研究成果の刊行に関する一覧表

22年度 雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kanezaki R, Toki T, Terui K, Xu G, Wang R, Shimada A, Hama A, Kanegane H, Kawakami K, Endo M, Hasegawa D, Kogawa K, Adachi S, Ikeda Y, Iwamoto S, Taga T, Kosaka Y, Kojima S, Hayashi Y, Ito E.	Down syndrome and GATA1 mutations in transient abnormal myeloproliferative disorder: mutation classes correlate with progression to myeloid leukemia.	Blood	116	4631-4638	2010
林 泰秀.	ダウン症候群に発症した transient abnormal myelopoiesis (TAM) への対応	周産期医学	40	937-941	2010
川村眞智子, 菊地 陽	特集: 新 WHO 分類 – MDS 委員会/白血病委員会 ダウン症候群に関連した骨髄増殖症 -2008 WHO 分類より-	日本小児血液学会雑誌	24	168-174	2010
Kawamura M, Kaku H, Ito T, Funata N, Taki T, Shimada A, Hayashi Y.	FLT3-internal tandem duplication, CD56 expression, and obstructive jaundice due to granulocytic sarcoma at relapse in a pediatric patient with t(8;21) acute myeloid leukemia.	Cancer Genet Cytogenet	203	292-296	2010
Taketani T, Taki T, Nakamura T, Ohyashiki K, Kobayashi Y, Fukuda S, Yamaguchi S, Hayashi Y.	High frequencies of simultaneous FLT3-ITD and WT1 mutations in myeloid leukemia with NUP98-HOX fusion genes.	Leukemia	24	1975-1977	2010
Shiba N, Kanazawa T, Park MJ, Okuno H, Shiba N, Kanazawa T, Park MJ, Okuno H, Tamura K, Tsukada S, Hayashi Y, Arakawa H.	NOTCH1 mutation in a female with myeloid /NK cell precursor acute leukemia.	Pediatr Blood Cancer	55	1406-1409	2010
Mizushima Y, Taki T, Shimada A, Yui Y, Hiraumi Y, Matsubara H, Watanabe M, Watanabe K, Kamitsuji Y, Hayashi Y, Tsukimoto I, Kobayashi R, Horibe K, Tawa A, Nakahata T, Adachi S.	Prognostic significance of the BAALC isoform pattern and CEBPA mutations in pediatric acute myeloid leukemia with normal karyotype: a study by the Japanese Childhood AML Cooperative Study Group.	Int J Hematol	91	831-837	2010

Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, Ueno T, Soda M, Hamada T, Hatura H, Takada S, Miyazaki Y, Kiyoi H, Ito E, Naoe T, Tomonaga M, Toyora M, Tajima S, Iwama A, Mano H.	Array-based genomic resequencing of human leukemia.	Oncogene	29	3723-3731	2010
Aikawa Y, Katsumoto T, Zhang P, Shima H, Shino M, Terui K, Ito E, Ohno H, Stanley RE, Singh H, Tenen DG and Kitabayashi I.	PU.1-mediated upregulation of M-CSFR is critical for MOZ-leukemia stem cell potential.	Nature Medicine	16	580-585	2010
Ogawa S, Shih LY, Suzuki T, Otsu M, Nakauchi H, Koeffler HP, Sanada M.	Deregulated intracellular signaling by mutated c-CBL in myeloid neoplasms.	Clin Cancer Res	16	3825-3831	2010
Ogawa S, Sanada M, Shih LY, Suzuki T, Otsu M, Nakauchi H, Koeffler HP.	Gain-of-function c-CBL mutations associated with uniparental disomy of 11q in myeloid neoplasms.	Cell Cycle	9	1051-1056	2010
Matsuda K, Sakashita K, Taira C, Tanaka -Yanagisawa M, Yanagisawa R, Shiohara M, Kanegane H, Hasegawa D, Kawasaki K, Endo M, Yajima S, Sasaki S, Kato K, Koike K, Kikuchi A, Ogawa A, Watanabe A, Sotomatsu M, Nonoyama S, Koike K.	Quantitative assessment of PTPN11 or RAS mutations at the neonatal period and during the clinical course in patients with juvenile myelomonocytic leukaemia.	Br J Haematol	148	593-599	2010
Muramatsu H, Makishima H, Jankowska AM, Cazzolli H, O'Keefe C, Yoshida N, Xu Y, Nishio N, Hama A, Yagasaki H, Takahashi Y, Kato K, Manabe A, Kojima S, Maciejewski JP.	Mutations of E3 ubiquitin ligase Cbl family members but not TET2 mutations are pathogenic in Juvenile Myelomonocytic Leukemia.	Blood	115	1969-1975	2010
Sugimoto Y, Muramatsu H, Makishima H, Prince C, Jankowska AM, Yoshida N, Xu Y, Nishio N, Hama A, Yagasaki H, Takahashi Y, Kato K, Manabe A, Kojima S, Maciejewski JP.	Spectrum of molecular defects in juvenile myelomonocytic leukaemia includes ASXL1 mutations.	Bri J Haematol	150	83-87	2010

Takagi M, Shinoda K, Piao J, Mitsuiki N, Takagi M, Matsuda K, Muramatsu H, Doisaki S, Nagasawa M, Morio T, Kasahara Y, Koike K, Kojima S, Takao A, Mizutani S.	Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation.	Blood	117	2887-2890	2010
山内芳忠、近藤裕一、長谷川久弥、平野慎也、藤村正哲	新生児希有疾患(病態)前方視的ｻｰﾍﾞｲﾗﾝｽ事業 趣旨、実施概略ならび登録状況	日本未熟児新生児学会雑誌	22巻1号		2010

23年度雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
村松秀城、菊地陽.	一過性骨髄異常増殖症(TAM)の治療戦略.	日本小児血液学会雑誌	25	179-184	2011
Yokoyama T, Toki T, Aoki Y, Kanezaki R, Park MJ, Kanno Y, Takahara T, Yamazaki Y, Ito E, Hayashi Y, Nakamura T.	Identification of TRIB1 R107L gain-of-function mutation in human acute megakaryocytic leukemia.	Blood	119	2608-2611	2012
Shiba N, Hasegawa D, Park MJ, Murata C, Matsubara A, Ogawa C, Manabe A, Arakawa H, Ogawa S, Hayashi Y.	CBL mutation in chronic myelomonocytic leukemia secondary to familial platelet disorder with propensity to develop acute myeloid leukemia(FPD/AML).	Blood	119	2612- 2614,	2012
Shiba N, Taki T, Park MJ, Nagasawa M, Kanazawa T, Takita J, Ohnishi H, Sotomatsu M, Arakawa H, Hayashi Y.	CBL mutation in childhood therapy-related leukemia.	Leukemia	25	1356-1358	2011
Shiba N, Park MJ, Taki T, Takita J, Hiwatari M, Kanazawa T, Sotomatsu M, Ishii E, Arakawa H, Ogawa S, Hayashi Y.	CBL mutations in infant acute lymphoblastic leukaemia.	Br J Haematol	156	672-674	2012
Oki K, Takita J, Hiwatari M, Nishimura R, Sanada M, Okubo J, Adachi M, Sotomatsu M, Kikuchi A, Igarashi T, Hayashi Y, Ogawa S.	IDH1 and IDH2 mutations are rare in pediatric myeloid malignancies.	Leukemia	25	382-384	2011
Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S, Ogawa S.	Frequent pathway mutations of splicing machinery in myelodysplasia.	Nature	478	64-69	2011

Kudo K, Terui K, Sasaki S, Kamio T, Sato T, Ito E.	CD7-positive acute myelomonocytic leukemia with trisomy 21 as a sole acquired chromosomal abnormality in two adolescents.	Leuk Res.	35	e167-8	2011
Yoshida K, Sanada M, Kato M, Kawahata R, Matsubara A, Takita J, Shih LY, Mori H, Koeffler HP, Ogawa S.	A nonsense mutation of IDH1 in myelodysplastic syndromes and related disorders.	Leukemia	25	184-186	2011
Nishio N, Takahashi Y, Tanaka M, Xu Y, Yoshida N, Sakaguchi H, Doisaki S, Hama A, Muramatsu H, Shimada A, Kojima S.	Aberrant phosphorylation of STAT5 by granulocyte-macrophage colony-stimulating factor in infant cytomegalovirus infection mimicking juvenile myelomonocytic leukemia.	Leuk Res	35	1261-1264	2011
Morishima T, Watanabe K, Niwa A, Fujino H, Matsubara H, Adachi S, Suemori H,Nakahata T, Heike T.	Neutrophil differentiation from human-induced pluripotent stem cells.	J Cell Physiol	226	1283-1291	2011
Sekimizu M, Sunami S, Nakazawa A, Hayashi Y, Okimoto Y, Saito AM, Horibe K, Tsurusawa M, Mori T.	Chromosome abnormalities in advanced stage T-cell lymphoblastic lymphoma of children and adolescents: a report from Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) and review of the literature.	Br J Haematol.	154	612-617	2011
Shiba N, Taki T, Park MJ, Shimada A, Sotomatsu M, Adachi S, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Arakawa H, Hayashi Y.	DNMT3A mutations are rare in childhood acute myeloid leukaemia, myelodysplastic syndromes and juvenile myelomonocytic leukaemia.	Br J Haematol	156 :	413-414	2012
Ismael O, Shimada A, Hama A, Sakaguchi H, Doisaki S, Muramatsu H, Yoshida N, Ito M, Takahashi Y, Akita N, Sunami S, Ohtsuka Y, Asada Y, Fujisaki H, Kojima S.	Mutations profile of polycythemia vera and essential thrombocythemia among Japanese children.	Pediat Blood Cancer		In press	2012

Kuramitsu M, Sato-Otsubo A, Morio T, Takagi M, Toki T, Terui K, RuNan Wang, Kanno H, Ohga S, Ohara A, Kojima S, Kitoh T, Goi K, Kudo K, Matsubayashi T, Mizue N, Ozeki M, Masumi A, Momose H, Takizawa K, Mizukami T, Yamaguchi K, Ogawa S, Ito E, Hamaguchi I.	Extensive gene deletions in Japanese patients with Diamond–Blackfan anemia.	Blood	119	2376-2384	2012
Hama A, Muramatsu H, Makishima H, Sugimoto Y, Szpurka H, Jasek M, O'Keefe C, Takahashi Y, Sakaguchi H, Doisaki S, Shimada A, Watanabe N, Kato K, Kiyoi H, Naoe T, Kojima S, Maciejewski JP.	Molecular lesions in childhood and adult acute megakaryoblastic leukaemia.	Brit J Haematol	156	316-325	2012

参考
＜21年度（フィジビリティスタディ期間） 雑誌＞

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kitoh T, Taki T, Hayashi Y, Nakamura K, Irino T, Osaka M.	Transient abnormal myelopoiesis in a Down syndrome newborn followed by acute myeloid leukemia: identification of the same chromosomal abnormality in both stages.	Cancer Genet Cytogenet.	188	99-102	2009
Ono R, Kumagai H, Nakajima H, Hishiya A, Taki T, Horikawa K, Takatsu K, Satoh T, Hayashi Y, Kitamura T, Nosaka T.	Mixed-lineage-leukemia (MLL) fusion protein collaborates with Ras to induce acute leukemia through aberrant Hox expression and Raf activation.	Leukemia	23	2197-2209	2009
Kurosawa H, Okuya M, Matsushita T, Kubota T, Endoh K, Kuwashima S, Hagisawa S,Sato Y, Fukushima K, Sugita K, Okada Y, Park MJ, Hayashi Y, Arisaka O.	JAK2V617F mutation-positive childhood essential thrombocythemia associated with cerebral venous sinus thrombosis.	J Pediatr Hematol Oncol.	31	678-680,	2009
Takita J, Motomura A, Koh K, Ida K, Taki T, Hayashi Y, Igarashi T.	Acute megakaryoblastic leukemia in a child with the MLL-AF4 fusion gene.	Eur J Haematol.	83	149-153	2009
Taketani T, Taki T, Nakamura H, Taniwaki M, Masuda J, Hayashi Y.	NUP98-NSD3 fusion gene in radiation-associated myelodysplastic syndrome with t(8;11)(p11;p15) and expression pattern of NSD family genes.	Cancer Genet Cytogenet. 1	90	108-112	2009
Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, Tamura A, Honda H, Sakata-Yanagimoto M, Kumano K, Oda H, Yamagata T, Takita J, Gotoh N, Nakazaki K, Kawamata N, Onodera M, Nobuyoshi M, Hayashi Y, Harada H, Kurokawa M, Chiba S, Mori H, Ozawa K, Omine M, Hirai H, Nakauchi H, Koeffler HP, Ogawa S.	Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms.	Nature.	460	904-908	2009

Watanabe-Okochi N, Oki T, Komeno Y, Kato N, Yuji K, Ono R, Harada Y, Harada H, Hayashi Y, Nakajima H, Nosaka T, Kitaura J, Kitamura T.	Possible involvement of RasGRP4 in leukemogenesis.	Int J Hematol.	89	470-481	2009
Mizoguchi Y, Fujita N, Taki T, Hayashi Y, Hamamoto K.	Juvenile myelomonocytic leukemia with t(7;11)(p15;p15) and NUP98-HOXA11 fusion.	Am J Hematol.	84	295-297	2009
Park MJ, Taki T, Oda M, Watanabe T, Yumura-Yagi K, Kobayashi R, Suzuki N, Hara J, Horibe K, Hayashi Y.	FBXW7 and NOTCH1 mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma.	Brit J Haematol	145	198-206	2009
Jo A, Tsukimoto I, Ishii E, Asou N, Mitani S, Shimada A, Igarashi T, Hayashi Y, Ichikawa H.	Age-associated difference in gene expression of pediatric acute myelomonocytic lineage leukemia (FAB M4 and M5 subtypes) and its correlation with prognosis.	Brit J Haematol	144	917-929	2009
Kato M, Sanada M, Kato I, Sato Y, Takita J, Takeuchi K, Niwa A, Chen Y, Nakazaki K, Nomoto J, Asakura Y, Muto S, Tamura A, Iio M, Akatsuka Y, Hayashi Y, Mori H, Igarashi T, Kurokawa M, Chiba S, Mori S, Ishikawa Y, Okamoto K, Tobinai K, Nakagama H, Nakahata T, Yoshino T, Kobayashi Y, & Ogawa S,	Frequent inactivation of A20 in B-cell lymphomas	Nature	459	712-6	2009
Yokoyama Y, Suzuki T, Sakata-Yanagimoto M, Kumano K, Higashi K, Takato T, Kurokawa M, Ogawa S, & Chiba S	Derivation of functional mature neutrophils from human embryonic stem cells	Blood	113	6584-92	2009
菊地　陽	Down症候群のTransient Abnormal Myelopoiesis (TAM) について.	日小血会誌	23	58-61	2009

Ⅶ. 研究成果の代表的論文

Down syndrome and *GATA1* mutations in transient abnormal myeloproliferative disorder: mutation classes correlate with progression to myeloid leukemia

*Rika Kanezaki,[1] *Tsutomu Toki,[1] Kiminori Terui,[1] Gang Xu,[1] RuNan Wang,[1] Akira Shimada,[2] Asahito Hama,[3] Hirokazu Kanegane,[4] Kiyoshi Kawakami,[5] Mikiya Endo,[6] Daisuke Hasegawa,[7] Kazuhiro Kogawa,[8] Souichi Adachi,[9] Yasuhiko Ikeda,[10] Shotaro Iwamoto,[11] Takashi Taga,[12] Yoshiyuki Kosaka,[13] Seiji Kojima,[3] Yasuhide Hayashi,[2] and Etsuro Ito[1]

[1]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [2]Department of Hematology/Oncology, Gunma Children's Medical Center, Gunma, Japan; [3]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Department of Pediatrics, Graduate School of Medicine, University of Toyama, Toyama, Japan; [5]Department of Pediatrics, Kagoshima City Hospital, Kagoshima, Japan; [6]Department of Pediatrics, Iwate Medical University, Morioka, Japan; [7]Department of Pediatrics, St Luke's International Hospital, Tokyo, Japan; [8]Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan; [9]Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [10]Department of Pediatrics, Aomori City Hospital, Aomori, Japan; [11]Department of Pediatrics, Mie University Graduate School of Medicine, Tsu, Japan; [12]Department of Pediatrics, Shiga University of Medical Science, Ohtsu, Japan; and [13]Department of Hematology and Oncology, Hyogo Children Hospital, Kobe, Japan

Twenty percent to 30% of transient abnormal myelopoiesis (TAM) observed in newborns with Down syndrome (DS) develop myeloid leukemia of DS (ML-DS). Most cases of TAM carry somatic *GATA1* mutations resulting in the exclusive expression of a truncated protein (GATA1s). However, there are no reports on the expression levels of GATA1s in TAM blasts, and the risk factors for the progression to ML-DS are unidentified. To test whether the spectrum of transcripts derived from the mutant *GATA1* genes affects the expression levels, we classified the mutations according to the types of transcripts, and investigated the modalities of expression by in vitro transfection experiments using *GATA1* expression constructs harboring mutations. We show here that the mutations affected the amount of mutant protein. Based on our estimates of GATA1s protein expression, the mutations were classified into GATA1s high and low groups. Phenotypic analyses of 66 TAM patients with *GATA1* mutations revealed that GATA1s low mutations were significantly associated with a risk of progression to ML-DS ($P < .001$) and lower white blood cell counts ($P = .004$). Our study indicates that quantitative differences in mutant protein levels have significant effects on the phenotype of TAM and warrants further investigation in a prospective study. (*Blood.* 2010;116(22):4631-4638)

Introduction

In children with Down syndrome (DS), the risk of developing acute megakaryocytic leukemia (AMKL) is estimated at 500 times higher than in children without DS. Interestingly, neonates with DS are at a high risk of developing a hematologic disorder referred to as transient abnormal myelopoiesis (TAM). It has been estimated that 5% to 10% of infants with DS exhibit the disorder, and in most cases, it resolves spontaneously within 3 months. However, approximately 20% of the severe cases are still subject to fatal complications and 20% to 30% of patients who escape from early death develop AMKL referred to as myeloid leukemia of DS (ML-DS) within 4 years.[1-4]

Recent studies found that high white blood cell (WBC) count, failure of spontaneous remission, early gestational age (EGA) and liver fibrosis or liver dysfunction are significantly associated with early death.[5-7] Most of the same covariates were found in all of the reports. However, the risk factors for the progression to ML-DS remain elusive.

Blast cells in most patients with TAM and ML-DS have mutations in exon 2 of the gene coding the transcription factor GATA1,[8-14] which is essential for normal development of erythroid and megakaryocytic cells.[15-18] The mutations lead to exclusive expression of a truncated GATA1 protein (referred to as GATA1s) translated from the second methionine on exon 3. These findings strongly suggest that the qualitative deficit of GATA1 contributes to the genesis of TAM and ML-DS. The analysis of megakaryocyte-specific knockdown of *GATA1* in vivo has revealed a critical role for this factor in megakaryocytic development. Reduced expression (or complete absence) of GATA1 in megakaryocytes leads to increased proliferation and deficient maturation as well as a reduced number of circulating platelets.[19,20] Mice harboring a heterozygous *GATA1* knockdown allele frequently develop erythroblastic leukemia.[21] These observations indicate that the expression levels of GATA1 are crucial for the proper development of erythroid and megakaryocytic cells and compromised GATA1 expression is a causal factor in leukemia.[22] Nevertheless, the impact of a quantitative deficit of the factor on the pathogenesis of TAM and ML-DS has not been examined.

In this study, we classified the *GATA1* mutations observed in TAM patients according to the types of transcripts, and investigated the modalities of gene expression by in vitro transfection assays using *GATA1* expression constructs. We report here that the spectrum of the transcripts derived from the mutant genes affects protein expression and the risk of progression from TAM to ML-DS.

Submitted April 30, 2010; accepted August 2, 2010. Prepublished online as *Blood* First Edition paper, August 20, 2010; DOI 10.1182/blood-2010-05-282426.

*R.K. and T.T. contributed equally to this work.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Methods

Patients

This study was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine, and all clinical samples were obtained with informed consent from the parents of all patients with TAM, in accordance with the Declaration of Helsinki. The following clinical data were collected: sex, gestational age, birth weight, time of diagnosis, symptom at diagnosis, and clinical presentation. The following laboratory data were obtained: a complete blood cell count at diagnosis including WBC and the percentage of blasts in the peripheral blood, coagulation parameters, liver enzymes (alanine aminotransferase and aspartate aminotransferase), and total bilirubin. The procedure for the detection of *GATA1* mutations was described previously.[13] Genomic DNA was directly extracted from peripheral blood or bone marrow with the QIAamp blood mini kit (QIAGEN). Total RNA was extracted from white blood cells prepared by removal of erythrocytes by hypotonic buffer treatment of peripheral blood. Clinical features, outcomes, and characteristics of *GATA1* mutations are indicated in Table 1.

Construction of GATA1 expression vectors

To construct *GATA1* minigene expression vectors, fragments of the normal human *GATA1* gene from a part of intron 1 to the stop codon located on exon 6 were amplified by polymerase chain reaction (PCR; Prime STAR HS: Takara Bio) and subcloned to mammalian expression vector pcDNA3.1 (+)/Neo (Invitrogen). To introduce mutations identical to those observed in TAM patients into the expression vector, the regions containing mutations were amplified by PCR from patient samples and inserted into the expression plasmid. To construct expression vectors carrying cDNA, we performed PCR using cDNA derived from baby hamster kidney 21 (BHK-21) cells transfected with *GATA1* minigene vectors. The PCR products were subcloned to pcDNA3.1(+)/Neo. Details of the sequence of each expression construct are described in Table 2.

Transfection

BHK-21, a baby hamster kidney fibroblast cell line, was cultured with Dulbecco modified Eagle medium supplemented 10% fetal bovine serum. *GATA1* expression vectors were transfected into BHK-21 cells using FuGENE HD transfection reagent (Roche Diagnostics) according to the manufacturer's methods. After 24 hours, protein and total RNA were extracted.

Western blot analysis

Lysates of transfected BHK-21 cells were transferred to Hybond-P (GE Healthcare) and processed for reaction with anti-GATA1 antibody M-20 (Santa Cruz Biotechnology) or anti-neomycin phosphotransferase II (NeoR) antibody (Millipore) as described previously.[23]

Northern blot analysis

Two micrograms of total RNA were transferred to Hybond-N+ (GE Healthcare) and hybridized with *GATA1* or *NeoR* DNA probe. Hybridization and detection were performed with the Gene Images AlkPhos Direct Labeling and Detection System (GE Healthcare) according to the manufacturer's instructions.

RT-PCR

To detect alternatively spliced transcripts derived from *GATA1* minigene constructs or from patients' peripheral blood mononuclear cells (obtained by Ficoll-Hypaque fractionation), we performed reverse transcription (RT)–PCR using primers T7: 5′ AATACGACTCACTATAG 3′ and GATA1 AS1, and GATA1 S1 and GATA1 AS1, respectively.[13] Densitometric analyses were performed by the Quantity-One software (Version 4.5.2; Bio-Rad Laboratories).

Statistical analysis

The cumulative incidence of the progression to ML-DS was analyzed with the Gray test. Differences in the distribution of individual parameters among patient subsets were analyzed using the Pearson χ^2 test or Fisher exact test for categorized variables and the Mann-Whitney U test for continuous variables. The univariate Cox proportional hazards model was used to obtain the estimates and the 95% confidence interval of the relative risk for prognostic factors.

Results

Patient characteristics and outcomes

From 2003 to 2008, we screened *GATA1* mutations in clinical samples obtained from 78 patients with TAM upon request from referring hospitals. Acquired *GATA1* mutations were detected in a total of 72 (92.3%) patients among them. Of the 72 patients, 6 harbored multiple *GATA1* mutant clones and were excluded from this study because we could not determine a dominant clone in these patients. Those 6 have not progressed to ML-DS. For the remaining 66 patients (32 male and 34 female), the clinical characteristics and laboratory data at diagnosis are described in Table 1 and summarized in Table 3. Early death within the first 6 months of life occurred in 16 patients (24.2%). The covariates correlated with early death were as follows: EGA, low birth weight, high WBC count at diagnosis, high percentage of peripheral blast cells, complication of effusions, and bleeding diatheses. These prognostic factors were identified in previous studies.[5-7] Eleven (16.7%) cases subsequently developed ML-DS. The median age at diagnosis of ML-DS was 396 days (range 221-747 days). Univariate analysis revealed no covariates correlated with progression to ML-DS except the low total bilirubin level at diagnosis ($P = .023$).

GATA1 mutations affect expression levels of GATA1s protein

We first asked whether the spectrum of transcripts derived from the mutant *GATA1* genes affected the expression levels of the translation products. The transcripts coding GATA1s protein were categorized into 3 groups as follows: loss of the first methionine, splicing errors, and premature termination codon (PTC). Furthermore, the PTC group was divided into 2 subcategories by the location of introduced PTC. In this report, we refer to the mutation that causes PTC before the second methionine at codon 84 as PTC type 1, and after codon 84 as PTC type 2. We constructed cDNA expression vectors for each class of mutations observed in TAM patients, and transfected these constructs into BHK-21 cells (Figure 1). The details of the *GATA1* mutations are described in Table 2. Western blot analysis revealed that GATA1s proteins were most abundantly expressed in mutants with splicing errors. The transcripts from mutants that had lost the first methionine were also efficiently translated. In contrast, in the cells expressing PTC type 1 or type 2 constructs, GATA1s expression levels were uniformly low. Note that the translation efficiency of the PTC type 2 transcripts was lowest among them.

To test the possibility that mutations in *GATA1* have an effect on the quantity of the transcripts, we next prepared human *GATA1* minigene expression vectors, and assessed the expression levels. Consistent with the results using cDNA expression vectors, Western blot analysis showed that the expression levels of GATA1s were lower in cells expressing PTC type 2 mutations, whereas the expression levels of the proteins from PTC type 1 mutations were not uniformly low (Figure 2Ai). Northern blot analysis revealed that the lowest expression levels of *GATA1* mRNAs were observed

Table 1. Clinical features and mutation characteristics in TAM patients with *GATA1* mutations

Patient No.	Sex	WBC, $\times 10^9$/L	Outcome	*GATA1* mutation*	Consequence of mutation	Mutation type
1[13,24]	F	63.9	CR	207 C>G	Tyr69stop	PTC 1-3′
2[13]	F	89.0	Early death	199 G>T	Glu67stop	PTC 1-3′
3[13]	F	NA	NA	174 ins 19 bp CAGCCACCGCTGCAGCTGC	Frame shift at codon58, stop at codon 73	PTC 1-3′
4[13]	F	128.8	CR	IVS1 to IVS2 del 1415 bp	Splice mutant	Splicing error
5[13]	F	NA	NA	49 C>T	Gln17stop	PTC 1-5′
6[13]	F	248.6	NA	Loss of 2nd exon	Splice mutant	Splicing error
7[13]	F	31.2	CR	Loss of 2nd exon	Splice mutant	Splicing error
8[13]	M	199.6	CR	−11 to +33 del 44 bp	No translation from Met1	Loss of 1st Met
9[13]	M	44.9	Early death	45 ins C	Frame shift at codon15, stop at codon 39	PTC 1-5′
10[13]	M	50.9	CR	37 G>T	Glu13stop	PTC 1-5′
11[13]	F	103.0	Early death	90-91 del AG	Frame shift at codon 30, stop at codon 38	PTC 1-5′
12[13]	F	14.6	Evolved to ML-DS	116 del A	Frame shift at codon 39, stop at codon 136	PTC 2
13[13]	M	423.0	CR	185 ins 22 bp GCTGCAGCTGCGGCACTGGCCT	Frame shift at codon 62, stop at codon 74	PTC 1-3′
14[13]	M	201.2	CR	189 C>A	Tyr63stop	PTC 1-3′
15[13]	M	NA	NA	1 A>G	No translation from Met1	Loss of 1st Met
16[13]	F	28.3	CR	189 C>A	Tyr63stop	PTC 1-3′
17[13]	M	203.0	Evolved to ML-DS	38-39 del AG	Frame shift at codon 13, stop at codon 38	PTC 1-5′
18[13]	M	31.3	CR	189 C>A	Tyr63stop	PTC 1-3′
19[13]	M	NA	NA	90-91 del AG	Frame shift at codon 30, stop at codon 38	PTC 1-5′
20[13]	F	114.0	Early death	187 ins T	Frame shift at codon 63, stop at codon 67	PTC 1-3′
21[25]	F	26.0	Evolved to ML-DS	194 ins 20 bp GGCACTGGCCTACTACAGGG	Frame shift at codon 65, stop at codon 143	PTC 2
22[25]	F	25.0	Evolved to ML-DS	194 ins 20 bp GGCACTGGCCTACTACAGGG	Frame shift at codon 65, stop at codon 143	PTC 2
23	F	49.9	CR	3 G>T	No translation from Met1	Loss of 1st Met
24	F	46.2	NA	IVS1 3′ boundary AG>AA	Splice mutant	Splicing error
25	F	10.5	CR	194 ins 19 bp GCACTGGCCTACTACAGGG	Frame shift at codon 65, stop at codon 73	PTC 1-3′
26[24]	F	244.0	Evolved to ML-DS	1 A>G	No translation from Met1	Loss of 1st Met
27	F	38.3	CR	Loss of 2nd Exon	Splice mutant	Splicing error
28[24]	F	34.6	CR	IVS1 to exon2 del 148 bp	Splice mutant	Splicing error
29	M	25.9	Evolved to ML-DS	160 ins TC	Frame shift at codon 54, stop at codon 137	PTC 2
30	F	52.3	Evolved to ML-DS	187 ins CCTAC	Frame shift at codon 63, stop at codon 138	PTC 2
31[24]	F	221.0	CR	183-193 del 11 bp CTACTACAGGG	Frame shift at codon 62, stop at codon 63	PTC 1-3′
32	M	149.7	CR	2 T>G	No translation from Met1	Loss of 1st Met
33[24]	M	132.3	Evolved to ML-DS	101-108 del 8 bp TCCCCTCT	Frame shift at codon 34, stop at codon 36	PTC 1-5′
34[24]	F	220.0	Early death	90-91 del AG	Frame shift at codon 30, stop at codon 38	PTC 1-5′
35[24]	M	166.0	Early death	IVS2 5′ boundary GT>CT	Splice mutant	Splicing error
36[24]	M	57.6	Early death	193-199 GACGCTG>TAGTAGT	Asp65stop	PTC 1-3′
37[24]	M	247.6	Early death	Exon2 to IVS2 del 218 bp	Splice mutant	Splicing error
38[24]	M	93.3	Early death	IVS1 3′ boundary AG>AA	Splice mutant	Splicing error
39[24]	M	290.8	Early death	186 ins 12 bp GGCACTGGCCTA	Tyr62stop	PTC 1-3′
40	F	7.8	CR	2 T>C	No translation from Met1	Loss of 1st Met
41[24]	M	136.6	Early death	IVS2 5′ boundary GT>GC	Splice mutant	Splicing error
42	M	33.1	Early death	187 ins 8 bp TGGCCTAC	Frame shift at codon 63, stop at codon 139	PTC 2
43	M	9.0	CR	22 ins G	Frame shift at codon 8, stop at codon 39	PTC 1-5′
44	M	24.1	Evolved to ML-DS	149 ins 20 bp AGCAGCTTCCTCCACTGCCC	Frame shift at codon 50, stop at codon 143	PTC 2
45[24]	F	53.3	CR	173 C>TGCTGCAGTGTAGTA	Frame shift at codon 58, stop at codon 141	PTC 2
46	F	119.0	CR	1 A>C	No translation from Met1	Loss of 1st Met
47	M	33.0	CR	189 C>A	Tyr63stop	PTC 1-3′
48	M	178.2	Early death	188 ins 22 bp GCAGCTGCGGCACTGGCCTACT	Frame shift at codon 63, stop at codon 74	PTC 1-3′
49	F	73.6	CR	3 G>A	No translation from Met1	Loss of 1st Met
50	F	12.9	CR	158 ins 7 bp AGCACAG	Frame shift at codon 53, stop at codon 69	PTC 1-5′
51	M	13.0	CR	154-161 del 8 bp ACAGCCAC	Frame shift at codon 52, stop at codon 64	PTC 1-5′
52	M	105.5	Early death	4 G>T	Glu2stop	PTC 1-5′
53	F	98.3	CR	4 G>T	Glu2stop	PTC 1-5′
54	F	356.9	CR	219 A>C	Splice mutant	Splicing error
55	F	25.8	Evolved to ML-DS	157 ins CA	Frame shift at codon 53, stop at codon 137	PTC 2
56	M	97.4	Evolved to ML-DS	185-188 del 4 bp ACTA	Frame shift at codon 62, stop at codon 135	PTC 2
57	F	97.3	Early death	3 G>A	No translation from Met1	Loss of 1st Met
58	M	NA	CR	3 G>A	No translation from Met1	Loss of 1st Met
59	M	20.2	CR	150 ins 5 bp TGGCT	Frame shift at codon 50, stop at codon 52	PTC 1-5′
60	M	133.4	CR	174 ins 19 bp CAAAGCAGCTGCAGCGGTG	Frame shift at codon 58, stop at codon 73	PTC 1-3′
61	M	NA	CR	220 G>T	Splice mutant	Splicing error
62	M	120.2	CR	220 G>A	Splice mutant	Splicing error
63	F	39.0	CR	97-139 del 43 bp	Frame shift at codon 33, stop at codon 122	PTC 2
64	F	NA	NA	156 ins C	Frame shift at codon 52, stop at codon 67	PTC 1-5′
65	F	32.4	CR	174 ins 7 bp CTGCAGC	Frame shift at codon 58, stop at codon 69	PTC 1-3′
66	M	69.4	Early death	174-177 GGCA>TGCGGTGG	Frame shift at codon 58, stop at codon 68	PTC 1-3′

We previously reported the *GATA1* mutations of the indicated patients.

F indicates female; M, male; CR, complete remission; NA, not available; and IVS, intervening sequence.

*For cDNA nucleotide numbering, nucleotide number 1 corresponds to the A of the ATG translation initiation codon in the reference sequence.

Table 2. GATA1 expression vectors used in this study

Name	Patient no.	*GATA1* mutation*	Last normal GATA1 amino acid	PTC	Mutation type
WG	–	–	Ser413	–	Normal
SP1	24, 38	intron1 3′ boundary AG>AA	Ser413	–	Splicing error
SP2	41	intron2 5′ boundary GT>GC	Ser413	–	Splicing error
L	46	1 A>C	(Met1 is replaced by Val1)	–	Loss of 1st Met
P1-1	11, 19, 34	90, 91 del AG	Gly31	38	PTC 1-5′
P1-2	14, 16, 18, 47	189 C>A	Tyr62	63	PTC 1-3′
P1-3	25	194 ins 19 bp	Arg64	73	PTC 1-3′
P1-4	17	38, 39 del AG	Ser12	38	PTC 1-5′
P1-5	33	101-108 del 8 bp	Phe33	36	PTC 1-5′
P1-6	50	158 ins 7 bp	Tyr52	69	PTC 1-5′
P1-7	3	174 ins 19 bp	Ala58	73	PTC 1-3′
P1-8	48	188 ins 22 bp	Try62	74	PTC 1-3′
P2-1	21, 22	194 ins 20 bp	Arg64	143	PTC 2
P2-2	44	149 ins 20 bp	Ala49	143	PTC 2
P2-3	29	160 ins TC	Ala53	137	PTC 2

– indicates not applicable.
*For cDNA nucleotide numbering, nucleotide number 1 corresponds to the A of the ATG translation initiation codon in the reference sequence.

in cells transfected with PTC type 2 constructs, whereas the mRNA levels in mutants that had lost the first methionine and PTC type 1 mutants were almost comparable to those of control minigene constructs harboring wild type *GATA1* gene (Figure 2Aiii). Thus, abundant proteins were produced from *GATA1* mRNAs in mutants with splicing errors and those that lost the first methionine. Conversely, relatively low levels of protein were produced by PTC type 2 mutants because of inefficient translation and reduced levels of message (Figure 2Ai,iii). However, in the case of PTC type 1 mutations, especially P1-1 and P1-4, we could find no correlation between the amount of transcripts or translation efficiency and the expression levels of GATA1s proteins (Figure 2Ai,iii).

GATA1s expression levels largely depend on the amount of the alternative splicing form

To investigate the precise relationship between PTC type 1 mutations and GATA1s protein levels, we examined more type 1 mutations using the minigene constructs. Western blot analysis showed relatively higher expression of the proteins in samples expressing P1-5, P1-7, P1-8, P1-2, and P1-3 than the other constructs (Figure 2Bi). Each mutation in the mutant minigene construct is described in Table 2. Interestingly, all samples that expressed higher levels of GATA1s protein exhibited intense signals at lower molecular weights than the dominant GATA1 signal (Figure 2Biii). Because the size of the lower molecular weight band was identical to that observed in the splicing error mutant (Figure 2Biii), we speculated that the signal might be derived from a transcript lacking exon 2 (Δexon 2) by alternative splicing. To examine that possibility, we attempted Northern blot analysis using the *GATA1* exon 2 fragment as a probe, and as expected, only the longer transcript was detected (Figure 2Biv). To confirm the correlation between the amount of Δexon 2 transcript and GATA1s protein, we performed a quantitative assessment by densitometric analysis. The results showed a strong correlation between Δexon 2 transcript and GATA1s protein

Table 3. Findings at diagnosis and during the course of TAM were significantly associated with early death and the progression to leukemia (univariate analysis)

Variable	Total (n = 66)	Early death (n = 16)	*P*	Progressed to ML-DS (n = 11)	*P*
Sex					
Male, n (%)	32 (48.5)	11 (68.8)		5 (45.5)	
Female, n (%)	34 (51.5)	5 (31.3)	.088	6 (54.5)	.947
Median gestational age, wk (range)	37.35 (30.0-40.6)	34.6 (30.0-38.4)		38.1 (32.6-40.6)	
Term versus preterm					
Term (≥ 37 weeks), n (%)	27 (58.7)	4 (30.8)		5 (71.4)	
Preterm (< 37 weeks), n (%)	19 (41.3)	9 (69.2)	.021	2 (28.6)	.465
Median birth weight, kg (range)	2.5 (1.4-3.5)	2.2 (1.6-2.7)		2.5 (1.6-3.5)	
Not LBW versus LBW					
Not LBW (≥ 2.5 kg), n (%)	24 (52.2)	3 (23.1)		3 (42.9)	
LBW (< 2.5 kg), n (%)	22 (47.8)	10 (76.9)	.025	4 (57.1)	.184
Median WBC, $\times 10^9$/L (range)	69.4 (7.8-423.0)	104.3 (33.1-290.8)		26 (14.6-244.0)	
WBC < 70 × 10^9/L vs WBC > 70 × 10^9/L					
WBC < 70 × 10^9/L, n (%)	30 (50.8)	4 (25.0)		7 (63.6)	
WBC > 70 × 10^9/L, n (%)	29 (49.2)	12 (75.0)	.020	4 (36.4)	.755
Median peripheral blasts, % (range)	56.0 (4.0-94.0)	78.0 (8.0-93.0)	.031	49.5 (6.0-66.0)	.752
Median AST, IU/L (range)	61 (16-4341)	79 (41-3866)	.620	51 (16-153)	.553
Median ALT, IU/L (range)	39 (4-653)	41 (7-473)	.455	12 (4-96)	.615
Median T-Bil mg/dL (range)	6.3 (0.6-46.0)	6.06 (2.4-16.5)	.922	3.01 (1.82-6.50)	.023
Effusions, n (%)	16 of 44 (36.4)	8 of 11 (72.7)	.007	1 of 7 (14.3)	.912
Bleeding diatheses, n (%)	13 of 45 (28.9)	8 of 12 (66.7)	.001	1 of 7 (14.3)	.123

Some clinical data were not available. We defined the number of patients for whom clinical data was available as (n).
LBW indicates low birth weight; AST, aspartate transaminase; ALT, alanine transaminase; and T-Bil, total bilirubin.

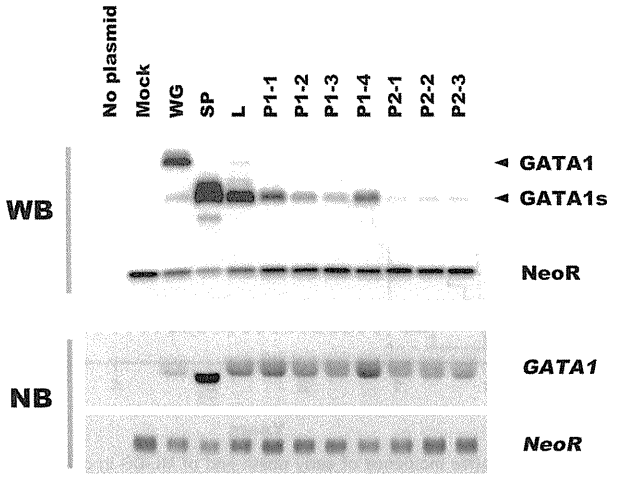

Figure 1. Effects of mutant transcripts of *GATA1* on the expression level of the truncated protein. The *GATA1* mutations observed in TAM patients are classified according to the types of transcripts. The translational efficiency of each transcript was assessed by Western blot analysis in BHK-21 cells transfected with *GATA1* cDNA expression vectors (top part of the panel) and Northern blot analysis (bottom part of the panel), respectively. WG indicates wild type GATA1; SP, splicing error mutation (Δexon 2); L, loss of first methionine mutation; P1, PTC type 1 mutation; P2, PTC type 2 mutation. The details of the *GATA1* mutations are summarized in Table 1. NeoR indicates Neomycin phosphotransferase II.

levels ($r = 0.892$, $P = .003$), but not with the long transcript containing exon2 nor total *GATA1* mRNA (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Next, we performed RT-PCR using primers recognizing both transcripts, and calculated the ratio of Δexon 2 to the long transcript (Figure 2Bvi-vii). The intensive short transcript was detected in all samples with higher expression of GATA1s (P1-5, P1-7, P1-8, P1-2, and P1-3; Figure 2Bvii). Interestingly, most of these mutations were clustered in the 3′ region of exon 2 (Table 2, Figure 2Bvii). These results suggest that the location of the mutation predicts the efficiency of alternative splicing and GATA1s expression levels.

To examine whether differential splicing efficiency could also be observed in TAM blasts with PTC type 1 mutations, RT-PCR analysis was performed using patients' clinical samples. Intense transcription of the short form was observed in the samples from the patients who had *GATA1* mutations located on the 3′ side of exon 2 (+169 to +218 in mRNA from the ATG translation initiation codon; Figure 3A-B). We refer to them as PTC type 1-3′ and the mutations located on the 5′ side of exon 2 as PTC type 1-5′.

Correlation of the phenotype and *GATA1* mutations in TAM patients

Based on these results, *GATA1* mutations were classified into 2 groups: a high GATA1s expression group (GATA1s high group) including the loss of first methionine type, the splicing error type, and PTC type 1-3′, and a low GATA1s expression group (GATA1s low group) including PTC type 1-5′ and PTC type 2. We classified TAM patients into these 2 groups in accordance with the GATA1s expression levels estimated from the mutations and compared their clinical data. High counts of WBC and blast cells were significantly associated with the GATA1s high group ($P = .004$ and $P = .008$, respectively; Table 4). Although high WBC count was correlated with early death, there were no significant differences in the cumulative incidence of early death between the 2 groups (Figure 4). Importantly, TAM patients in the GATA1s low group had a significantly higher risk for the development of leukemia ($P < .001$; Figure 4). Of 11 TAM patients who progressed to ML-DS, 10 belonged to the GATA1s low group. Notably, 8 patients among them had PTC type 2 mutations (Tables 1, 5).

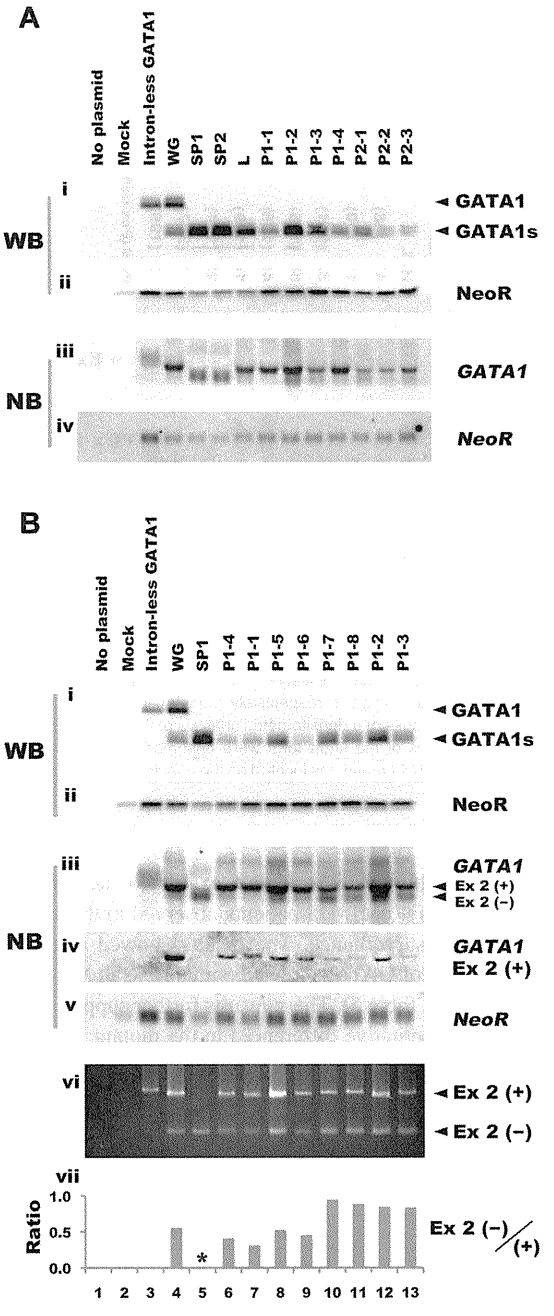

Figure 2. GATA1 mutations affect the expression level of the truncated protein. (A) The expression levels of GATA1s protein and mRNA were assessed in BHK-21 cells transfected with human *GATA1* minigene expression vectors carrying mutations observed in TAM patients. Western blot analysis was performed with anti-GATA1 (i) or anti-NeoR antibody (ii). Northern blot analysis was carried out with *GATA1* exon 3-6 fragment (iii) or *NeoR* cDNA (iv) as probe. (B) The expression levels of GATA1s protein and mRNA in BHK-21 cells transfected with human *GATA1* minigene expression vectors with PTC type 1 mutation. Levels were assessed by Western blot analysis with anti-GATA1 antibody (i), anti-NeoR antibody (ii). Northern blot analysis was performed with GATA1 exon 3-6 (iii), exon 2 (iv), or NeoR cDNA (v). To detect the transcripts derived from the human *GATA1* minigene expression construct, RT-PCR analysis was carried out using primers described in "RT-PCR" (vi). Ex 2(+) and Ex 2(−) indicate PCR products or transcripts with or without exon 2, respectively. Ratio of Ex 2(−)/(+) was calculated from the results of a densitometric analysis of the RT-PCR. The asterisk denotes unavailable data (vii).

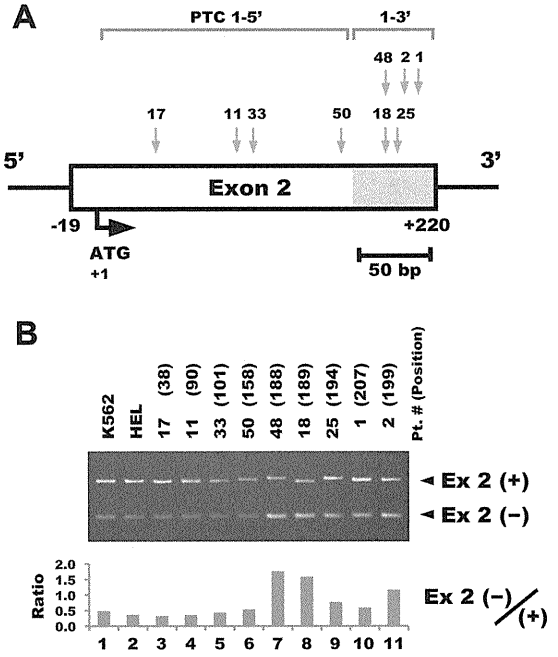

Figure 3. The location of the PTC type 1 mutation affects the efficiency of alternative splicing in TAM blast cells. (A) The location of the *GATA1* mutation in each TAM patient. Details of the mutation in each sample are described in Table 1. (B) RT-PCR analysis of *GATA1* in TAM blast cells harboring PTC type 1 mutations. RT-PCR was performed using primers recognizing both the long transcript including exon 2 and Δexon 2 (top). All of the patient samples consisted of mononuclear cells from peripheral blood. The numbers in parentheses indicate the number of nucleotides in mRNA from the translation initiation codon. Ex 2(+) and Ex 2(−) indicate PCR products with or without exon 2, respectively (middle). Ratio of Ex 2(−)/(+) was calculated from the results of a densitometric analysis of the RT-PCR (bottom). Note that the intense bands of the short form were observed in the samples from the patients who have *GATA1* mutations located on the 3′ side of exon 2 (lanes 7-11).

To validate this observation, we examined the proportion of mutation types in 40 ML-DS patients observed in the same period of time as this surveillance. The results showed a significantly higher incidence of GATA1s low type mutations in ML-DS than in TAM ($P = .039$; Table 5). These results further support the present findings that quantitative differences in the mutant protein have a significant effect on the risk of progression to ML-DS.

Table 4. Correlations between patient covariates and GATA1 expression levels

	GATA1s expression group		
	High (n = 40)	Low (n = 26)	*P*
Sex: male/female, n	19/21	13/13	.843*
Gestational age, wk	37.3 (30.0-40.0)	37.9 (32.6-40.6)	.487
Birth weight, kg	2.5 (1.6-3.3)	2.5 (1.4-3.5)	.698
WBC, $\times 10^9$/L	105.65 (7.8-423.0)	39.0 (9.0-220.0)	.004
Number of blasts, $\times 10^9$/L	72.1 (0.42-301.6)	13.4 (0.45-189.2)	.008
AST, IU/L	68.5 (23-501)	46.5 (16-4341)	.113
ALT, IU/L	41.0 (5-407)	12.5 (4-653)	.075
T-Bil mg/dL	6.7 (0.6-15.3)	4.65 (1.82-46.0)	.270
Effusions, n (%)	11 of 27 (40.7)	5 of 17 (29.4)	.447†
Bleeding diatheses, n (%)	8 of 29 (27.6)	5 of 16 (31.3)	.528†

Values are given as the median (range). *P* values estimated by Mann-Whitney *U* test.

*Pearson χ^2 test.

†Fisher exact test.

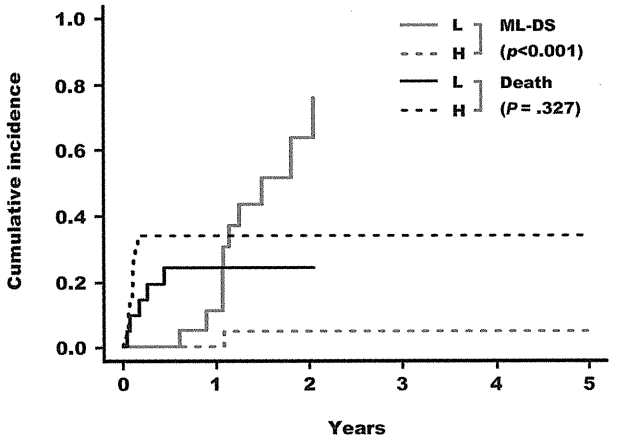

Figure 4. Cumulative incidence of early death and of ML-DS in children with TAM. Based on the estimated GATA1s expression levels, patients were classified in 2 groups: GATA1s high and low groups. TAM patients in the GATA1s low group had a significantly higher risk for the development of leukemia (P (gray) $< .001$).

Discussion

In TAM, *GATA1* mutations lead to the expression of proteins lacking the N-terminal transactivation domain. In addition to this qualitative change, we showed here that the mutations affect the expression level of the truncated protein. The mutations were classified into 2 groups according to the estimated GATA1s expression level. Comparison of the clinical features between the 2 groups revealed that GATA1s low mutations were significantly associated with a high risk of progression to ML-DS and lower counts of both WBC and blast cells. These results suggest that quantitative differences in protein expression caused by *GATA1* mutations have significant effects on the phenotype of TAM.

GATA1s was shown previously to be produced from wild-type *GATA1* through 2 mechanisms: use of the alternative translation initiation site at codon 84 of the full-length transcript and alternative splicing of exon 2.[12,26] However, the translation efficiencies of GATA1s from the full-length of mRNA and short transcripts have not been investigated. Our results clearly showed that the Δexon 2 transcript produced GATA1s much more abundantly than did the full-length transcript. The translation efficiencies of GATA1s from full-length transcripts containing PTC were also lower than the alternative spliced form. These results support our contention that GATA1s expression levels largely depend on the amount of the Δexon 2 transcript. Thus, one cannot predict the expression level of GATA1s protein from the total amount of the transcript.

The differences in the quantities of GATA1s proteins expressed by PTC type 1-5′ and -3′ mutations revealed the importance of the location of the mutation for splicing efficiency and protein expression. The splicing efficiency is regulated by *cis*-elements located in exons and introns (referred to as exonic and intronic splicing enhancers or silencers), and transacting factors recognizing these elements.[27,28] The PTC type 1-3′ mutations induced efficient skipping of exon 2 (Figures 2Bvi-vii, 3A-B). These mutations might affect exonic splicing enhancers or silencers located in exon 2. To predict the splicing pattern from the mutations more accurately, the elucidation of *cis*-elements and transacting splicing factors, which regulate the splicing of exon 2 of *GATA1*, will be very important.

Table 5. Summary of outcomes and *GATA1* mutation types in TAM patients

	Outcome of TAM				TAM		ML-DS	
Mutation type	CR	Early death	Evolved to ML-DS	NA	Total (n = 66)		Total (n = 40)	
High group								
Loss of 1st Met, n (%)	7	1	1	1	10 (15.2)		3 (7.5)	
Splicing error, n (%)	7	4	0	2	13 (19.7)	40 (15.2)	6 (15.0)	16 (40.0)
PTC 1-3′, n (%)	10	6	0	1	17 (25.8)		7 (17.5)	
Low group								
SPTC 1-5′, n (%)	6	4	2	3	15 (22.7)	26 (39.4)	14 (35.0)	24 (60.0)
PTC 2, n (%)	2	1	8	0	11 (16.7)		10 (25.0)	

The nonsense mediated RNA decay pathway (NMD), a cellular mechanism for detection of PTC and prevention of translation from aberrant transcripts,[29,30] might regulate the expression of GATA1s protein derived from PTC type 2 mutations, which contained PTCs after the second methionine at codon 84. We consistently detected low amounts of transcripts of *GATA1* in samples expressing PTC type 2 mutations, whereas the expression levels of *GATA1* mRNA from PTC type 1 mutations were comparable with that from wild-type *GATA1* (Figure 2Aiii). These results suggest that the location of PTC relative to alternative translation initiation sites is important for effective NMD surveillance.

Available evidence indicates that acute leukemia arises from cooperation between one class of mutations that interferes with differentiation (class II mutations) and another class that confers a proliferative advantage to cells (class I mutations).[31] Recent reports showed that introducing high levels of exogenous GATA1 lacking the N-terminus did not reduce the aberrant growth of GATA1-null megakaryocytes, but instead induced differentiation.[32,33] This observation suggested that abundant GATA1s protein functions like a class I mutation in TAM blasts. In contrast, reducing GATA1 expression leads to differentiation arrest and aberrant growth of megakaryocytic cells.[19,20] The present data suggest that GATA1s is expressed at very low levels in TAM blasts with GATA1s low mutations. These levels may not be sufficient to provoke normal maturation. Together, these findings suggest that the low expression of GATA1s might function like class II mutations in TAM blasts. Additional class I mutations or epigenetic alterations might be more effective in the development of leukemia in blast cells expressing GATA1s at low levels.

In the present study, we identified a subgroup of TAM patients with a higher risk of developing ML-DS. Of 66 children, 11 (16.7%) with TAM subsequently developed ML-DS and 10 of them belonged to the GATA1s low group harboring the PTC type 2 or PTC type 1-5′ mutations. Surprisingly, 8 of 11 patients (73%) with the PTC type 2 mutations developed ML-DS (Tables 1, 5), whereas 2 of 15 patients (13.3%) with PTC type 1-5′ mutations developed leukemia. The estimated expression levels of GATA1s from PTC type 2 mutations were lower than those from PTC type 1-5′ mutations (Figures 1, 2Ai). These results suggest that the type 2 mutations may be a more significant risk factor for developing ML-DS (supplemental Figure 2). However, our classification of *GATA1* mutations mainly rested on extrapolation from in vitro transfection experiments (Figures 1-2) and RT-PCR analyses of a small number of patient samples (Figure 3). The stability of the transcripts and the splicing efficiency of the second exon of *GATA1* will be regulated through complex mechanisms. To confirm our findings, precise mapping of the mutations that affect the expression levels of GATA1s and a prospective study with a large series of TAM patients are necessary.

Finally, we proposed the hypothesis that the quantitative differences in GATA1s protein expression caused by mutations have a significant effect on the phenotype of TAM. The observations described here provide valuable information about the roles of *GATA1* mutations on multistep leukemogenesis in DS patients. Moreover, the results might have implications for management of leukemia observed in DS infants and children. Because the blast cells in both TAM and subsequent ML-DS appear highly sensitive to cytarabine,[34-39] the preleukemic clone could be treated with low-dose cytarabine without severe side effects, and elimination of the preleukemic clone might prevent progression to leukemia.

Acknowledgments

We thank Dr Tetsuo Mitsui (Yamagata University School of Medicine), Shingo Morinaga (National Hospital Organization Kumamoto Medical Center), Takahide Nakano (Kansai Medical University), Masahiro Migita (Japan Red Cross Kumamoto Hospital), Hiroshi Kanda (Kurume University School of Medicine), Koji Kato (The First Nagoya Red Cross Hospital), and Takahiro Uehara (Kameda Medical Center) for providing patient samples. We thank Dr Eiki Tsushima, Ko Kudo (Hirosaki University Graduate School of Medicine), and Ms Hitomi Iwabuchi for statistical analysis, helpful discussions, and technical assistance, respectively.

This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and Health and Labor Sciences Research Grants (research on intractable diseases) the Ministry of Health, Labor, and Welfare of Japan.

Authorship

Contribution: R.K. and T. Toki designed, organized, and performed research, analyzed data, and wrote the paper; K.T. designed research and collected and analyzed clinical data; G.X. and R.W. performed mutation screening; A.S., H.K., K. Kawakami, M.E., D.H., K. Kogawa, S.A., Y.I., S.I., T. Taga, Y.K., and Y.H. provided clinical samples and data; A.H. and S.K. performed mutation screening and provided clinical samples and data; and E.I. designed and organized research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Etsuro Ito, Department of Pediatrics, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori, 036-8563, Japan; e-mail: eturou@cc.hirosaki-u.ac.jp.

References

1. Zipursky A, Poon A, Doyle J. Leukemia in Down syndrome: a review. *Pediatr Hematol Oncol.* 1992;9(2):139-149.
2. Hasle H, Niemeyer CM, Chessells JM, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. *Leukemia.* 2003;17(2):277-282.
3. Hitzler JK. Acute megakaryoblastic leukemia in Down syndrome. *Pediatr Blood Cancer.* 2007; 49(7):1066-1069.
4. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. *Blood.* 2009; 113(12):2619-2628.
5. Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. *Blood.* 2006;107(12):4606-4613.
6. Klusmann JH, Creutzig U, Zimmermann M, et al. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. *Blood.* 2008;111(6):2991-2998.
7. Muramatsu H, Kato K, Watanabe N, et al. Risk factors for early death in neonates with Down syndrome and transient leukaemia. *Br J Haematol.* 2008;142(4):610-615.
8. Wechsler J, Greene M, McDevitt MA, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat Genet.* 2002;32(1):148-152.
9. Greene ME, Mundschau G, Wechsler J, et al. Mutations in GATA1 in both transient myeloproliferative disorder and acute megakaryoblastic leukemia of Down syndrome. *Blood Cells Mol Dis.* 2003;31(3):351-356.
10. Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. *Blood.* 2003;101(11):4301-4304.
11. Mundschau G, Gurbuxani S, Gamis AS, Greene ME, Arceci RJ, Crispino JD. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. *Blood.* 2003;101(11):4298-4300.
12. Rainis L, Bercovich D, Strehl S, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. *Blood.* 2003;102(3):981-986.
13. Xu G, Nagano M, Kanezaki R, et al. Frequent mutations in the GATA-1 gene in the transient myeloproliferative disorder of Down syndrome. *Blood.* 2003;102(8):2960-2968.
14. Groet J, McElwaine S, Spinelli M, et al. Acquired mutations in GATA1 in neonates with Down's syndrome with transient myeloid disorder. *Lancet.* 2003;361(9369):1617-1620.
15. Weiss MJ, Orkin SH. Transcription factor GATA-1 permits survival and maturation of erythroid precursors by preventing apoptosis. *Proc Natl Acad Sci U S A.* 1995;92(21):9623-9627.
16. Morceau F, Schnekenburger M, Dicato M, Diederich M. GATA-1: friends, brothers, and coworkers. *Ann N Y Acad Sci.* 2004;1030:537-554.
17. Ferreira R, Ohneda K, Yamamoto M, Philipsen S. GATA1 function, a paradigm for transcription factors in hematopoiesis. *Mol Cell Biol.* 2005;25(4): 1215-1227.
18. Gutierrez L, Tsukamoto S, Suzuki M, et al. Ablation of Gata1 in adult mice results in aplastic crisis, revealing its essential role in steady-state and stress erythropoiesis. *Blood.* 2008;111(8):4375-4385.
19. Shivdasani RA, Fujiwara Y, McDevitt MA, Orkin SH. A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. *EMBO J.* 1997;16(13):3965-3973.
20. Vyas P, Ault K, Jackson CW, Orkin SH, Shivdasani RA. Consequences of GATA-1 deficiency in megakaryocytes and platelets. *Blood.* 1999;93(9):2867-2875.
21. Shimizu R, Kuroha T, Ohneda O, et al. Leukemogenesis caused by incapacitated GATA-1 function. *Mol Cell Biol.* 2004;24(24):10814-10825.
22. Shimizu R, Engel JD, Yamamoto M. GATA1-related leukaemias. *Nat Rev Cancer.* 2008;8(4): 279-287.
23. Xu G, Kanezaki R, Toki T, et al. Physical association of the patient-specific GATA1 mutants with RUNX1 in acute megakaryoblastic leukemia accompanying Down syndrome. *Leukemia.* 2006; 20(6):1002-1008.
24. Toki T, Kanezaki R, Adachi S et al. The key role of stem cell factor/KIT signaling in the proliferation of blast cells from Down syndrome-related leukemia. *Leukemia.* 2009;23(1):95-103.
25. Shimada A, Xu G, Toki T et al. Fetal origin of the *GATA-1* mutation in identical twins with transient myeloproliferative disorder and acute megakaryoblastic leukemia accompanying Down's syndrome. *Blood.* 2004;103(1):366.
26. Calligaris R, Bottardi S, Cogoi S, Apezteguia I, Santoro C. Alternative translation initiation site usage results in two functionally distinct forms of the GATA-1 transcription factor. *Proc Natl Acad Sci U S A.* 1995;92(25):11598-11602.
27. Pozzoli U, Sironi M. Silencers regulate both constitutive and alternative splicing events in mammals. *Cell Mol Life Sci.* 2005;62(14):1579-1604.
28. Wang Z, Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. *RNA.* 2008;14(5):802-813.
29. Neu-Yilik G, Kulozik AE. NMD: multitasking between mRNA surveillance and modulation of gene expression. *Adv Genet.* 2008;62:185-243.
30. Shyu AB, Wilkinson MF, van Hoof A. Messenger RNA regulation: to translate or to degrade. *EMBO J.* 2008;27(3):471-481.
31. Deguchi K, Gilliland DG. Cooperativity between mutations in tyrosine kinases and in hematopoietic transcription factors in AML. *Leukemia.* 2002; 16(4):740-744.
32. Kuhl C, Atzberger A, Iborra F, Nieswandt B, Porcher C, Vyas P. GATA1-mediated megakaryocyte differentiation and growth control can be uncoupled and mapped to different domains in GATA1. *Mol Cell Biol.* 2005;25(19):8592-8606.
33. Muntean AG, Crispino JD. Differential requirements for the activation domain and FOG-interaction surface of GATA-1 in megakaryocyte gene expression and development. *Blood.* 2005; 106(4):1223-1231.
34. Taub JW, Matherly LH, Stout ML, Buck SA, Gurney JG, Ravindranath Y. Enhanced metabolism of 1-beta-D-arabinofuranosylcytosine in Down syndrome cells: a contributing factor to the superior event free survival of Down syndrome children with acute myeloid leukemia. *Blood.* 1996;87(8):3395-3403.
35. Taub JW, Huang X, Matherly LH, et al. Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. *Blood.* 1999;94(4): 1393-1400.
36. Frost BM, Gustafsson G, Larsson R, Nygren P, Lonnerholm G. Cellular cytotoxic drug sensitivity in children with acute leukemia and Down's syndrome: an explanation to differences in clinical outcome? *Leukemia.* 2000;14(5):943-944.
37. Zwaan CM, Kaspers GJ, Pieters R, et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. *Blood.* 2002;99(1):245-251.
38. Ge Y, Stout ML, Tatman DA, et al. GATA1, cytidine deaminase, and the high cure rate of Down syndrome children with acute megakaryocytic leukemia. *J Natl Cancer Inst.* 2005;97(3):226-231.
39. Taub JW, Ge Y. Down syndrome, drug metabolism and chromosome 21. *Pediatr Blood Cancer.* 2005;44(1):33-39.

―新生児救急疾患―

ダウン症候群に発症した transient abnormal myelopoiesis（TAM）への対応

林　泰秀

はじめに

ダウン症候群（DS）の新生期には一過性骨髄増殖症（transient abnormal myelopoiesis：TAM）[1]または transient myeloproliferative disorder（TMD）[2]が約10％の頻度でみられ，生後4年以内にその約20％が急性巨核球性白血病（acute megakaryoblastic leukemia：AMKL）に進展する[2~4]。TAMは新保，長尾ら[1]により一つの疾患概念として提唱され，自然寛解する例が多いとされてきたが，近年致死的な肝線維症などを合併することがあり，予後良好な疾患ではないことがクローズアップされ[5~8]，一過性白血病（transient leukemia）と呼ぶべきであるとの報告もある[9]。最近の Pediatric Oncology Group（POG）や I-BFM によって行われた前方視的研究や我が国の後方視的研究でも約20％の早期死亡があり，治療成績が著しく改善された AMKL より予後不良である[10,11]。

TAM 発症の分子機構

DS の AMKL では赤血球系と巨核球系の転写因子 *GATA1* の遺伝子変異が，白血病の多段階発症過程の早期に起こっていることが明らかされた[12]。すなわち，*GATA1* 遺伝子の後天的な変異が，AMKL だけでなく TAM でもほとんどの症例で検出され，この遺伝子変異の結果，TAM の細胞では約50 kD の完全長の GATA1 蛋白が発現せず，N 末端転写活性化ドメインを欠く約40 kD の変異 GATA1 蛋白（GATA1s）のみが発現していた[3,4,13]。さらに，一卵性双生児の TAM 症例の解析から胎児期に *GATA1* 遺伝子の変異が生じていることが明らかにされた[14]。しかし，*GATA1* 変異による TAM 発症の仕組みは未だ解明されていない[3,4]。

DS における21トリソミーの構成は TAM を合併する DS と合併しない DS では異なり，通常第2減数分裂時の染色体不分離による片親性ダイソミー（uniparental disomy）の DS における頻度は30％以下と考えられているが，TAM を合併した症例ではそのほとんどが片親性ダイソミーを認めると報告されている[15]。*GATA1* 変異のほかに，エピジェネティックな転写制御機構などが TAM 発症に重要な役割を果たしている可能性が示唆されている[3,4]。

一般的な TAM の臨床像と診断

DS の児に合併する TAM の頻度は約10％と考えられているが，多数の DS 例での疫学的研究の報告はないので実際の頻度は明らかではない。TAM の芽球は AMKL の芽球と類似しており，区別することができない。

TAM の診断については，DS の児で肝脾腫，白血球増多，血小板減少などがあれば末梢血の血液像から芽球の有無を確認する。骨髄中よりも末梢血中のほうが芽球の割合が多いため，骨髄穿刺は診断に必須ではない。芽球の表面マーカーの検索により TAM の芽球は AMKL と同様に CD7，33，34，41，42b，61，glycophorinA が高率に陽性であり，電子顕微鏡による血小板ペルオキシダーゼ（PPO）反応が陽性である。芽球は形態学的に細胞質が好塩基性で辺縁に偽足様の突起（bleb）を有し，ペルオキシダーゼ染色は陰性である。モザイ

はやし やすひで　群馬県立小児医療センター
〒377-8577　群馬県渋川市北橘町下箱田779
E-mail address：hayashiy-tky@umin.ac.jp

ク型DSにもTAMは合併することがあるため[16]，患児の表現型が正常であってもTAMの可能性があり，体細胞の染色体検査が必要である。ほとんどのTAMは21トリソミー以外の染色体異常を有さないが[2]，稀に付加的染色体異常を伴う症例の報告があり[17]，白血病との異同が問題となる。先天性白血病と鑑別するためにも，TAMの芽球の染色体検査は必須である[2]。致死的経過をたどるものは肝機能異常，播種性血管内凝固症候群(DIC)，全身性浮腫を合併することが多く[18,19]，DICや閉塞性黄疸，肝トランスアミナーゼの上昇を伴う症例は治療のタイミングを逃さないよう慎重な経過観察が必要である。

TAMにおける高サイトカイン血症

TAMの病態には高サイトカイン血症が関与[20]し，肝線維症などの病態を反映しているものと考えられる。特に致死例では高サイトカイン血症の制御が臨床上重要で，今後TAMにおける高サイトカイン血症を起こす病態の解明が必要と考えられる。

重症型のTAM症例

さまざまなタイプのTAMが報告されている。致死的経過をたどる劇症タイプの一つに肝機能異常を伴うものがある。これらは循環不全やDICを伴っており，剖検では多臓器へのTAM芽球の浸潤と高度の肝線維症が特徴的である。こういった症例は肝機能異常を伴い，肝生検では肝の線維化と中心静脈の閉塞，肝実質細胞の脱落，芽球の浸潤が報告されているが，芽球の浸潤を認めない症例もある。肝線維化のマーカーであるプロコラーゲンⅢペプチド(P-Ⅲ-P)やⅣ型コラーゲン，ヒアルロン酸が高値を示し，芽球から産生されるtransforming growth factor-β(TGF-β)，platelet-derived growth factor(PDGF)などのサイトカインが肝の線維化の原因と推察されている[18,19,21]。また，予後不良の劇症タイプとして胎児水腫による胎児死亡例やDIC，腎不全，全身性の浮腫を伴う症例があり，その機序は明らかではない。実際に肝不全で死亡する症例以外にも芽球が消失せず，増加傾向となり死亡する症例がある。これらは出生時に*GATA1*以外の遺伝子異常によりすでに白血病として発症している可能性が示唆される。今後*GATA1*以外のsecond hit(遺伝子変異)が何であるのか解明が待たれる。

劇症タイプとは対照的に症状がなく，先天性心疾患や消化器疾患のために採血し，末梢血に芽球がみられるため診断される症例もある。AMKLの中にはTAMの既往歴が明らかでない症例があり，これらは臨床症状がはっきりしていなかったため診断に至らず，芽球も自然消失した可能性がある。TAMの既往歴が明らかでないAMKLの症例でもガスリー斑の血痕から抽出したDNAより，AMKLの時と同じ*GATA1*遺伝子変異が確認されている。

我が国のTAMの多数症例の検討

塚本ら[22]の全国のアンケート調査では，250施設中185施設(74%)から回答があり，約1/3の施設で重症TAMの経験があり，そのうち約1/4の施設では化学療法を試みられていた。重症TAM 38例の頻度はNICU入院1,000例に約1例で，症状として初診時，胎児期から肝脾腫を認めた。死亡例は38例中25例(66%)で，低出生体重児が多く，初診時に特にLDHが高く，経過中に肝障害，閉塞性黄疸が重篤化していた。化学療法施行例では生存率が53%(15例中8例)で，非施行例の21%(23例中5例)に比べ生存率がやや高い傾向があったが依然として低値であった[22]。予後不良群を識別する指標として直接ビリンビンが8 mg/dL以上，LDH 4,000 IU/L以上，GOT 250 U/L以上と報告している。

Muramatsuら[23]は1992～2006年に14施設の70例(男児46例，女児24例)についての後方視的検討を行い，16例(22.9%)が早期死亡し，死因は肝不全10例，腎不全3例，心不全2例，PPHN 1例であった。12例(17.1%)で後にAMKLを発症し，うち1例が再発死亡し，最終的には全生存率は75.7%であった。多変量解析では，短い在胎週数と直接ビリルビン値が最も生命予後と関係する結

表　白血球数と在胎週数による生存率(菊地, 2009)[24]

	WBC 10 万未満	WBC 10 万以上
満期産	92.1%(35/38)	50%(3/6)
早期産	85.7%(12/14)	26.7%(4/15)

満期産：在胎 37 週以上，早期産：在胎 37 週未満，WBC：白血球数

果であった。

菊地[24]は，日本小児血液学会会員施設に対するアンケート調査を行い，2003～2005 年に我が国で発症した TAM 73 例をまとめて報告した。44 例に先天性心疾患がみられ，MDS が 2 例に，AMKL が 11 例に発症した。19 例が死亡し，死因は肝不全，腎不全の臓器不全が多かった。予後不良因子は(**表**)，在胎週数 37 週未満，初診時白血球数 10 万/μL 以上，直接ビリルビンの最高値 5 mg/dL 以上，全身浮腫の存在であった。

POG 9481 スタディ

米国の POG は 1996～1999 年に前方視的に 48 例の TAM の解析を行い[10]，生後 9 カ月未満の早期死亡を 47 例中 8 例(17%)に認め，早期死亡した 8 例全例で肝機能異常と DIC を認めた。腹水，心嚢水，胸水の合併も多く，2 例で末期に少量キロサイドを投与したが，無効であったと報告している。早期死亡と関連のある因子としては診断時の白血球数増多($p<0.001$)，肝トランスアミナーゼの高値(ALT $p=0.001$，AST $p=0.005$)，末梢血中からの芽球が消失しない($p<0.001$)などをあげている。また，21 トリソミー以外の染色体異常をもつ TAM はその後 AMKL を発症するリスクが高いと報告している。

BFM スタディ

ヨーロッパの BFM グループは，146 例の TAM (transient leukemia と命名)の前方視的研究を行い，5 年全生存率が 85±3%，無イベント生存率が 63±4%と報告した[11]。白血球増多，血小板減少または肝不全の 28 例にシタラビン(0.5～1.5 mg/kg)を投与し，投与しない群に比べて有意に 5 年無イベント生存率がよかった(52±12%対 28±11%，$p=0.02$)と報告した。29 例(23%)が AMKL になり，TAM の既往のある患者は有意に予後良好であったと報告した。

治　療

古典的タイプの TAM で全身状態がよい場合は経過観察を行う。予後不良例は 10～20%にみられ，支持療法とともに早期治療介入が必要である。治療としては，交換輸血，ステロイドにより一時的に芽球の数が減少し，症状が緩和されることもあるが，無効であることが多い。全身性浮腫，肝機能障害が増悪傾向である場合，抗癌剤の投与を考慮する。少量シタラビン(Ara-C)10 mg/m^{2}を 1 日 2 回投与することにより肝不全が軽快，治癒する症例も報告されている[25]。そのほかシタラビン投与の報告が散見する[6,7,26]。今後前方視的研究を行い，治療の時期や治療法を確立する必要がある。また，BFM ではシタラビン 0.5～1.5 mg/kg 7 日間投与法を推奨している。我が国でも重症例の定義と標準的治療を確立する必要がある。

登録事業(図)と今後の方向性

これまで TAM は一般小児科，未熟児新生児科，遺伝科，循環器科，血液腫瘍科とさまざまな科で診察，治療されてきた。そのため全体像の把握が困難で，一部に予後不良例があることが知られていたが，全体としては予後良好な疾患とされてきた。近年の POG の報告や我が国の研究で約 20%の予後不良例が存在することが明らかになり，全数登録をして前方視的研究をする必要性が明らかになってきた。幸い 2007 年から日本小児血液学会で疾患登録事業が開始されて登録が可能になった。日本小児血液学会員の ID 番号とパスワードが必要なので，TAM の診断がついた際には近くにいる日本小児血液学会会員に連絡をして登録していただくことが望まれる(図)。平成 21 年度から厚生労働省の「ダウン症候群でみられる一過性骨髄異常増殖症の重症度分類のための診断基準と治