Table 2 Infusion related toxicities

	Phase I		Phase II	
	Course 1 ($N = 20$) G 3+4/G 1–4	Course 2 ($N = 13$) G 3+4/G 1–4	Course 1 ($N = 20$) G 3+4/G 1–4	Course 2 ($N = 18$) G 3+4/G 1–4
Gastro-intestinal				
Nausea	2/12	0/5	4/14	1/6
Vomiting	1/7	0/5	0/9	1/5
Hepatic				
Bilirubin	0/1	0/0	0/1	0/1
γ-GTP	0/1	0/0	0/0	0/0
GOT	1/3	0/0	1/1	0/0
GPT	12	0/0	1/1	0/0
Metabolic/laboratory				
Hyperglycemia	0/1	0/0	1/2	0/0
Elevated LDH	0/2	0/0	0/2	0/0
Hypoalbuminema	0/3	0/0	0/2	0/1
General conditions				
Chilliness	0/11	0/3	0/5	0/2
Headache	0/4	0/3	0/6	0/0
Fever	1/16	0/8	0/15	0/5
Hematological				
Leucopenia	4/4	1/1	1/1	0/0
Lymphopenia	2/5	0/2	3/4	0/1
Thrombocytopenia	4/4	0/0	6/6	0/0
Coagulopathy				
Elevated D-dimer	0/2	0/0	0/5	0/1
aPTT	0/1	0/0	0/2	0/0
PT	0/1	0/0	0/0	0/1

γ-GTP gamma-glutamyl transpeptidase, *GOT* glutamic-oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *DH* lactose dehydrogenase

including high dose ara-C, and another patient had received five courses of induction therapy.

Responders were not limited to the first relapse. In the phase I part, a patient with an M3 subtype who was at the third relapsed and was resistant to all-trans-retinoic acid (ATRA) achieved blast clearance. A patient with an M4 subtype who relapsed after high-dose cytosine arabinoside (ara-C) and standard induction regimens achieved blast clearance. Another patient with an M4 subtype who failed standard induction therapy and relapsed five times also achieved blast clearance.

Cases with poor karyotype also responded. In the phase I part, a patient with an M4 subtype with complex karyotype (45, XY, -14, add(17)(p11), add(5)(q31)) achieved CR. In the phase II part, a patient with a M4 subtype with t(3;15)(p25;q22) achieved CRp and one with an M5 subtype who had an unfavorable karyotype (47, XX, +8) achieved blast clearance.

Overall survival (OS) was calculated using the Kaplan–Meier method (Fig. 1). The median OS was 305 days for the phase I part (not shown), and 420 days for the phase II part. The 95% confidence interval was from 74 days (lower limit) to 444 days (upper limit) for the phase I part. The lower limit of 95% confidence interval was 248 days and the upper limit has not been reached yet.

RFS of the two patients who achieved CR in the phase I part was 104 days and 62 months plus, in which "plus" means the patient lived longer than the follow-up interval. RFS for the six patients who achieved CR or CRp in the phase II part was 142 days, 161 days, 71 months, 50-plus months, 47-plus months, 47-plus months, and 44-plus months. The five long-term survivors in the phase II part included three with an M3 subtype, one with an M4 subtype, and one with an M5 subtype.

4 Discussion

In this study, the recommended dose of GO for Japanese patients with AML was 9 mg/m^2, given as two intravenous infusions separated by approximately 14 days, and is the

Table 3 Summary of hp67.6 pharmacokinetic parameters by dose group for dose periods 1 and 2

GO (mg/m^2)	Dose period	N	C_{max} (ng/ml)	$AUC_{0\sim t}$ (mg h/l)	AUC_{inf} (mg h/l)	$t_{1/2}$ (h)	λ_2 (1/h)	CL (l/h)	V_z (l)	V_{ss} (l)
6.0	1	6	1837 ± 237	63.5 ± 15.6	67.1 ± 16.6	62.78 ± 7.90	0.0112 ± 0.0014	0.151 ± 0.045	13.64 ± 4.29	7.54 ± 2.02
	2	5	1934 ± 569	90.0 ± 44.1	109.1 ± 42.9	310.51 ± 514.76	0.0084 ± 0.0055	0.097 ± 0.037	37.58 ± 54.98	23.23 ± 29.75
7.5	1	3	2497 ± 832	103.2 ± 79.8	108.7 ± 81.1	54.08 ± 28.38	0.0151 ± 0.0068	0.152 ± 0.089	11.73 ± 8.66	6.93 ± 4.84
	2	2	2808	170.0	180.9	52.24	0.0136	0.084	5.91	3.79
9.0	1	11	3248 ± 1195	123.6 ± 95.5	133.4 ± 94.0	50.94 ± 24.72	0.0164 ± 0.0078	0.188 ± 0.139	12.89 ± 11.78	6.71 ± 4.99
	2	6	3640 ± 859	217.4 ± 134.4	223.1 ± 135.9	59.06 ± 36.03	0.0140 ± 0.0046	0.082 ± 0.037	5.99 ± 2.00	3.95 ± 1.13

(Mean ± SD)

Table 4 Summary of total and unconjugated calicheamicin pharmacokinetic parameters by dose group for dose periods 1 and 2

GO (mg/m^2)	Dose period	N	C_{max} (ng/ml)		$AUC_{0\sim t}$ (mg h/l)		AUC_{inf} (mg h/l)		$t_{1/2}$ (h)		λ_2 (1/h)	
			Total	Unconjugated	Total	Unconjugated	Total	Unconjugated	Total	Unconjugated	Total	Unconjugated
6.0	1	6	45 ± 8	4 ± 3	0.95 ± 0.24	0.17 ± 0.28	1.07 ± 0.27	0.28 ± 0.51	15.31 ± 4.34	54.76 ± 69.94	0.0481 ± 0.0123	0.0306 ± 0.0212
	2	5	40 ± 5	5 ± 4	1.53 ± 0.69	0.49 ± 0.57	1.79 ± 0.77	1.52 ± 1.65	60.31 ± 46.85	1880.56 ± 1836.56	0.0224 ± 0.0191	0.0021 ± 0.0031
7.5	1	3	56 ± 20	5 ± 0	2.23 ± 1.99	0.14 ± 0.07	2.35 ± 2.05	0.15 ± 0.08	21.70 ± 14.49	23.49 ± 19.92	0.0408 ± 0.0199	0.0435 ± 0.0248
	2	2	59	7	3.60	0.25	4.05	0.27	42.46	28.15	0.0208	0.0310
9.0	1	11	83 ± 25	6 ± 4	2.50 ± 1.91	0.21 ± 0.16	2.72 ± 1.98	0.35 ± 0.32	24.39 ± 15.88	169.86 ± 270.02	0.0463 ± 0.0445	0.0272 ± 0.0321
	2	6	107 ± 18	9 ± 5	5.83 ± 1.45	0.43 ± 0.19	6.16 ± 1.57	0.79 ± 0.78	48.67 ± 13.40	609.51 ± 1221.08	0.0151 ± 0.0035	0.0088 ± 0.0061

(Mean ± SD)

Table 5 Number of CR, CRp and blast clearance: phase I and II

	Phase I (mg/m^2)			Phase II	Total
	6	7.5	9		
CR	0	0	2	5	7
CRp	0	0	0	1	1
Blast clearance	1	2	2	3	8
No remission	5	1	7	11	13
Total	6	3	11	20	40

same as that described in the product label, where approved [8]. In the phase I part, the dose level of 9 mg/m^2 was tolerable and two CRs were achieved.

However, one patient who received 9 mg/m^2 GO during the initial dose escalation developed pulmonary bleeding after infusion. This could be a manifestation of the infusion-related syndrome, which already has been reported in a post-marketing study performed in the US. It has been reported that eight patients experienced pulmonary events immediately after or within 24 h of the GO administration; six of the eight had high leucocyte counts of more than 60,000/μl. This manifestation appears to be similar to the presentation of pulmonary leukostasis or to the acute pulmonary events that have been reported after administration of other monoclonal antibodies [8].

To examine this hypothesis, other patients were monitored for coagulopathy after the fatal hemorrhage occurred and we found a subset of patients who developed coagulopathy upon infusion of GO. In the phase II part, in which collection of coagulation data was required, thrombocytopenia (six patients), hyperfibrinogenemia (two patients), and increased levels of fibrinogen degradation product (five patients) were observed. The pulmonary toxicity we observed could be the combined effects of these two toxicities, infusion-related toxicity and coagulopathy after infusion of GO. Patients with high leucocyte counts should be given GO with caution.

Including this pulmonary toxicity, all the toxicity profiles observed in our study were comparable to the earlier papers [6, 8, 9]. Among them hepatic toxicities were reported. In the earlier phase II studies [6], 23% of patients treated with the 9 mg/m^2 doses experienced grade 3–4 bilirubin elevation; however, this elevation was transient. Although cases with prior HSCT were not excluded from our study, all the patients lacked history of HSCT, and had chance to observe the incidence of hepatic toxicities attributed to the agent alone. And in patients with no history of HSCT, we observed such hepatic toxicities. Grade 3–4 elevations of GOT and GPT were observed in two cases in the phase I part, which was judged to be DLT, and four cases in phase II part. Despite these elevations of hepatic enzymes, they were transient and no cases developed jaundice. It has been reported that the incidence of sinusoidal obstructive syndrome/veno-occlusive disease is not specific to GO, and the rate of this toxicity was the same as that of conventional therapy [10].

The patterns of pharmacokinetic data of Japanese patients were almost the same as those of earlier studies [8]. Regarding the half-lives ($t_{1/2}$) of hP67.6, the data as well as the tendency to prolongation of clearance during the subsequent dose periods were the same as the earlier study. In our study of 9 mg/m^2, half-lives were 51 ± 25 and 59 ± 36 h from the first dose to the second dose. In earlier studies, half-lives were 67 ± 37 and 88 ± 58 h, respectively [8]. In our study, AUC_{inf} for the first and second doses was 133 ± 94 and 223 ± 136 mg h/l, respectively, while it was 132 ± 136 and 243 ± 198 mg h/l, respectively, in earlier studies [8].

The parameters of half-lives and AUC_{inf} of total calicheamicin also showed the same pattern (Table 4). In our study, for 9 mg/m^2, half-lives were 24 ± 16 and 48 ± 13 h for the first and second infusions, respectively. In earlier studies, they were 39 ± 25 and 63 ± 63 h for the first and second infusions, respectively [8]. Again, in our study, for 9 mg/m^2, AUCs of total calicheamicin were 2.7 ± 2.0 and 6.2 ± 1.6 mg h/l, for the first and the second infusions, respectively; in the earlier studies, they were 2.1 ± 1.8 and 4.7 ± 4.1 mg h/l for the first and the second infusions, respectively. The AUC_{inf} of unconjugated calicheamicin was also higher after the second infusion than after the first infusion (Table 4), and it was again comparable with the earlier studies.

This observation suggests that there are no race-specific differences in the pharmacokinetics of GO. Similarly, it has been reported that no age or gender differences exist in the pharmacokinetics of this agent [11]. The similarity of the pharmacokinetic data for Japanese and non-Japanese patients might translate into similar efficacy of GO. In the phase I part, two patients had CRs at the dose level of 9 mg/m^2, whereas none had CR or CRp at the dose levels of 6 and 7.5 mg/m^2. In the phase II part, at the dose level of 9 mg/m^2, the overall remission rate was 30.0% (95% CI, 14.0–50.8%). In the earlier phase II studies, the overall remission rate was 30% (95% CI, 22–38%). Thus, there was reproducible efficacy of GO in Japanese and non-Japanese patients and this result contributed to the approval of this agent in Japan.

This study included cases with multiple relapses, and even in those patients we were able to observe responses. Our study showed that relapse cases are likely to achieve responses as compared with the refractory cases; patients with earlier longer remission prior to this study responded better than with the shorter one; the number is small, and parameters contributing to the response should be obtained

Table 6 Summary of remission rates by demographic and baseline characteristics: Phase II

Characteristic	CR	CRp	Neither CR nor CRp	Indeterminable	Total	OR[a] (%)
Sex						
Male	3	1	10	0	14	4/14 (28.6)
Female	2	0	4	0	6	2/6 (33.3)
Age						
<60	5	1	9	0	15	6/15 (40.0)
≥60	0	0	5	0	5	0/5 (0.0)
Number of relapse times						
0	3	0	9	0	12	25.0 (3/12)
1	0	1	3	0	4	25.0 (1/4)
2	1	0	1	0	2	50.0 (1/2)
3	1	0	0	0	1	100.0 (1/1)
4	0	0	1	0	1	0.0 (0/1)
FAB subtypes (at initial presentation)						
M1	0	0	2	0	2	0/2 (0.0)
M2	0	0	6	0	6	0/6 (0/0)
M3	2	0	0	0	2	2/2 (100.0)
M4	0	1	1	0	2	1/2 (50.0)
M4E0	2	0	1	0	3	2/3 (66.7)
M5	1	0	3	0	4	1/4 (25.0)
M6	0	0	1	0	1	0/1 (0.0)
FAB subtypes (at prestudy screening)						
M1	0	0	3	0	3	0/3 (0.0)
M2	0	1	5	0	6	1/6 (16.7)
M3	2	0	0	0	2	2/2 (100.0)
M4	1	0	2	0	3	1/3 (33.3)
M4E0	1	0	1	0	2	1/2 (50.0)
M5	1	0	2	0	3	1/3 (33.3)
M6	0	0	1	0	1	0/1 (0.0)
Prognostic category (at initial presentation)						
Favorable	3	0	3	0	3	0/3 (0.0)
Intermediate	2	0	10	0	12	2/12 (16.7)
Poor	0	0	3	0	3	3/3 (100.0)
Not available	0	1	1	0	2	1/2 (50.0)
Prognostic category (at prestudy screening)						
Favorable	20	0	1	0	3	0/3 (0.0)
Intermediate	2	1	4	0	7	3/7 (42.9)
Poor	0	0	3	0	3	2/3 (66.7)
Not available	1	0	6	0	7	1/7 (14.3)
Duration of first remission						
<1 year	1	0	5	0	6	1/6 (16.7)
≥1 year	4	1	9	0	14	5/14 (35.7)
Disease status at pretreatment						
Relapse	5	1	12	0	18	6/18 (33.3)
Refractory	0	0	2	0	2	0/2 (0.0)
HiDAC						
Without	2	0	9	0	11	2/11 (18.2)
With	3	1	5	0	9	4/9 (44.4)

HiDAC high dose ara-C

[a] Overall remission (%) = (CR plus CRp)/$N \times 100$

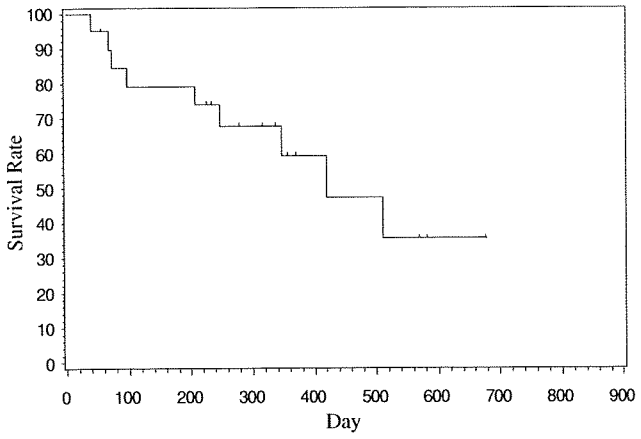

Fig. 1 Overall survival in the phase II part

in the future. Patients who achieved CR also had included those cases poor karyotypes. It has been reported that there is no correlation between karyotype and achievement of CR [12].

Three long-term survivors had the M3 subtype. It should be noted that all of 3 M3 patients achieved CR. This marker is unique as blasts containing M3 on their surface are abundant with CD33. Due to the availability of other highly effective agents (ATRA, arsenic trioxide), relatively few M3 patients have been treated thus far with GO, and their follow-up is still short [13]. As the earlier study did not include M3 cases, this is the first report of GO benefit to M3 patients.

In conclusion, our study confirmed the efficacy and safety of GO when administered as monotherapy. Further studies of GO used in combination with other agents are warranted.

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Short communication

Transient abnormal myelopoiesis in a Down syndrome newborn followed by acute myeloid leukemia: identification of the same chromosomal abnormality in both stages

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Abstract A transient abnormal myelopoiesis was observed in a newborn with Down syndrome. Cytogenetic study revealed multiple oligoclonal abnormalities: 47,XY,inv(6)(p23q21),+21c[3]/47,XY,der(7)t(1;7)(q25;p15),+21c[1]/47,XY,del(13)(q?),+21c[1]/47,XY,+21c[15]. Ten months after the patient achieved remission, the transient abnormal myelopoiesis evolved to an acute megakaryoblastic leukemia. Cytogenetic study revealed only a single clonal abnormality, 47,XY,der(7)t(1;7)(q25;p15),+21c, identical to one of the structural changes seen at birth. Sequence analysis of the *GATA1* gene revealed a deletion–insertion mutation within the exon 2 introducing a stop codon after Arg 64. It may be that the der(7)t(1;7)(q25;p15) abnormality played some selective role in the development of acute megakaryoblastic leukemia in this patient. To our knowledge, the present case is unique in demonstrating a subclone with der(7)t(1;7)(q25;p15) evolving to acute leukemia.

1. Introduction

Approximately 10% of patients with Down syndrome are born with transient abnormal myelopoiesis (TAM) [1,2]. Of these cases, ~20% recur as acute megakaryoblastic leukemia (AMKL) [3]. Acquired mutations in *GATA1* in the leukemic blasts are detected in virtually all of these cases [4–7], but the second hit for the full expression of AMKL is still a matter of discussion [6,7]. GATA1 is a transcription factor that regulates megakaryocytic differentiation, and the mutations observed are considered to cause accumulation of poorly differentiated megakaryocytic precursors [4]. Strikingly, *GATA1* is located on chromosome X; therefore, genetic interaction of *GATA1* with one or more genes on other chromosomes presumably contributes to the development of AMKL in Down syndrome.

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in Down syndrome often demonstrate chromosomal abnormalities in addition to the constitutional trisomy 21 [8–12]. These include both numerical and structural abnormalities, mostly complete or partial trisomy of a specific chromosome; reciprocal translocations are relatively rare. The role of these chromosomal translocations in developing leukemia in Down syndrome is also unknown. Here, we report the case of a Down syndrome patient who developed AMKL showing der(7)t(1;7)(q25;p15) following TAM at birth.

2. Case report

This Down syndrome patient was a boy born at 39 weeks gestational age, weighing 3,235 g. Thrombocytopenia was revealed after birth. At 5 days after birth, he was diagnosed as having TAM. Initial white blood cell count was 13,200/μL with 7% blasts, hemoglobin was 15.0 g/dL, and platelet count was 52,000/μL. Chromosomal analysis of bone marrow cells at diagnosis revealed the

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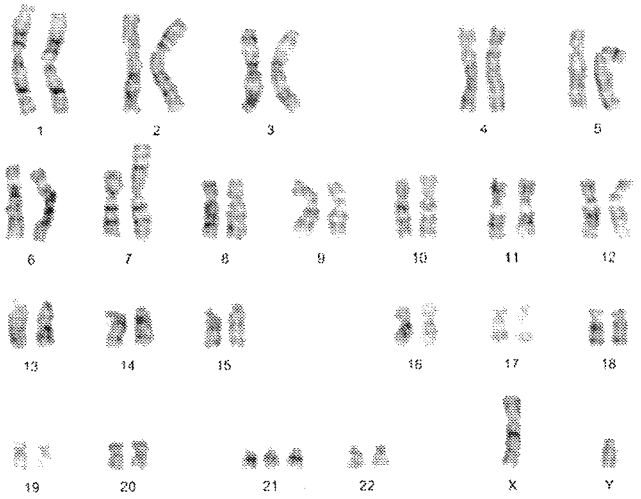

Fig. 1. Karyogram of the blast in leukemic phase showing 47,XY,der(7)t(1;7)(q25;p15),+21c.

karyotype 47,XY,inv(6)(p23q21),+21c[3]/47,XY,der(7)t(1;7)(q25;p15),+21c[1]/47,XY,del(13)(q?),+21c[1]/47,XY,+21c[15]/. Although he was not treated, his white blood cell count gradually decreased within 2 months, and at 7 months after birth his platelet count spontaneously recovered to within the normal range (although he suffered from thrombocytopenia due to an unknown viral infection). At 10 months after birth, the blasts increased suddenly.

Persistent thrombocytopenia was noted after platelet transfusions twice weekly. A bone marrow aspirate showed excessive myelofibrosis. Immunophenotyping of peripheral blasts showed $CD7^+$, $CD13^+$, $CD33^+$, $CD38^+$, $CD41^+$, $CD42b^+$, $CD56^+$, $CD157^+$, and $HLA\text{-}DR^+$. Chromosome analysis showed a der(7)t(1;7)(q25;p15) abnormality, in addition to the constitutional trisomy 21 (Fig. 1). Spectral karyotyping further confirmed the presence of the t(1;7) translocation, expressed as 47,XY,der(7)t(1;7)(q25;p15),+21c (Fig. 2), resulting in partial trisomy of 1q.

We analyzed the *GATA1* mutation in the peripheral blood sample, after written informed consent was obtained from his parents. Genomic DNA was extracted, and then polymerase chain reaction (PCR) was performed. Subcloning and nucleotide sequencing of PCR products were performed as described previously [6]. Sequence analysis of *GATA1* gene revealed a deletion–insertion mutation within exon 2, introducing premature stop codon after Arg 64 (Fig. 3).

A trephine biopsy revealed the presence of a typical megakaryocyte proliferation and prominent fibrosis. The final diagnosis of AMKL led to the initiation of combination therapy of pirarubicin HCl (25 mg/m^2 per day for 2 days), cytosine arabinoside (100 mg/m^2 per day for 7 days), and etoposide (150 mg/m^2 per day for 3 days) [13]. Complete remission was achieved after two courses of the therapy. Continuation of the consolidation therapy was uneventful, and six cycles of the same regimen were completed. As of writing, the patient had been in continuous complete remission without marked side effects for 5 years after initiation of the therapy.

3. Discussion

Recent collaborative studies on AML in Down syndrome children have established that the most frequent cytogenetic abnormality in AML or MDS in Down syndrome is trisomy 8 or partial trisomy of 1q [2,14–17]. Because AML and MDS with Down syndrome have distinct biologic and clinical features, the identification of Down syndrome patients with a mild or normal phenotype in the AML/MDS population is of fundamental importance for clinical diagnosis and management. Partial trisomy of 1q

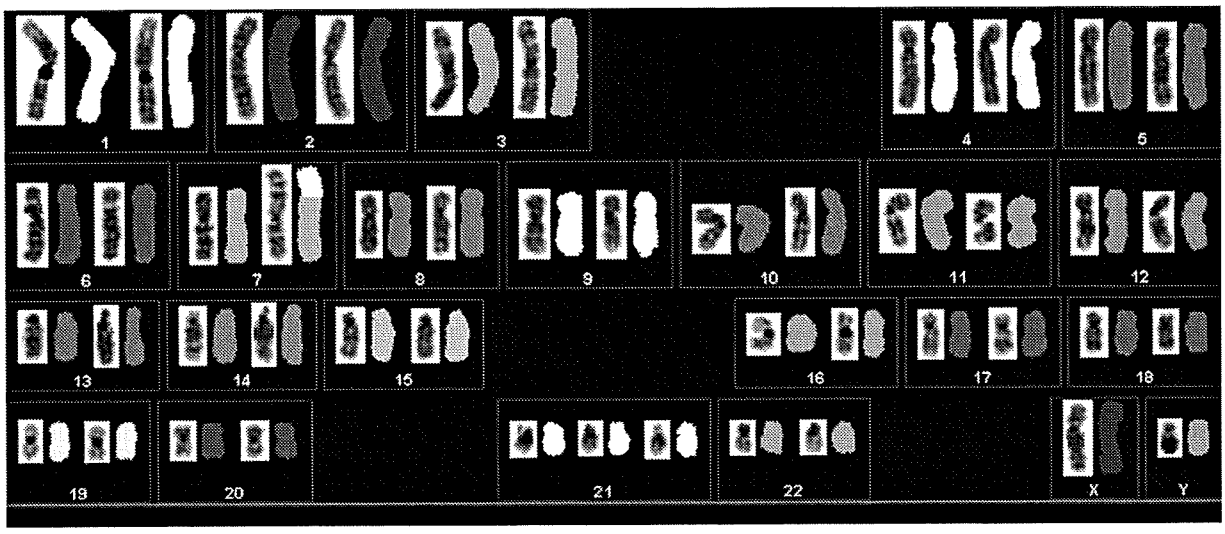

Fig. 2. Spectral karyotyping showing 47,XY,der(7)t(1;7)(q25;p15),+21c.

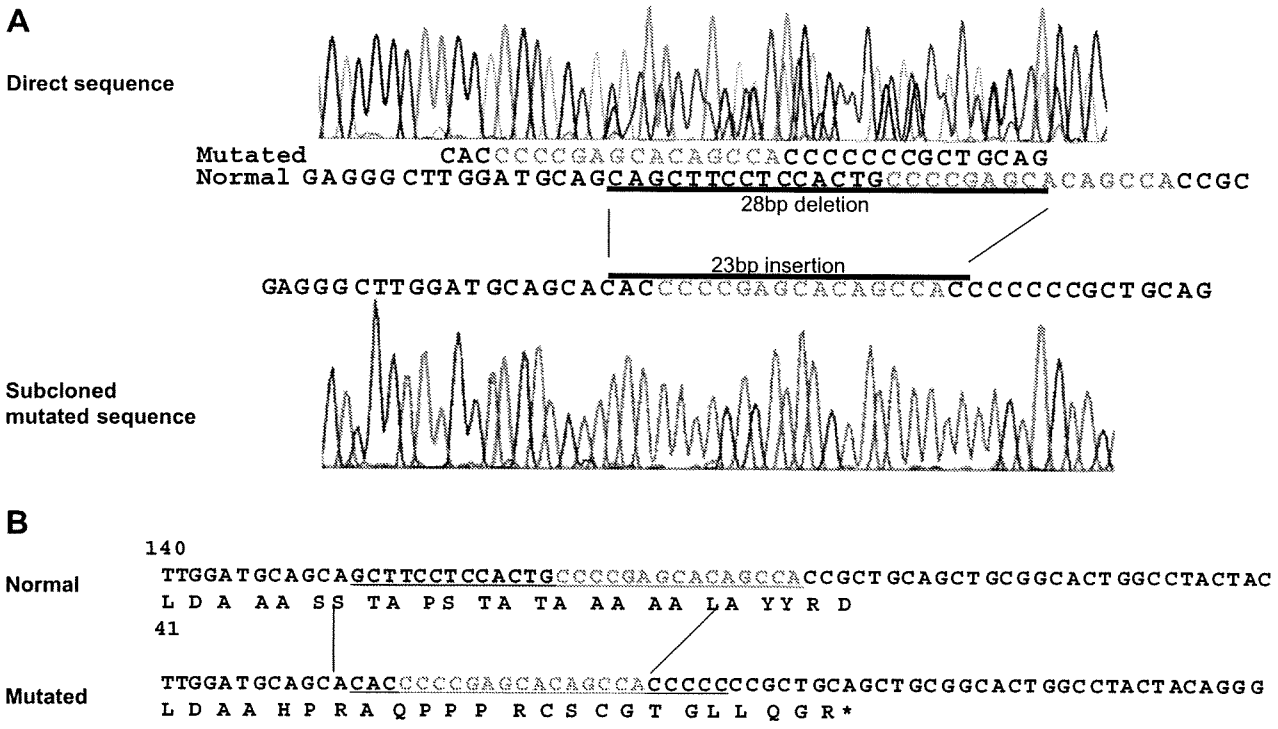

Fig. 3. Mutational analysis of the *GATA1* gene. (A) Sequence analysis was performed directly and using subcloned polymerase chain reaction product and showed a deletion–insertion mutation (28-bp deletion and 23-bp insertion) between 152 and 179 within exon 2. (B) This deletion–insertion mutation introduced a premature stop codon after Arg64. Numbers represent nucleotides from the 5′ end of exon 2.

has been reported by several authors and appears to represent a nonrandom chromosomal abnormality in patients with MDS/AML and Down syndrome [14,17]. Partial trisomy of 7q [8] or monosomy 1 [18,19] have also been reported. Unbalanced translocation t(1;7) in childhood myelodysplasia has been reported [20]. It is also possible that the t(1;7) played some role in the development of the MDS [21]. The mechanism of formation of the der(7)t(1;7) and its role in leukemogenesis are still unclear. Given that der(7)t(1;7) results in partial trisomy of 1q and partial monosomy of 7q, the increased dosage of the oncogenes located at 1q or the loss of the tumor suppressor genes located at 7q (or both factors) may be implicated in leukemogenesis of MDS and AML with der(7)t(1;7).

Cases of TAM usually have no karyotypic abnormality [1], but AMKL is associated with chromosomal abnormalities, including 8 trisomy and 19 trisomy [2]. Rare TAM cases have had chromosome abnormalities that were also observed in developing AMKL [22]. As for the *GATA1* gene, the deletion–insertion mutations within exon 2 in our patient have been reported previously in only two cases of TAM [5,7]. Reciprocal translocations are rare in TAM with Down syndrome. In the present case, a der(7)t(1;7) with partial trisomy of 1q, which is among the most frequently observed abnormalities in Down syndrome, might contribute to evolution to acute leukemia. The present report contributes insight into the mechanism of leukemic transformation from TAM in Down syndrome.

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bjh research paper

FBXW7 and *NOTCH1* mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma

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Summary

Mutation analysis of *FBXW7* and *NOTCH1* genes was performed in 55 T cell acute lymphoblastic leukaemia (T-ALL) and 14 T cell non-Hodgkin lymphoma (T-NHL) patients who were treated on the Japan Association of Childhood Leukaemia Study (JACLS) protocols ALL-97 and NHL-98. *FBXW7* and/or *NOTCH1* mutations were found in 22 (40·0%) of 55 T-ALL and 7 (50·0%) of 14 T-NHL patients. *FBXW7* mutations were found in 8 (14·6%) of 55 T-ALL and 3 (21·4%) of 14 T-NHL patients, and *NOTCH1* mutations in 17 (30·9%) of 55 T-ALL and 6 (42·9%) of 14 T-NHL patients. Three (5·4%) T-ALL and two (1·4%) T-NHL patients had mutations in both *FBXW7* and *NOTCH1*. *FBXW7* mutations included one insertion, one deletion, one deletion/insertion and nine missense mutations. *NOTCH1* mutations were detected in the heterodimerization domain (HD) in 15 cases, in the PEST domain in seven cases, and in both the HD and PEST domains in one case. Five-year event-free survival and overall survival for patients with *FBXW7* and/or *NOTCH1* mutations were 95·5% (95% CI, 71·9–99·4%) and 100% respectively, suggesting that T-ALL patients with *FBXW7* and/or *NOTCH1* mutation represent a good prognosis compared to those without *FBXW7* and/or *NOTCH1* mutations (63·6%, $P = 0·007$ and 78·8%, $P = 0·023$, respectively).

Keywords: ALL, childhood, prognostic factors, genetic analysis, T cells, molecular diagnosis.

The outcomes of paediatric T cell acute lymphoblastic leukaemia (T-ALL) have improved in recent years as a result of intensified therapies, with 5-year relapse-free survival rates in the range of about 60–85% (Gaynon *et al*, 2000; Maloney *et al*, 2000; Moghrabi *et al*, 2007; Pui *et al*, 2004), which are relatively low compared to those of B-precursor ALL. A stringent assessment of the risk of relapse is critical in determining which patients need to receive more effective therapy. In T-ALL, it has been reported that the abnormal expression of *TLX1* (*HOX11*) is associated with a favourable prognosis, although the prognostic significance of this finding has yet to be determined (Ferrando *et al*, 2004; Ferrando *et al*, 2002). On the other hand, a few reports have suggested that microarray analysis could distinguish high-risk cases in T-ALL (Ferrando & Look 2003; Winter *et al*, 2007).

Recently, activating mutations of *NOTCH1* gene have been reported in more than half of T-ALL cases (Weng *et al*, 2004). *NOTCH1*, previously termed *TAN1*, was discovered as a

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partner gene in T-ALL with a *t*(7;9)(q34;q34·3), and was found in <1% of T-ALLs (Ellisen *et al*, 1991). A good clinical outcome for T-ALL patients with *NOTCH1* mutations was reported in the paediatric ALL-BFM 2000 study (Breit *et al*, 2006). On the contrary, other papers reported that *NOTCH1* mutations were not associated with good clinical outcome in T-ALL (van Grotel *et al*, 2008; Zhu *et al*, 2006). Thus, clinical significance of *NOTCH1* mutation in T-ALL still remains controversial.

F-box and WD40 domain protein 7 (FBXW7; previously termed FBW7, CDC4, or Archipelago), is also considered a candidate prognostic factor in T-ALL. FBXW7 was originally isolated as a Lin12/NOTCH-negative regulator in Caenorhabditis elegans (Hubbard *et al*, 1997), and plays a critical role in intracellular NOTCH1 degradation which depends on an intact NOTCH1 PEST domain (Fryer *et al*, 2004; Tetzlaff *et al*, 2004). Recently, it was reported that the *FBXW7* gene was mutated in various tumours including breast, ovarian, and endometrial cancers and T-ALL cell lines (Moberg *et al*, 2001).

In this study, we analyzed the frequencies and clinical significance of *FBXW7* and *NOTCH1* mutations in paediatric T-ALL and T cell non-Hodgkin lymphoma (T-NHL). *FBXW7* as well as *NOTCH1* was found to be frequently mutated in paediatric T-ALL and T-NHL. We firstly described that mutations of either *FBXW7* or *NOTCH1* genes, rather than *FBXW7* or *NOTCH1* alone, were associated with good clinical outcome in T-ALL.

Methods

Patients and treatments

All children with T-ALL or T-NHL, aged under 15 years were enrolled into the Japan Association of Childhood Leukaemia Study (JACLS) protocol ALL-97 between 1997–2001 and JACLS trial NHL-T98 between 1998–2002 (Oda *et al*, 2006) (Fig S1). All T-NHL patients were pathologically diagnosed as having lymphoblastic lymphoma. Patients who failed to obtain complete remission (CR) with risk adapted induction chemotherapy were scheduled to undergo F-protocol at 6 weeks following the start of their initial induction chemotherapy. Samples from 55 newly diagnosed T-ALL and 14 T-NHL patients were examined in this study. At the time of diagnosis, bone marrow (BM) and/or peripheral blood (PB) cells were obtained from T-ALL patients and lymph nodes and/or pleural effusions were obtained from T-NHL patients. T-lineage immunophenotypic subtype was defined as simultaneous expression of two or more T-lineage associated molecules including CD2, CD3, CD5, CD7, and CD8, on at least 20% of lymphoblasts. T-ALL was characterized by definition as the presence of more than 25% bone marrow involvement of lymphoblasts. Cytogenetic studies were performed on 60 patients by using regular G-banding method. Advanced stage (stages 3 and 4) T-NHL patients were enrolled in this protocol, and the histopathology of specimens was reviewed by central pathology reviewers. A total of 69 patients were included in the present study; 49 were male and 20 female; 55 were children diagnosed with T-ALL (median age of 9·5 years; range: 2·0–15·0 years) and 14 with T-NHL (median age of 11·0 years; range: 3·7–15·0 years). The basic clinical and immunological characteristics of this patient subgroup did not differ from those of the entire group. The two-year treatment regimen consisted of induction therapy (vincristine sulfate, high dose-methotrexate, cytarabine, prednisone, L-asparaginase), five drug consolidation therapy A and B including high doses of L-asparaginase, and maintenance therapy with block-rotated treatment using the drugs listed above. Informed consent was obtained from the patients or their parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Centre approved this project.

DNA and RNA preparation

DNA and RNA were prepared from samples of BM, PB, lymph nodes, and pleural effusions containing tumour cells of patients with primary T-ALL and T-NHL, by using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA).

Detection of FBXW7 and NOTCH1 mutations

Mutation detection was performed by polymerase chain reaction (PCR)-based denaturing high-performance liquid chromatography (dHPLC) using a WAVE DNA fragment analysis system (Transgenomic, Omaha, NE, USA) equipped with a DNASep HT cartridge (Weng *et al*, 2004). The PCR products of positive cases detected by PCR-based dHPLC were purified using the QIAquick PCR Purification Kit (Qiagen). Sequencing by means of fluorescent-dye chemistry was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Shimada *et al*, 2006; Taketani *et al*, 2004). For further confirmation of insertion and deletion mutations, the purified PCR products were subcloned using a TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and then sequenced (Taketani *et al*, 2004). *FBXW7* mutations were screened from exons 2 to 12 using primers described previously (Cassia *et al*, 2003). *NOTCH1* mutations in the N-terminal region and the C-terminal region of the HD domain (exons 26 and 27), the transcriptional activation domain (TAD) (exon 34), and the PEST domain (exon 34) were screened by using primers described previously (Weng *et al*, 2004).

Statistical analyses

Proportional differences between groups were analyzed by chi-squared or Fisher's exact tests. The Kaplan–Meier method was used to estimate survival rates. Differences in prognosis between groups were evaluated using the log-rank test. Event-free survival (EFS) was measured from the time of diagnosis

to the time of analysis or first event. Failure to achieve remission, relapse or death that occurred during continuous complete remission were evaluated as events. Overall survival (OS) was defined as the time from diagnosis to death. Multivariate survival analysis was performed using the Cox proportional-hazards model. A P value of less than 0·05 (two-sided) was considered statistically significant. All statistical analyses were performed using STATA 8·1 (STATACORP LP, College Station, TX, USA).

Results

FBXW7 and NOTCH1 mutations in T-ALL and T-NHL patients

FBXW7 and/or *NOTCH1* mutations were found in 22 (40·0%) of 55 T-ALL and 7 (50·0%) of 14 T-NHL patients (Tables I–III). *FBXW7* mutations were found in 8 (14·6%) of 55 T-ALL and 3 (21·4%) of 14 T-NHL patients, and *NOTCH1*

Table I. *FBW7* and *NOTCH1* mutations in T-ALL and T-NHL patients.

	FBXW7 mutation		*NOTCH1* mutation	
Patient no.	Nucleotide*	Amino acid	Nucleotide†	Amino acid
T-ALL 4	–	–	4778T > C	L1593P
T-ALL 5	1662C > T	R505C	4817_4818insGCCCCC	1606delinsLPP
T-ALL 8	1450_1451ins AGCTGTT GTCTCTCATCATATG CCTTCTCAC	435AVVSHHMPSHHfsX	–	–
T-ALL 20	1542C > T	R465C	4847T > A	I1616N
T-ALL 22	–	–	4775_4776insGAC	1592delinsLT
T-ALL 23	–	–	7355_7356insCTGGC	2453WRCTLFCPRKAPPCP RRCHPRWSHPfsX
T-ALL 26	–	–	4818_4819insCTTTATCTC	1606_1607insHYL
T-ALL 30	–	–	4732_4734del	1578delV
T-ALL 31	–	–	4732_4734del	1578delV
T-ALL 32	2029T > C	V627A	–	–
T-ALL 33	–	–	4754T > C	L1585P
T-ALL 34	1543G > A 715_718delinsGAC	R465H 189RPQNIQVPLGLYHV QQHQQLLGTSEQPM AKGNNDAELHLSSHL QASRNGfsX	–	–
T-ALL 35	–	–	7412delinsAG	S2471X
T-ALL 37	–	–	4732-4734del	1578delV
T-ALL 38	1585G > A	R479Q	–	–
T-ALL 41	–	–	4754T > C	L1585P
T-ALL 46	–	–	7318C > T	Q2440X
T-ALL 49	1585G > A	R479Q	4732-4734del	1578delV
T-ALL 50	–	–	7330C > T	Q2444X
T-ALL 65	–	–	4814_4815delinsCCCCCCCCGA CCATAAGCC	1606PPDHKPSVTHTASRfsX
T-ALL 67	1543G > A	R465H	–	–
T-ALL 75	–	–	4818_4822delinsAGCACACCA GCCCAAGC	1606delinsLAHQP
T-NHL 18	–	–	4709_4718del	1570_1573delinsVDK
T-NHL 25	–	–	7541_7542del	2515RVPfsX
T-NHL 54	–	–	4793G>C	R1598P
			7541_7542del	2515RVPfsX
T-NHL 55	1543G > A	R465H	–	–
T-NHL 58	1543G > A	R465H	4845_4847delinsCCCCTCGAA	1615_1617delinsIPSNF
T-NHL 59	–	–	7326_7327insCGCGGAGGTGC	2443RGGACSHWAPAAWRC TLFCPRRAPPCP RRCHPR WSHPfsX
T-NHL 61	2107del	653RVNLFETfsX	7403_7404insGGGGG	2469GGHPRWSHPfsX

*Nucleotide number is according to the GenBank accession number NM_033632.
†Nucleotide number is according to the GenBank accession number NM_017617.

Table II. Association of *NOTCH1* and *FBXW7* mutations with clinical characteristics in 55 T-ALL patients.

	NOTCH1			*FBXW7*			*FBXW7* and/or *NOTCH1*		
	Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)	
Patient characteristics	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*
Overall	17	38		8	47		22	33	
Gender									
Male	12 (70·6)	25 (65·8)	0·726	7 (87·5)	30 (63·8)	0·250	16 (72·7)	21 (63·6)	0·481
Female	5 (29·4)	13 (34·2)		1 (12·5)	17 (36·2)		6 (27·3)	12 (36·4)	
Age at diagnosis (years)				3 (37·5)	27 (57·4)	0·295	12 (54·5)	18 (54·5)	1·0
<10	10 (58·8)	20 (52·6)	0·670	5 (62·5)	20 (42·6)		10 (45·5)	15 (45·5)	
≥10	7 (41·2)	18 (47·4)							
Presenting at diagnosis WBC ($x10^9$/l)									
<100	12 (70·6)	18 (47·4)	0·110	17 (89·5)	26 (72·2)	0·238	16 (72·7)	14 (42·4)	0·027
≥100	5 (29·4)	20 (52·6)		5 (9·1)	13 (36·1)		6 (27·3)	19 (57·6)	
Mediastinal involvement									
Yes	12 (70·6)	22 (57·9)	0·371	4 (50·0)	30 (63·8)	0·464	14 (63·6)	20 (60·6)	0·821
No	5 (29·4)	16 (42·1)		4 (50·0)	17 (36·2)		8 (36·4)	13 (39·4)	
T cell immunophenotype									
Pro and Pre	3 (17·6)	5 (13·2)	0·665	0 (0)	8 (17·0)	0·287	3 (13·6)	5 (15·2)	0·164
Cortical	8 (47·1)	14 (36·8)		5 (62·5)	17 (36·2)		12 (54·5)	10 (30·3)	
Mature	6 (35·3)	19 (50·0)		3 (37·5)	22 (46·8)		7 (31·8)	18 (54·5)	
Chromosomal abnormalities*									
No	11 (68·8)	21 (55·3)	0·749	8 (100·0)	24 (52·2)	0·031	16 (76·2)	16 (48·5)	0·172
Yes									
Abnormalities involving TCR locus† (+)	1 (6·3)	5 (13·2)		0 (0·0)	6 (13·0)		4 (19·0)	12 (36·4)	
Abnormalities involving TCR locus (−)	4 (25·0)	12 (31·6)		0 (0·0)	16 (34·8)		1 (4·8)	5 (15·2)	
Relapse									
Yes	0 (0)	10 (26·3)	0·022	1 (12·5)	9 (19·1)	1·0	1 (4·5)	9 (27·3)	0·039
No	17 (100)	28 (73·7)		7 (87·5)	38 (80·9)		21 (95·5)	24 (72·7)	

Pro and Pre ($CD7^+$ and $CD1^-$), Corical ($CD1^+$), Mature ($CD1^+$, $sCD3^+$).

P, χ^2 or Fisher's exact test; TCR, T cell receptor.

*Total $n = 54$.

†Chromosomal abnormalities including 14q11, 7p15, and 7q35.

mutations in 17 (30·9%) of 55 T-ALL and 6 (42·3%) of 14 T-NHL patients. Three (5·4%) T-ALL and two (1·4%) T-NHL patients presented mutations in both *FBXW7* and *NOTCH1* (Table I).

The 12 *FBXW7* mutations detected included nine missense mutations, one 31 bp insertion, one single nucleotide deletion, and one deletion/insertion mutation (Table I). Seven of nine missense mutations were clustered in a 'hot spot' encoding arginines 465 and 479 residues which are highly conserved in the WD40 (tryptophan–aspartic-acid) repeat of FBXW7 (Fig S2A). Of the 12 identified *FBXW7* mutations, one insertion (T-ALL 8), one deletion/insertion (T-ALL 34), and one single nucleotide deletion (T-NHL 61) have not been previously described in T-ALL or other cancers (Fig S2B–D). *FBXW7* missense mutation encoding V627A (T-ALL 32) was also a novel mutation. V627 of FBXW7 is evolutionarily conserved, and V627A was not detected in normal lymphocytes from 20 healthy volunteers. One patient (T-ALL 34) had a *FBXW7* deletion/insertion mutation and a missense mutation that encoded FBXW7 residue R465H (Table I, Fig S2C).

Of the 24 *NOTCH1* mutations detected in 23 cases, 16 (66·7%) were located in sequences encoding the HD domain, 8 (33·3%) in the PEST domain (Table I). In one case (T-NHL 54), mutations were detected in both the HD and PEST domains. Of these 24 mutations, 17 (70·9%) were short in-frame insertion or deletions, 5 (20·8%) were missense mutations, and 2 (8·3%) were nonsense mutations in sequences encoding the HD or PEST domains, respectively. Furthermore, a single nucleotide polymorphism C5097T was observed in the sequence encoding the C-terminal region of the HD domain in 63 (91·3%) of 69 patients, as previously reported for Japanese adult patients with mature T cell malignancies (Shimizu *et al*, 2007).

Table III. Association of *NOTCH1* and *FBXW7* mutations with clinical characteristics in 14 T-NHL patients.

	NOTCH1			*FBXW7*			*FBXW7* and/or *NOTCH1*		
	Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)	
Patient characteristics	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*
Overall	6	8		3	11		7	7	
Gender									
Male	5 (83·3)	7 (87·5)	1·0	2 (66·7)	10 (90·9)	0·396	6 (85·7)	6 (85·7)	1·0
Female	1 (16·7)	1 (12·5)		1 (33·3)	1 (9·1)		1 (14·3)	1 (14·3)	
Age at diagnosis (years)									
<10	4 (66·7)	2 (25·0)	0·277	2 (66·7)	4 (36·4)	0·538	4 (57·1)	2 (28·6)	0·592
≥10	2 (33·3)	6 (75·0)		1 (33·3)	7 (63·6)		3 (42·9)	5 (71·4)	
Mediastinal involvement									
Yes	0 (0·0)	1 (12·5)	1·0	0 (0·0)	1 (9·1)	1·0	0 (0·0)	1 (14·3)	1·0
No	6 (100·0)	7 (87·5)		3 (100·0)	10 (90·9)		7 (100·0)	6 (85·7)	
T cell immunophenotype									
Pro and Pre	0 (0·0)	0 (0·0)	1·0	0 (0·0)	0 (0·0)	1·0	0 (0·0)	0 (0·0)	1·0
Cortical	2 (33·3)	2 (28·6)		1 (33·3)	3 (30·0)		2 (28·6)	2 (33·3)	
Mature	4 (66·7)	5 (71·4)		2 (66·7)	7 (70·0)		5 (71·4)	4 (66·7)	
Chromosomal abnormalities*									
No	4 (66·7)	3 (42·9)	0·755	3 (100·0)	4 (40·0)	0·217	5 (71·4)	2 (33·3)	0·470
Yes									
Abnormalities involving TCR locus† (+)	1 (16·7)	1 (14·3)		0 (0·0)	2 (20·0)		1 (14·3)	1 (16·7)	
Abnormalities involving TCR locus (−)	1 (16·7)	3 (42·9)		0 (0·0)	4 (40·0)		1 (14·3)	3 (50·0)	
Relapse									
Yes	0 (0·0)	2 (25·0)	0·473	0 (0·0)	2 (18·2)	1·0	0 (0·0)	1 (14·3)	1·0
No	6 (100·0)	6 (75·0)		3 (100·0)	9 (81·8)		7 (100·0)	6 (85·7)	

Pro and Pre ($CD7^+$ and $CD1^-$), Corical ($CD1^+$), Mature ($CD1^+$, $sCD3^+$).
P, χ^2 or Fisher's exact test; TCR, T cell receptor.
*Total n = 13.
†Chromosomal abnormalities including 14q11, 7p15, or 7q35.

Clinical characteristics of FBXW7 and NOTCH1 mutations

The clinical and biological characteristics of the patients in this study are shown in Tables II and III. *FBXW7* and/or *NOTCH1* mutations were associated only with white blood cell (WBC) counts. *FBXW7* and/or *NOTCH1* mutations, but not *FBXW7* or *NOTCH1* alone, were found more frequently in T-ALL patients with low WBC count, $<10 \times 10^9$/l, than in those with higher WBC count, $>10 \times 10^9$/l ($P = 0{\cdot}027$). *FBXW7* mutations, but not *NOTCH1* mutations, were negatively associated with chromosome abnormalities in both T-ALL and T-NHL. All T-ALL and T-NHL patients having *FBXW7* mutation lacked a chromosome abnormality (100% vs. 52·2%, $P = 0{\cdot}031$ in T-ALL and 100% vs. 40·0%, $P = 0{\cdot}217$ in T-NHL).

Prognostic significance of FBXW7 and NOTCH1 mutations

We next analyzed the correlation between *FBXW7* and/or *NOTCH1* mutations and clinical outcome. T-ALL patients with *NOTCH1* mutation had a better clinical outcome than those without *NOTCH1* mutation {100% vs. 65·8% [95% confidence interval (CI), 48·5–78·5%]; $P = 0{\cdot}008$ for 5-year EFS and 100% vs. 81·6% [95% CI, 65·2–90·8%]; $P = 0{\cdot}065$ for 5-year OS, respectively} (Fig S3), while the prognostic difference between patients with and without *FBXW7* mutation was not significant [87·5% (95% CI, 38·7–98·1%) vs. 74·5% (95% CI, 59·4–84·6%); $P = 0{\cdot}400$ for 5-year EFS and 100% vs. 85·1% (95% CI, 71·3–92·6%); $P = 0{\cdot}259$ for 5-year OS, respectively] (Fig S4). The 5-year EFS and OS for T-ALL patients with *FBXW7* and/or *NOTCH1* mutations were extremely high, suggesting a good prognosis for patients with *FBXW7/NOTCH1* mutation compared to those without [95·5% (95% CI, 71·9–99·4%) vs. 63·6% (95% CI, 45·0–77·5%); $P = 0{\cdot}007$ and 100% vs. 78·8% (95% CI, 60·6–89·3%); $P = 0{\cdot}023$, respectively] (Fig 1). Notably, all three patients with both *FBXW7* and *NOTCH1* mutations were alive without relapse.

Multivariate analysis of prognostic factors adjusted for gender, age at diagnosis, and WBC count presented at diagnosis revealed that *FBXW7* and/or *NOTCH1* mutation status, risk group for treatment, and chromosomal abnormalities retained

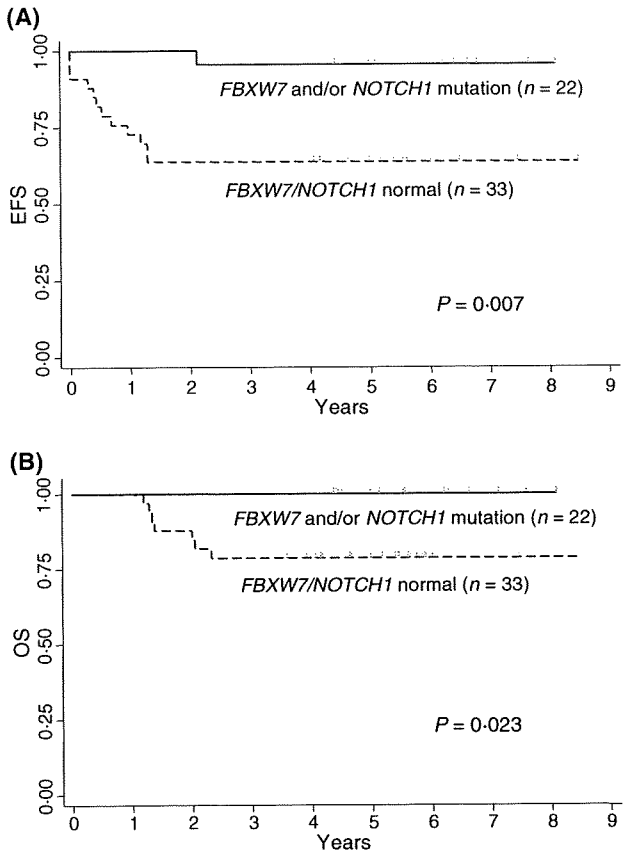

Fig 1. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *FBXW7* and/or *NOTCH1* mutation.

their significant effects on EFS (Table IV). On the other hand, multivariate analysis adjusted for *NOTCH1* and/or *FBXW7* mutation status, risk group for treatment, and chromosomal abnormalities, in addition to gender, age at diagnosis, and WBC count presented at diagnosis, revealed that none of them retained EFS significance (Table IV).

In T-NHL, patients with *NOTCH1* and/or *FBXW7* mutation also had a good prognosis, although the differences in 5-year EFS and OS for patients with and without *NOTCH1* and/or *FBXW7* mutations were not significant [EFS, 85·7% (95% CI, 33·4–97·9%) vs. 57·1% (95% CI, 17·2–83·7%), $P = 0·313$; OS, 85·7% (95% CI, 33·4–97·9%) vs. 53·6% (95% CI, 13·2–82·5%), $P = 0·286$].

Discussion

In this study, we found 14·6% *FBXW7* mutations and 30·9% *NOTCH1* mutations in T-ALL patients, and 21·4% *FBXW7* mutations and 42·3% *NOTCH1* mutations in T-NHL patients. Frequencies of *FBXW7* and *NOTCH1* mutations in T-ALL in this study were similar to those in other recent studies (8·6–30·8% for *FBXW7* mutations, and 30·8–70·8% for *NOTCH1* mutations) (Akhoondi *et al*, 2007; Lee *et al*, 2005; Malyukova *et al*, 2007; Mansour *et al*, 2006; O'Neil *et al*, 2007; Thompson *et al*, 2007; van Grotel *et al*, 2008). This is the first report describing high frequencies of *FBXW7* and *NOTCH1* mutations in T-NHL as well as in T-ALL. The types of mutations identified were similar in T-ALL and T-NHL patients (Table I), although it was previously reported that gene expression profiling revealed intrinsic differences between T-ALL and T-NHL (Raetz *et al*, 2006).

Our results demonstrated that *FBXW7* and/or *NOTCH1* mutations as well as *NOTCH1* mutations alone had a good prognosis in T-ALL patients. The *P* value regarding the significant difference in prognosis for patients with *FBXW7* and/or *NOTCH1* status ($P = 0·007$ for EFS) was less than for those with *NOTCH1* status alone ($P = 0·008$), although the difference in prognosis for *FBXW7* status alone was not significant ($P = 0·397$). All T-ALL and T-NHL patients with

Table IV. Multivariate analysis of effects of *FBXW7* and/or *NOTCH1* mutations on EFS in 55 T-ALL patients.

	Crude HR		Adjusted HR1*		Adjusted HR2†	
	(95% CI)	*P*‡	(95% CI)	*P*‡	(95% CI)	*P*‡
FBXW7 and/or *NOTCH1* mutation						
Negative	1·00§		1·00§		1·00§	
Positive	0·10 (0·01–0·78)	0·028	0·10 (0·01–0·77)	0·027	0·24 (0·05–1·13)	0·071
Chromosomal abnormalities						
No	1·00§		1·00§		1·00§	
Yes						
Abnormalities involving TCR locus (+)	5·99 (1·55–23·22)	0·010	6·04 (1·54–23·70)	0·010	6·41 (1·35–30·58)	0·020
Abnormalities involving TCR locus (−)	7·63 (1·53–38·11)	0·013	10·80 (2·03–57·57)	0·005	3·22 (0·89–11·67)	0·076

HR, hazard ratio; CI, confidence interval.

*Adjusted for sex, age at diagnosis and presenting white blood cell count (categorical: see Table I).

†Adjusted for sex, age at diagnosis, presenting white blood cell count, *FBXW7* and/or *NOTCH1* mutations category, determined risk and chromosomal abnormalities) (categorical: see Table I).

‡*P*, X^2 test.

§Reference category.

FBXW7 mutations, with the exception of one T-ALL patient, have survived without relapse. One patient (T-ALL 38) had an isolated CNS relapse; however, the patient had survived 2 years after the relapse episode.

The paediatric ALL-BFM 2000 study reported the good clinical outcome for T-ALL patients with *NOTCH1* mutations (Breit *et al*, 2006), however, other two reports described results that were not compatible with this (van Grotel *et al*, 2008; Zhu *et al*, 2006). One possible explanation for this discrepancy of prognostic impact is the different treatment protocols used; the survival rates reported in other papers were apparently lower [28·8% 3-year relapse free survival (Zhu *et al*, 2006) and 65% 5-year disease-free survival (van Grotel *et al*, 2008)] for T-ALL patients with *NOTCH1* mutation than those of the ALL-BFM 2000 study (90% relapse-free survival) and our study (100% 5-year EFS). On the other hand, there was no statistically significant impact of *NOTCH1* mutations on prognosis in T-NHL patients, perhaps because the number of T-NHL patients was small in this study. Further study of T-NHL patients is needed to clarify the association of *FBXW7* and *NOTCH1* mutations with T-NHL prognosis.

Four novel mutations were found, and two of the four, V627A in T-ALL 32 and a frame shift mutation at codon 653 in T-NHL 61, were positioned outside of a 'hot spot' region. Codon 627 is localized in the seventh β-propeller blade (β-PB7) of FBXW7 (Orlicky *et al*, 2003), and a R689W mutation in the β-PB8 was also reported in T-ALL cases (Malyukova *et al*, 2007). C-terminal truncation of FBXW7 observed in T-NHL 61 was also reported in an endometrial tumour (nonsense mutation of codon 658) (Akhoondi *et al*, 2007), and these mutations result in the absence of a portion of β-PB7 and all of β-PB8. These findings suggested that a structural change of any β-propeller blades may have similar effects on FBXW7 function. Furthermore, it was also demonstrated that *Fbxw7* deficiency in adult haematopoietic cells leads to T-ALL in mice (Matsuoka *et al*, 2008), suggesting that inactivation of FBXW7 plays a critical role in T-ALL leukaemogenesis.

Chromosomal abnormalities of the *TLX3* (5q35) and *TLX1* (10q24) locus have been reported to be associated with poor and good outcome (van Grotel *et al*, 2008). In this study, chromosomal abnormalities involving the *TLX1* locus were found in one patient and chromosomal abnormalities involving the breakpoint at 5q35·1 (*TLX3*) were not found in any patients. *t*(10;11)(q13;q14) [*PICALM-MLLT10* (previously termed *CALM-AF10*)] was not found. The prognostic significance of these cytogenetic abnormalities was not clear because the number of patients was small.

Notably, *FBXW7* mutations were only observed in T-ALL and T-NHL patients lacking chromosomal abnormalities. *FBXW7* is considered to be a haplo-insufficient tumour suppressor gene (Mao *et al*, 2004). Inactivation of FBXW7 has been reported to cause chromosomal instability in karyotypically stable colorectal cancer cells, resulting in a striking phenotype associated with micronuclei and chromosomal instability (Rajagopalan *et al*, 2004). On the contrary, *FBXW7* mutation has been reported to lack association with chromosomal instability in colorectal cancer (Kemp *et al*, 2005), which was compatible with the present results for T-ALL. Further studies are needed to clarify this issue.

In conclusion, *FBXW7* and *NOTCH1* are functionally related each other, and the mutations of either *FBXW7* or *NOTCH1* genes rather than *FBXW7* or *NOTCH1* alone were associated with good clinical outcome in T-ALL, suggesting that the status of both *FBXW7* and *NOTCH1*, rather than *FBXW7* or *NOTCH1* alone, is a useful prognostic factor in T-ALL.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Treatment plan for JACLS ALL T-97 protocol.

Fig S2. Newly identified *FBW7* mutations in T-ALL patients.

Fig S3. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *NOTCH1* mutation.

Fig S4. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *FBW7* mutation.

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Immunologically silent cancer clone transmission from mother to offspring

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Rare cases of possible materno-fetal transmission of cancer have been recorded over the past 100 years but evidence for a shared cancer clone has been very limited. We provide genetic evidence for mother to offspring transmission, in utero, of a leukemic cell clone. Maternal and infant cancer clones shared the same unique *BCR-ABL1* genomic fusion sequence, indicating a shared, single-cell origin. Microsatellite markers in the infant cancer were all of maternal origin. Additionally, the infant, maternally-derived cancer cells had a major deletion on one copy of chromosome 6p that included deletion of HLA alleles that were not inherited by the infant (i.e., foreign to the infant), suggesting a possible mechanism for immune evasion.

fetus | fusion gene | leukemia

Rare cases of melanoma or hemopoietic malignancies in infants have been recorded that may have been of maternal origin (1). Genetic evidence for a shared, materno-fetal clone of cancer cells has, however, to date, been sparse and based upon limited karotype information (1). Unambiguous attribution of transmission of a cancer clone should be achievable by genetic fingerprinting, the most striking precedent for which is canine transmissible venereal sarcoma (CTVS) in which multiple cases worldwide derive from a single clone (2). Leukemia fusion genes, generated by chromosome translocations, have patient-specific or idiosyncratic genomic sequences at the fusion breakpoints and are frequently early or initiating events (3). They therefore provide stable, specific, and sensitive clonal markers and can unambiguously identify a single-cell origin in different individuals as documented with monozygotic twins with concordant leukemia (4). We report here equivalent genetic scrutiny of a case of concordant maternal and infant ALL/lymphoma with the *BCR-ABL1* fusion gene.

Results

The Mother. The Japanese mother was 28 years old at her child's delivery. No hematological abnormalities had been identified during the pregnancy, and the birth was uncomplicated. Thirty-six days after the delivery, the mother experienced vaginal bleeding. On day 39, she developed fever, and on day 43, bleeding became uncontrollable. Blood showed leukocytosis (206,800/μL) with 97% lymphoblasts, anemia (hemoglobin level: 3.5g/dL), and thrombocytopenia (platelet count: $0.2 \times 10^4/\mu$L). Bone marrow aspiration revealed peroxidase-negative lymphoblasts (99.6% of nucleated cells), which were positive for CD10, CD19, CD20, CD34, TdT, and CD79a. Chromosomal G-banding showed 46,XX,t (9, 22)(q34;q11), and 3.2×10^5 copies/μgRNA of p190-type *BCR-ABL1* mRNA were detected by RT-PCR. She was diagnosed as having B-cell precursor Ph+ ALL (see *SI Text* for clinical treatment).

The Infant. The 11-month-old female offspring of the above mother was admitted to hospital with right cheek swelling. MRI revealed a mass in the cheek (Fig. S1*A*) and a pleural effusion of the lung. There was no lymph node swelling or organomegaly. She was born with normal delivery at 40 weeks, 5 days gestation. There was no history of prenatal abnormalities including intrauterine growth retardation, and she showed normal growth and development until admission.

Laboratory Findings on Infant Samples. Laboratory analyses of the maternal and infant samples was carried out with full ethical approval in accordance with the Declaration of Helsinki (Local ethics approval # CCR2285) and with informed consent of the family (father). Biopsy of the primary jaw tumor showed the presence of small round blue cell tumor with large nucleus/cytoplasm ratio, which diffusely proliferated with partial hyalinization of stroma. A large antibody panel was used to distinguish a sarcoma from lymphoma. LCA, CD10, CD20, CD79a, TdT, CD34, and MIC2 were positive by immunohistochemical staining, and CD3, CD5, CD56, desmin, HHF35, S100, GFAP, chromogranin, and synaptophysin were all negative. No cytogenetic analysis was performed but subsequent FISH analysis revealed positivity for the *BCR-ABL1* gene (Fig. S1*B*).

Cells (48.2%) in the pleural fluid were positive for CD10, CD19, CD34, and HLA-DR and p190-type *BCR-ABL1* chimeric mRNA was detected (9.5×10^4 copies/μgRNA) by quantitative RT-PCR (Q-PCR).

Blood count findings on the infant were as follows; WBC 10,100/μL (segment forms 22%; lymphocytes 72%; monocytes 5%; eosinophil 1%), hemoglobin level 12.5 g/dL, platelet count $38.4 \times 10^4/\mu$L. No blast cells were detected in the cerebrospinal fluid, and there was no morphological evidence of tumor infiltration in bone marrow. Bone marrow aspirates were negative for *BCR-ABL1* chimeric mRNA by Q-PCR. The patient's neoplastic cells had the same immunophenotype and abnormal genotype (*BCR-ABL1* fusion) as her mother's ALL but, in light of the presentation features, she was diagnosed as having B-cell precursor lymphoblastic lymphoma stage III by the St. Jude Staging System (see *SI Text* for clinical treatment of infant).

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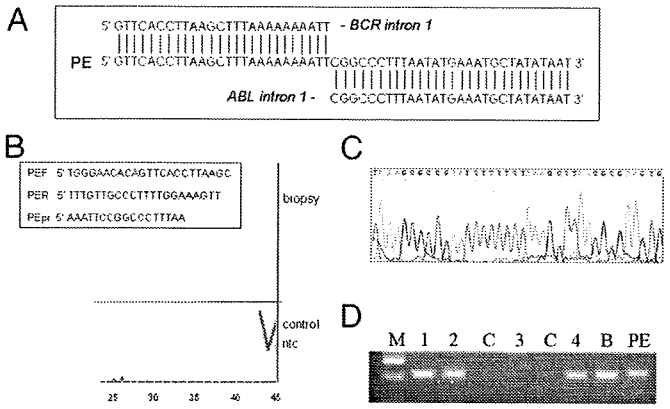

Fig. 1. Characterization of the *BCR-ABL1* fusion gene. (*A*) Comparison of the pleural effusion (PE) *BCR-ABL1* breakpoint DNA sequence with *BCR* intron 1 (NM_004327) and *ABL1* intron 1 (NM_007313) sequences. (*B*) Q-PCR amplification of the *BCR-ABL1* breakpoint in WGA DNA from mother's bone marrow biopsy. The primers and probe shown were chosen to span the PE fusion sequence obtained from the child's PE DNA. (*C*) Reverse strand DNA sequence of DNA from the mother's biopsy showing the same *BCR-ABL1* fusion sequence as found in the child. (*D*) PCR of the *BCR-ABL1* breakpoint in DNA from the neonatal blood spot confirming presence of the *BCR-ABL1* fusion gene. Lanes 1–4: slices from the child's card (1, 2, and 4 positive), C: DNA from control neonatal blood spot, B: mother's marrow biopsy DNA. PE: child's pleural effusion DNA. M: marker.

***BCR-ABL1* Genomic Fusion Sequencing.** We first cloned the *BCR-ABL1* genomic breakpoint region from the infant's pleural effusion (PE) (see *Materials and Methods*). DNA was Whole Genome Amplified (GenomiPhi, GE Healthcare), according to the manufacturer's instructions. The breakpoint was designated as a fusion between *BCR* intron 1 (46110 bp from ATG: NM_004327) and *ABL1* intron 1 (118930 bp from ATG: NM_007313) (Fig. 1*C*).

DNA from the mother's bone marrow was isolated by scraping cells from a formalin fixed paraffin embedded (FFPE) biopsy slide (the only sample available) using Recoverall (Ambion) as suggested by the manufacturer. Fragmented FFPE DNA was then subjected to whole genome amplification (WGA), and 2 μL amplified DNA subjected to 45 cycles Q-PCR with primers designed by Primer 3 software (5) and described in Fig. 1*B* and a FAM-labeled probe that spanned the specific *BCR-ABL1* breakpoint sequence. After successful Q-PCR (Fig. 1*B*), the product was purified and sequenced using the reverse *ABL1* primer. The fusion sequence in the mother's biopsy was verified as identical to that obtained from the pleural effusion of the child (Fig. 1*C*).

The archived neonatal blood spot (Guthrie card) of the infant was screened for the clonotypic *BCR-ABL1* genomic sequence using specific primers and as previously described for other fusion genes (6). Three out of four blood spot slices were positive (Fig. 1*D*), indicating that the cancer clone was present in the blood at birth.

Microsatellite Markers. Short tandem repeat (STR) microsatellite analysis of the DNA extracted from the jaw biopsy showed one predominant population (>95% of alleles) that did not correspond to the DNA profile obtained from the paternal sample (Fig. 2). The STR profile shows that it shared one allele with the patient's germline DNA for all of the 15 STR markers studied which was different from the paternally-inherited alleles, demonstrating that the jaw tumor sample was of maternal origin

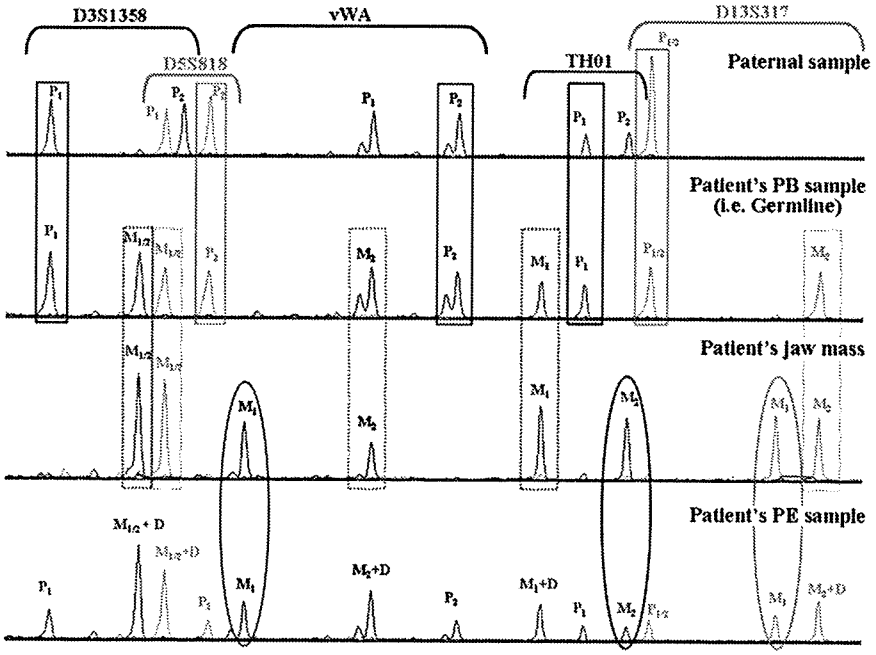

Fig. 2. STR typing for the paternal sample and patient's peripheral blood (PB), lymphoma (from the jaw mass) and pleural effusion samples. Figure shows typing for five out of the 15 STR markers analyzed. The STR profile of patient's jaw mass shows the sharing of one allele with the patient's germline DNA for all markers while the other allele is not present in the paternal DNA, demonstrating that the lymphoma DNA obtained from the jaw mass contained >95% of maternal cells. For each STR marker the Paternal (P1, P2) and Maternal (M1, M2) alleles are indicated. P and M alleles contributing to the patient's germline DNA are contained within solid and dotted rectangles, respectively. The PE sample shows a mixture of different alleles, with some markers showing up to three different alleles (vWA, TH01, and D13S317, contained in ovals), indicating heterozygosity for the maternal genotype on these markers. The remaining maternal alleles contributing to the daughter's genotype in this sample are indicated as M + D. These former markers were used for calculation of the percentage of maternal cells present in the PE sample (≈50–60%) by comparing the areas of the maternal-only peaks (M) versus the paternal peaks (corresponding to the patient's cells). This proportion of maternal cells approximates with the percentage tumor infiltration identified by immunophenotype (48.2%).