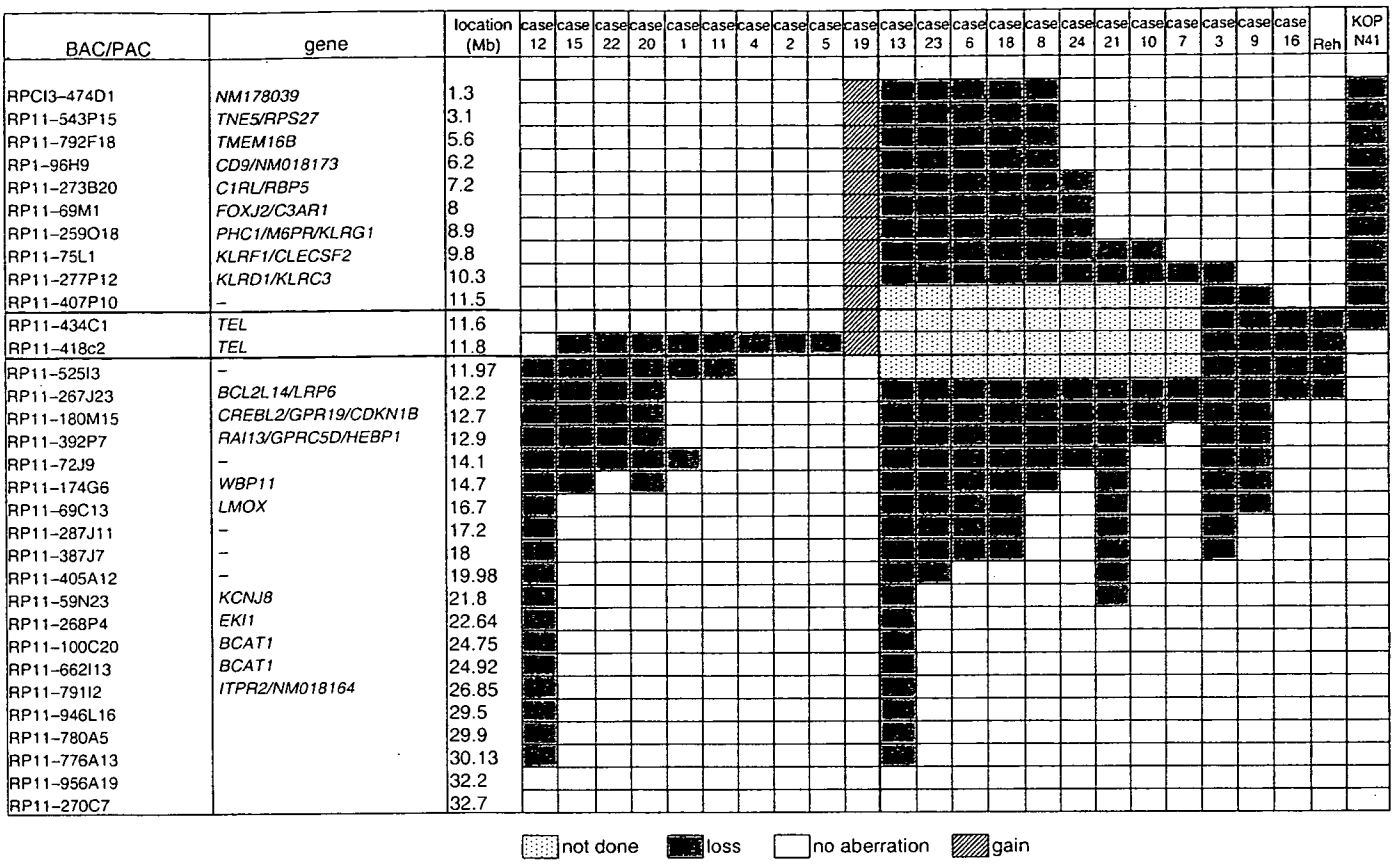


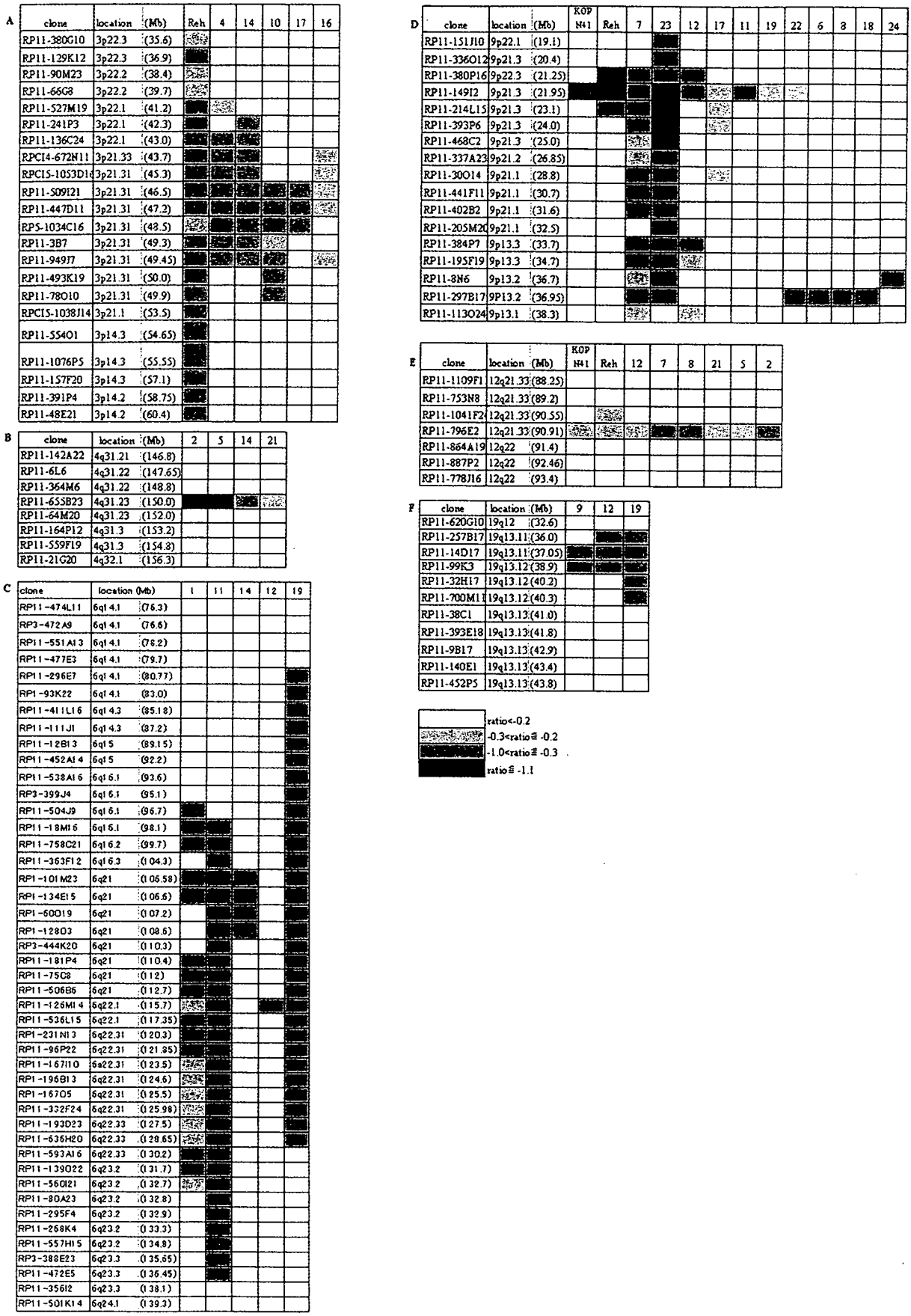
Fig. 3. Common region of loss at 12p13.2 region. Bacteria artificial chromosome and P1-derived artificial chromosome (BAC/PAC) clones, their location, and the absence or presence of loss are shown in each case and cell line. Filled-in and hatched boxes represent losses and gains, respectively. Dotted boxes indicate clones not tested.

leukemia. Deletion of the *TEL* gene, or part of it, found in our contig array could therefore be located on the allele involved in the *TEL–AML1* translocation. However, given that deletion of the *TEL* gene or part of it is on the allele involved in the *TEL–AML1* translocation, exons 1–5 should be retained as these exons are contained in the *TEL–AML1* fusion gene. Because BAC RP11-434C1 and RP11-418C2 contain exon 1 and exons 3–8, respectively, RP11-434C should be retained in this case. Cases 9 and 16, as well as the Reh and KOPN41 cell lines, which exhibit deletion of BAC-434C1, can thus be assumed to have lost the *TEL* gene or part of it on the allele not involved in the *TEL–AML1* translocation. Along the same lines, the cases mentioned earlier that showed large-scale deletions (cases 3, 6, 7, 8, 10, 13, 18, 21, 23 and 24) were judged to have lost the region including *TEL* on the allele not involved in the *TEL–AML1* translocation. Altogether, 12 of the 24 cases (cases 3, 6, 7, 8, 9, 10, 13, 16, 18, 21, 23 and 24) and the Reh and KOPN41 cell lines exhibited loss of the region including the *TEL* gene on the allele not involved in the *TEL-AML1* translocation. The loss, *ipso facto*, is unlikely to have coincided with the translocation. It should be noted that the KOPN41 cell line, unlike cases 9 and 16 and Reh, did not show deletion of BAC RP11-418C2, which contains exons 3–8 of the *TEL* gene, but exhibited deletion of BAC RP11-434C1 and its telomeric region (Fig. 3). Thus, the minimum region of deletion at 12p13 occurring independently of *TEL–AML1* translocation in both the samples and cell lines analyzed was most likely the *TEL* gene. Cases 2, 4 and 5 exhibited loss of a region at BAC RP11-418C2, but not at adjacent regions (BAC RP11-434C1 and RP11-525I3). These cases may represent intragenic deletion of the *TEL* gene on the allele not involved in the *TEL–AML1* translocation,[8] and could thus be included in the cases with *TEL* deletion not accompanying the translocation.

Consensus regions deleted at 3p21.3, 4q31.23, 6q21, 9p13.2, 9p21.3, 11q22.1-q23.3, 12q21.33 and 19q13.11-q13.12. Our BAC-array-CGH analysis showed, in addition to the TEL region, common regions of loss at 3p21.3, 4q31.23, 6q21, 9p13.2, 9p21.3, 11q22.1-q23.3, 12q21.33 and 19q13.11-q13.12.

3p21.3. Loss of the 3p21.31 region has been reported in many solid tumors.[24–26] In our array-CGH analysis, the minimum common region of loss was flanked by BAC RPCI5-1053D16 and RP5-1034C14 (Fig. 4a). This region contains a variety of genes, including *LIMD1* (LIM domains-containing protein 1), *LZTFL1* (leucine zipper transcription factor-like 1) and *FYCO1* (FYVE and coiled-coil domain containing 1), in addition to genes that code for several chemokines, *SMARCC1* (SWI/SNF-related matrix associated actin-dependent regulator of chromatin subfamily member 1), *CDC25A*, *ZNF589* (zinc finger protein 589), *LRRC2* (leucine rich repeat containing 2) and *TDGF1* (teratocarcinoma-derived growth factor 1 presursor). Among these, the *LIMD1* product is capable of inhibiting tumor growth by interacting with the retinoblastoma protein and suppressing E2F1-mediated transcription.[27] *LRRC2*[28] is a relative of ras suppressor protein 1 (*RSP-1*), which inhibits ras-mediated transformation of NIH3T3 cells. These findings suggest that the 3p21.31 region deleted in our patient samples contained tumor suppressors.

4q31.23. Loss of the 4q31 region has been reported in solid tumors.[29,30] Four of the cases included in our study exhibited loss of the region flanked by BAC RP11-364M6 and RP11-64M20 (Fig. 4b). This region contains genes including *EDNRA* (endothelin-1 receptor precursor), *TMEM34* (transmembrane protein 34), *ARHGAP10* (Rho GTPase activating protein 10), *NR3C2*



A

clone	location	(Mb)	Reh	4	14	10	17	16
RP11-380G10	3p22.3	(35.6)						
RP11-129K12	3p22.3	(36.9)						
RP11-90M23	3p22.2	(38.4)						
RP11-66G8	3p22.2	(39.7)						
RP11-527M19	3p22.1	(41.2)						
RP11-241P3	3p22.1	(42.3)						
RP11-136C24	3p22.1	(43.0)						
RPCI4-672N11	3p21.33	(43.7)						
RPCI5-1053D16	3p21.31	(45.3)						
RP11-509I21	3p21.31	(46.5)						
RP11-447D11	3p21.31	(47.2)						
RP5-1034C16	3p21.31	(48.5)						
RP11-3B7	3p21.31	(49.3)						
RP11-949J7	3p21.31	(49.45)						
RP11-493K19	3p21.31	(50.0)						
RP11-78O10	3p21.31	(49.9)						
RPCI5-1038J14	3p21.1	(53.5)						
RP11-554O1	3p14.3	(54.65)						
RP11-1076P5	3p14.3	(55.55)						
RP11-157F20	3p14.3	(57.1)						
RP11-391P4	3p14.2	(58.75)						
RP11-48E21	3p14.2	(60.4)						

B

clone	location	(Mb)	2	5	14	21
RP11-142A22	4q31.21	(146.8)				
RP11-6L6	4q31.22	(147.65)				
RP11-364M6	4q31.22	(148.8)				
RP11-655B23	4q31.23	(150.0)				
RP11-64M20	4q31.23	(152.0)				
RP11-164P12	4q31.3	(153.2)				
RP11-559F19	4q31.3	(154.8)				
RP11-21G20	4q32.1	(156.3)				

C

clone	location	(Mb)	1	11	14	12	19
RP11-474L11	6q14.1	(76.3)					
RP3-472A9	6q14.1	(76.6)					
RP11-551A13	6q14.1	(78.2)					
RP11-477E3	6q14.1	(79.7)					
RP11-296E7	6q14.1	(80.77)					
RP1-93K22	6q14.1	(83.0)					
RP11-411L16	6q14.3	(85.18)					
RP11-111J1	6q14.3	(87.2)					
RP11-128I13	6q15	(89.15)					
RP11-452A14	6q15	(92.2)					
RP11-538A16	6q16.1	(93.6)					
RP3-399J4	6q16.1	(95.1)					
RP11-504J9	6q16.1	(96.7)					
RP11-18M16	6q16.1	(98.1)					
RP11-758C21	6q16.2	(99.7)					
RP11-363F12	6q16.3	(104.3)					
RP1-101M23	6q21	(106.58)					
RP1-134E15	6q21	(106.6)					
RP1-60O19	6q21	(107.2)					
RP1-128O3	6q21	(108.6)					
RP3-444K20	6q21	(110.3)					
RP11-181P4	6q21	(110.4)					
RP11-75G8	6q21	(112)					
RP11-506B6	6q21	(112.7)					
RP11-126M14	6q22.1	(115.7)					
RP11-536L15	6q22.1	(117.35)					
RP1-231N13	6q22.31	(120.3)					
RP11-96P22	6q22.31	(121.85)					
RP11-167I10	6q22.31	(123.5)					
RP1-196B13	6q22.31	(124.6)					
RP1-167O5	6q22.31	(125.5)					
RP11-332F24	6q22.31	(125.98)					
RP11-193D23	6q22.33	(127.5)					
RP11-636H20	6q22.33	(128.65)					
RP11-593A16	6q22.33	(130.2)					
RP11-139O22	6q23.2	(131.7)					
RP11-560I21	6q23.2	(132.7)					
RP11-80A23	6q23.2	(132.8)					
RP11-295F4	6q23.2	(132.9)					
RP11-268K4	6q23.2	(133.3)					
RP11-557H15	6q23.2	(134.8)					
RP3-388E23	6q23.3	(135.65)					
RP11-472E5	6q23.3	(136.45)					
RP11-356I2	6q23.3	(138.1)					
RP11-501K14	6q24.1	(139.3)					

D

clone	location	(Mb)	KOPN41	Reh	7	23	12	17	11	19	22	6	8	18	24
RP11-151J10	9p22.1	(19.1)													
RP11-336O12	9p21.3	(20.4)													
RP11-380P16	9p22.3	(21.25)													
RP11-149I2	9p21.3	(21.95)													
RP11-214L15	9p21.3	(23.1)													
RP11-393P6	9p21.3	(24.0)													
RP11-468C2	9p21.3	(25.0)													
RP11-337A23	9p21.2	(26.85)													
RP11-30O14	9p21.1	(28.8)													
RP11-441F11	9p21.1	(30.7)													
RP11-402B2	9p21.1	(31.6)													
RP11-205M20	9p21.1	(32.5)													
RP11-384P7	9p13.3	(33.7)													
RP11-195F19	9p13.3	(34.7)													
RP11-8N6	9p13.2	(36.7)													
RP11-297B17	9P13.2	(36.95)													
RP11-113O24	9p13.1	(38.3)													

E

clone	location	(Mb)	KOPN41	Reh	12	7	8	21	5	2
RP11-1109F1	12q21.33	(88.25)								
RP11-753N8	12q21.33	(89.2)								
RP11-1041F2	12q21.33	(90.55)								
RP11-796E2	12q21.33	(90.91)								
RP11-864A19	12q22	(91.4)								
RP11-887P2	12q22	(92.46)								
RP11-778J16	12q22	(93.4)								

F

clone	location	(Mb)	9	12	19
RP11-620G10	19q12	(32.6)			
RP11-257B17	19q13.11	(36.0)			
RP11-14D17	19q13.11	(37.05)			
RP11-99K3	19q13.12	(38.9)			
RP11-32H17	19q13.12	(40.2)			
RP11-700M11	19q13.12	(40.3)			
RP11-38C1	19q13.13	(41.0)			
RP11-393E18	19q13.13	(41.8)			
RP11-9B17	19q13.13	(42.9)			
RP11-140E1	19q13.13	(43.4)			
RP11-452P5	19q13.13	(43.8)			

ratio<-0.2
-0.3<ratio≦-0.2
-1.0<ratio≦-0.3
ratio≦-1.1

Fig. 4. Common region of loss at 3p21.3, 4q31.23, 6q21, 9p13.2, 9p21.3, 12q21.33 and 19q13.11-q13.12 regions. Bacteria artificial chromosome and P1-derived artificial chromosome (BAC/PAC) clones, their location, and the $\log_2$ ratios of signals of case/cell line versus normal control are presented as indicated.

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(mineralocorticoid receptor), *DCAK2-HUMAN* (serine/threonine-protein kisase DCAMKL2) and *LRBA* (lipopolysaccharide-responsive and beige-like anchor protein). It is speculated that deregulated intracellular signaling through loss of these genes may facilitate the development of leukemia.

6q21. Deletion of the 6q21 region has been found in lymphoid malignancies,[31–38] and contains genes including *AF6q21* (*FOXO3A*) implicated in cell cycle and apoptosis,[39] a putative tumor suppressor *AIM1* (absent in melanoma protein 1),[38] and *BLIMP1* (B lymphocyte-induced maturation protein 1),[40] implicated in plasma cell differentiation. Among these, *AIM1* and *BLIMP1* were included in the region commonly lost in our patient samples and flanked by BAC RP11-363F12 and RP1-60O19 (Fig. 4c). Case 12 showed a loss only at BAC RP11-126M14, which was shared by cases 1, 11 and 19; however, this BAC contains no known cancer-related genes, and does not fall in the reported regions of loss or LOH in lymphoid leukemia.[32–36]

9p13.2. Results of an LOH study of small lung cancers suggest that the 9p13 region contains tumor suppressors.[41,42] Our array-CGH analysis revealed that six cases showed loss of the region flanked by BAC RP11-8N6 and RP11-113O24 (Fig. 4d). This region contains the *PAX5* gene that encodes the transcription factor B cell-specific activating protein (BSAP), essential for B cell lineage commitment,[40] whereas overexpression of Pax5 has been linked to the development of lymphoma.[43,44] In our study, this region containing *PAX5* was unexpectedly deleted. The region also contains miscellaneous genes including *ZCCHC7* (zinc finger CCHC domain containing 7), *ZBTB5* (zinc finger and BTB domain containing protein 5), *FRMPD1* (FERM and PDZ domain containing 1), *RG9MTD3* (RNA guanine-9-methyl transferase domain containing 3), *WDR32* (WD repeat domain 32) and *SHB* (Src homology 2 domain containing adoptor protein B), although their involvement in relation to leukemia remains unknown.

9p21.3. Seven cases and two cell lines exhibited loss of 9p21.3, and the common region of loss was flanked by BAC RP11-380P16 and RP11-214L15 (Fig. 4d). This region contains genes for the interferon alpha precursors *CDKN2A* (cyclin dependent kinase 4 inhibitor A; *INK4a*) and *CDKN2B* (cyclin dependent kinase 4 inhibitor B; *INK4b*). The latter two genes are frequently deleted or inactivated in many tumors.[45]

11q22.1-q23.3. Three cases in our study exhibited loss of the 11q22.1-q23.3 region spanning approximately 15 Mb. The limited number of cases available for analysis made it impossible to identify the respective genes in this large chromosomal area, although this region overlaps with the region deleted in some lymphoid malignancies,[46,47] and contains the *ATM* gene as a candidate tumor suppressor. Mutations of the *ATM* gene have also been implicated in the development of acute lymphoblastic leukemia.[48–50]

12q21.33. Six cases and two cell lines exhibited loss of the region flanked by BAC RP11-1041F24 and RP11-864A19 (Fig. 4e). This region contains *BTG1* as the only known gene. *BTG1* belongs to the anti-proliferative (APRO) gene family, the product of which is capable of inhibiting cell proliferation.[51–53]

19q13.11-q13.12. Loss of the 19q13 region has been reported in some cancers.[54,55] Three of our cases showed loss of this region flanked by BAC RP11-257B17 and RP11-32H17 (Fig. 4f). This region contains miscellaneous genes including *PDCD5* (programmed cell death protein 5), the product of which is capable of enhancing apoptosis,[56] *ANKRD27* (ankyrin repeat domain 27), *ULE1B-HUMAN* (ubiquitin-like 2 activating enzyme E1B) and *RHPN2* (Rhophilin 2). Loss of these genes may lead to resistance to apoptosis and deregulated cellular signaling.

Regions of loss presented here are relatively large in size, thus precluding accurate delineation with the BAC array-CGH method of the genes responsible in their respective regions. We therefore next used the oligonucleotide array-CGH method for selected samples (cases 2, 5, 11, 12, 14, 17, and cell lines Reh and KOPN41) for further narrowing down of genes responsible. Because 3p21.31, 6q16 and 19q13.11 exhibited large regions of loss, it was unlikely that the genes responsible could be singled out there. We therefore focused on the 4q21.23, 9p21.3 and 12q21.33 regions. Figure 5 illustrates the oligonucleotide array-CGH profiles of each of these regions. The results showed that *NR3C* and *ARHGAP10* in 4q31.23, *BTG1* in 12q21.33, and *CDNK2A*, *CDNK2B* and *MTAP* in 9p21.3 were the genes responsible for the respective region of loss. Among these, deletion of *NR3C* in cases 2 and 5, and *MTAP/CDNK2A/CDNK2B* in Reh and KOPN41, were homozygous, as indicated by their respective log2 ratios.

Enforced expression of TEL and BTG1 is capable of limiting proliferation of Reh cells. Finally, we sought to determine which of the deleted genes are likely to be associated with the development of leukemia. To this end, the products of the genes involved were expressed using a retroviral expression system. We focused on *TEL* and *BTG1*, as these genes were found to be single genes commonly deleted in 12p13.2 and 12q21.3, respectively. Reh cells were infected with retrovirus for *TEL* or *BTG1* that coexpress GFP by virtue of the ires-GFP cassette (Fig. 6a), and the percentage GFP expression in the bulk culture was then monitored by flow cytometry; we were unsuccessful in our efforts to infect KOPN41 cells. Arf and Ink4a, often deleted in many cancers including *TEL–AML1* leukemia, were also included in the analysis. Expression of exogenously transduced genes was confirmed by western blotting (not shown). Figure 6b shows typical FACS data immediately following infection and 4 weeks thereafter. Reh cells infected with GFP-only control virus displayed 18.5% GFP immediately following infection, and remained largely unchanged over 4 weeks. In contrast, Reh cells infected with Arf- and Ink4a-virus, as expected, resulted in decreased GFP expression over time, suggesting that enforced expression of Arf and Ink4a compromise cell growth. Of particular note, enforced expression of *TEL*, and to a lesser extent of *BTG1*, also compromised cell growth. The time-course of GFP expression is summarized in Fig. 6c. Because Arf and Ink4a are well-established tumor suppressors, we next focused on TEL to further examine its effect on cell growth. Reh cells were infected with TEL- and GFP-only control viruses, cell membranes were stained with PKH-26 dye, and GFP expression monitored together with dye fluorescence (Fig. 6d). Reh cells infected with the GFP-only control virus displayed equal PKH-26 fluorescence intensity over time when comparing GFP-positive and GFP-negative fractions, suggesting equivalent cell division in both fractions. In contrast, in the case of TEL-virus-infected Reh cells, the GFP-positive fraction displayed higher PKH-26 fluorescence compared with the GFP-negative fraction, although it was noted that fluorescence levels were equivalent immediately following infection. Cell death as detected by 7-AAD (amino-actinomycin D) staining was similar over time in TEL-virus-infected cells (not shown). These findings suggest that inhibited cell proliferation, but not enhanced cell death, is the primary cause of the compromised cell growth observed.

Discussion

Postnatal secondary genetic events have been postulated as being crucial for *TEL–AML1*-harboring clones to become overtly leukemic.[1,2] FISH analysis using probes for *TEL* and *AML1* has frequently detected deletion of non-translocated *TEL* and amplification of *AML1*, suggesting that these genomic alterations are candidates for the secondary genetic events.[6,14]

In a search for additional genomic abnormalities, detailed cytogenetic studies and CGH analyses have been conducted, albeit with low resolution, thus making the detection of chromosomal imbalances limited. Here we used a genome-wide array-CGH method with proven higher resolution to identify genomic alterations in 24 *TEL–AML1* patient samples. Our findings

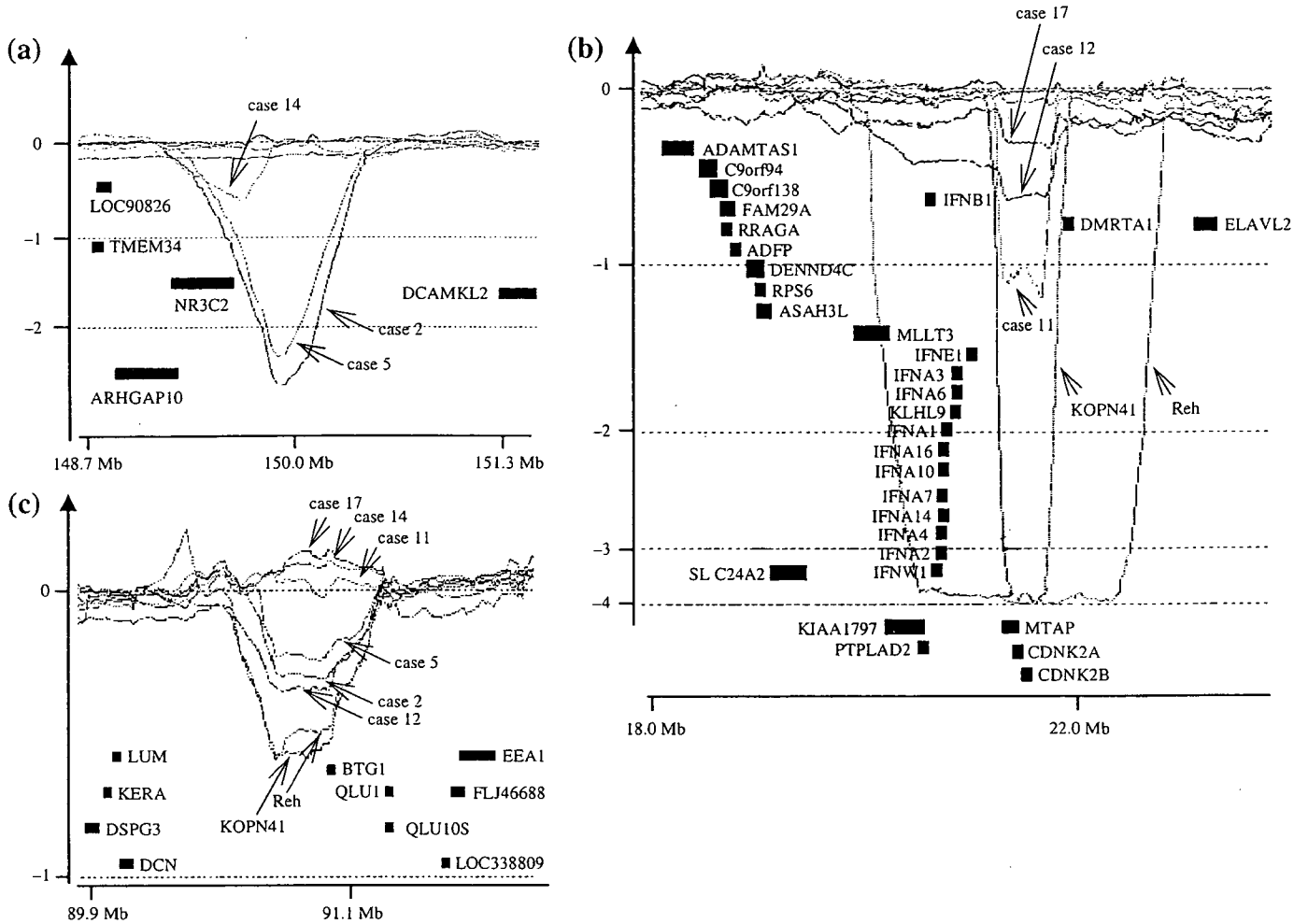

Fig. 5. Oligonucleotide array-comparative genomic hybridization (CGH) profiles. DNA from cases 2, 5, 11, 12, 14, 17, and cell lines Reh and KOPN41 were used for the oligonucleotide array-CGH analysis of the 4q21.23, 9p21.3 and 12q21.33 regions. Horizontal and vertical axes, respectively, represent the positions of the oligonucleotide probes and $\log_2$ ratios of the hybridized signals. Locations of genes are presented as solid bars, with the genes contained in the minimum region of loss shown in red.

showed that: (1) all patients had, in addition to the *TEL–AML1* translocation, at least two chromosomal imbalances, including gain of chromosomes 10 and 21q and loss of chromosomes 3p21, 4q31, 9p13, 9p21, 12p13, 12q21 and 19q13; (2) 12 out of 24 patients (50%) showed deletion of the *TEL* gene on the allele not involved in the *TEL–AML1* translocation; and (3) genes responsible for cell cycle regulation were also frequently deleted (45.8%).

Using a conventional CGH method, Ma *et al.* (two cases)[6] and Kanerva *et al.* (eight cases)[7] demonstrated the loss of 6q, 8p, 9p, 11q21qter and 12p, and gain of 7p15-pter, 8q22-qter, 10p12-pter, Xq23-q26 and 21 in *TEL–AML1* leukemia samples. CGH analysis of our patient samples also identified loss of 6q (four cases), 9p (one case), 11q (three cases), 12p (nine cases), and gain of 21q (six cases) and Xq25-qter (two cases). Loss of 7q32-qter (one case), 1p34 (one case), 16p (one case) and 17p (one case), and gain of 16 (one cases), 10 (four cases) and Xp (one case) were additionally identified in our CGH study, although some of these were reported by cytogenetic analyzes.[22,23] Six cases had no abnormalities, as indicated by the CGH method used.

Our array-CGH analysis revealed chromosomal imbalances in more detail than was possible using conventional CGH. The region encompassing the *TEL* gene on the allele not involved in the *TEL–AML1* translocation is reportedly deleted in *TEL–AML1* leukemia. The findings of LOH studies using PCR-mediated detection of microsatellite markers and expression analysis of genes involved in the deleted region of leukemia samples suggest that *TEL* is most likely the gene in the minimum common region of deletion, although these studies are not necessarily confined to *TEL–AML1* leukemia.[6–14] In the study presented here, we used *TEL–AML1* leukemia samples and cell lines exclusively for the array-CGH analysis and found that TEL is indeed the gene in question. It is known that chromosomal translocations such as the *TEL–AML1* fusion often accompany deletion of the chromosomal regions adjacent to the break point on the allele involved in the translocation. Indeed, through detailed FISH analyses of Reh and KOPN41 cell lines (S. Tsuzuki and S. Karnan, unpublished observations, May 2005), we found that in the case of the Reh cell line, *TEL* on the allele not involved in the *TEL–AML1* translocation was translocated to chromosome 5, which then became associated with the partial deletion of *TEL*. The other *TEL* allele in Reh cells was translocated to the *AML1* gene, and was accompanied by deletion of regions centromeric to the *TEL* gene (RP11-525I3 and RP11-267J23). Similarly, in the case of KOPN41, *TEL* not involved in the *TEL–AML1* translocation was found to be fused to material of chromosome 21 (and did not generate a TEL–AML1 chimera), which accompanied deletion of regions telomeric to the break point, although the *TEL* gene and its neighboring genes on the allele involved in the *TEL–AML1* translocation remained undeleted. Thus, both the Reh and KOPN41 cell lines showed

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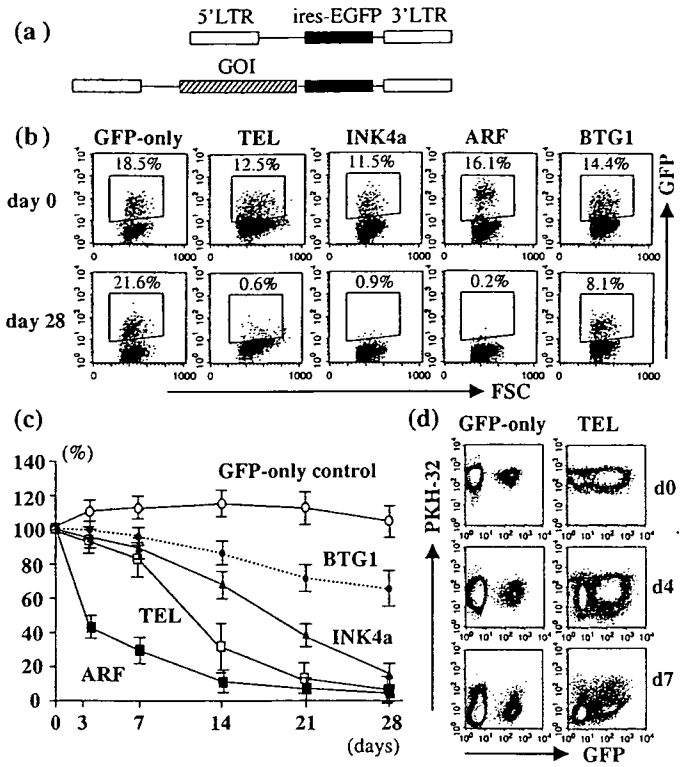

Fig. 6. Effect of enforced expression of *TEL* and *BTG1* on growth of Reh cells. (a) Schematic drawing of the retroviral vectors used (not to scale). (b) Typical FACS profile showing green fluorescent protein (GFP) expression of Reh cells infected with the indicated virus. Note that the GFP-control essentially displayed constant GFP expression over 28 days, in contrast to TEL-, INK4a-, Arf- and BTG1-virus infected cells, where GFP expression decreased with time. (c) Time-course of GFP expression. % GFP expression relative to that of day 0 is shown. Typical data of five independent experiments is presented together with the mean and SD of triplicate samples. (d) Inhibited cell division of TEL-expressing Reh cells. Reh cells were infected with GFP-only control-viruses or TEL-viruses, and immediately stained with PKH26 fluorescent dye. FACS profiles for GFP and PKH-26 fluorescence of infected cells are shown. Two independent experiments yielded similar results.

partial deletion of the *TEL* gene and its adjacent chromosomal regions on the allele not involved in the *TEL–AML1* translocation. The overlapping region of the deletion was in BAC RP11-434C1, which contained exon 1 of the *TEL* gene exclusively. When combined with the findings of our patient sample analysis, these results suggest that the minimum common region of loss at 12p13.3 is the *TEL* gene. Cases 2, 4 and 5 may feature intragenic deletions of the *TEL* gene on the allele not involved in the *TEL–AML1* translocation, as previously reported.[8] Importantly, loss of *TEL* is not confined to leukemia, but has also been reported for a subset of solid tumors, such as prostate cancer.[57,58] These findings suggest that *TEL* might act as a tumor suppressor.

Along the same lines, it should be noted that most of the chromosomal losses found in our study are also found in non-hematopoietic malignancies.[59] In addition to the *INK4a/ARF* and *ATM* regions, the 3p21.3, 4q31.23, 6q21, 9p21.2 and 19q13.12 regions are often found to be deleted in many tumors. It is not clear which genes are responsible for tumor development in these regions, but *LIMD1*[27] and *LRRC2*[28] in 3p21.31, *AIM*[38] in 6q21 and *PDCD5* in 19q13.11-q13.12[56] are plausible candidates. Detailed analyses to determine the minimum common region of deletion should provide a better understanding of the genes and mechanisms involved in the development of leukemia.

The development of leukemia is thought to require at least two mutations comprising class I mutations, which deregulate cell proliferation, and class II mutations, which restrict cell differentiation.[60] The *TEL–AML1* translocation has been classified as a class II mutation with inhibitory effects on B cell differentiation.[4] As for class I mutations, gain-of-function mutations of tyrosine kinases, such as c-kit and flt-3, have frequently been found in myeloid leukemia,[60] but not in *TEL–AML1* leukemia.[61] Thus, loss of cell cycle inhibitor genes observed in our study may represent a class I mutation; *LIMD1*, *INK4a/ARF*, *BTG1* and *TEL* are included in the region of loss. Of these, we focused on *TEL* and *BTG1*, which are both single genes known to be located in the minimum common region of loss, and set out to examine effects on the cell growth of *TEL–AML1*-harboring Reh cells. Results show that TEL and BTG1 inhibit cell growth, just as the well-established tumor suppressors Ink4a/Arf; the ability of BTG1 to inhibit cell growth was less marked compared with other inhibitors, but was reproducible.[62,63] These findings suggest that deletion of *TEL* and *BTG1* could partly account for the class I mutation in *TEL–AML1* leukemia.

In conclusion, the study presented here demonstrated that, in addition to previously described abnormalities such as deletion of *TEL* and amplification of the *AML1* gene, ALL with t(12;21) contains a limited number of non-random chromosomal imbalances. The chromosomal imbalances found in our study suggest pathways for tumor development that are also involved in cancers other than *TEL–AML1* leukemia. Identification of some of the genes located in each region associated with the chromosomal imbalances was possible using array-CGH, with its attendant high resolution, coupled with functional analysis. The data presented here should help to further our understanding of the molecular mechanisms leading to the development of leukemia.

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原　著

小児がん長期フォローアップ調査報告

久留米大学小児科[1]，愛媛大学小児科[2]，愛知県心身障害者コロニー中央病院児童精神科[3]，新潟県立がんセンター小児科[4]，国立国際医療センター小児科[5]，日本医科大学小児科[6]，国立成育医療センター総合診療部[7]，長野県立こども病院血液腫瘍科[8]，東北大学小児科[9]，あけぼの小児クリニック[10]，国立病院機構名古屋医療センター臨床研究センター[11]

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要　旨

近年の小児がん治癒率向上の一方で，治療や原疾患に伴う合併症が長期に及んだり，治療終了後遠隔期に合併症を発症する症例が報告されている．このような小児がんの晩期合併症は今後増加してゆくものと考えられ，長期フォローの重要性は高まっている．2005年4月に結成されたJPLSG長期フォローアップ委員会は，初期の活動として全国的な現状を把握し，小児がん経験者の長期フォローのあり方を提言する目的で施設アンケートを行った．対象はJPLSG登録211施設で，そのうち145（68.7%）施設から回答を得た．施設の概要，長期フォローに当たる小児科の状況，コメディカルとの連携の体制，系統別に晩期合併症の経験の有無と，各合併症の診療体制，長期間受診のない症例への対応，患者が成人した後の診療体制，治療経過の総括方法や情報開示の現状，今後期待される長期フォローのあり方などを評価した．結果より各施設が限られた体制で多様な晩期合併症に対処している現状が浮き彫りとなった．今後委員会活動の方向性として，①患者が受けた個別の抗がん治療に応じたリスクベースケア（危険因子に基づく管理）の導入と指針作成，②治療や合併症の基本情報が共有できる「治療総括ひな型」の作成，③長期フォローアップセンターを土台としたネットワークの確立と一次診療医や患者家族への教育，④公費負担のあり方に関して国への提言などが示唆された．

キーワード：小児がん，長期フォローアップ，晩期合併症，キャリーオーバー，
リスクベースケア（危険因子に基づく管理）

はじめに

小児がん医療における治療終了後の長期フォローアップは，小児がんを克服したいわゆる「経験者」たちの増加に伴いその重要性が増している．成長著しい小児期において化学療法を中心とした抗がん治療の与える影響を，治療が終了した後も医療者が追跡し，小児がん経験者が身体的・心理的・社会的な健康を保つことができるよう継続的に支援することが「真の治癒」に欠かせない．治療後一定期間を経て発症するいわゆる「晩期合併症」は治療終了直後や数年以内に発症するものから数十年経過して顕在化するものまで発症時期が多様であり，また疾患も内分泌・循環器・呼吸器さらに二次がんをはじめとした身体的なものから進学・結婚・就職等心理社会的な分野まで幅広く及ぶ[1]．海外の大規模研究によると[2]，18歳以上（平均26.6歳）の小児がん経験者において，全体の62.3%に1領域以上の健康上の問題が認められることが明らかにされた．わが国においてこれらの晩期合併症に対する長期フォローアップはほとんどが小児血液腫瘍専門医の外来診療で行われているが，担当する医師の知識や経験に依存する面が大きく，多様な合併症に対応するに当たり，施設や地域における格差が存在することも事実である．さらに，小児特定疾患の制度改正に伴い治療終了後5年経過した患者に対しての医療負担が増加したため，患者自身の受診動機を維持することも今後より困難になることが予測される．2005年4月に結成された日本小児白血病リンパ腫研究グループ（JPLSG）長期フォローアップ委員会は，初期の活動として全国的な現状を把握し，小児がん経験者の長期フォローのあり方を提言する目的で施設アンケートを行った．

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