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Masashi Kiguchi has studied various optical measurements: nonlinear spectroscopy, time-resolved spectroscopy, near-field spectroscopy, and near-infrared spectroscopy, and his background is physics and laser spectroscopy. He has studied the problems related to the principle of near-infrared spectroscopy (NIRS) measurement and has been taking the lead in the development of new techniques for observing brain activities to open new research fields and in basic studies for putting them to practical use.

Queries

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Mutations of the *GATA2* and *CEBPA* genes in paediatric acute myeloid leukaemia

Hereditary *GATA2* mutations show predisposition to acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) (Hahn *et al*, 2011). These mutations have also been reported in chronic myeloid leukaemia (Zhang *et al*, 2008) and monocytopenia and mycobacterial infection (MonoMAC) syndrome (Hsu *et al*, 2011). More recently, *GATA2* mutations have been identified in *de novo* AML, especially in adult patients with biallelic *CEBPA* mutations (Greif *et al*, 2012; Green *et al*, 2013). GATA2 and CEBPA are transcription factors that are crucial for haematopoietic development. These findings prompted us to identify possible *GATA2* and *CEBPA* mutations in patients with various paediatric leukaemias.

Direct Sequencing of *GATA2* was performed in 157 *de novo* AML patients, including 13 patients with acute promyelocytic leukaemia (APL; French–American–British type-M3) and 10 with Down syndrome (DS; Table S1), 22 secondary AML patients, 40 juvenile myelomonocytic leukaemia (JMML) patients, 50 acute lymphoblastic leukaemia (ALL) patients, 70 cell lines (25 B-cell precursor-ALL, 15 T-cell-ALL, 22 AML, and 8 neuroblastomas), and 60 healthy subjects. *GATA2* mutation analysis was performed by direct sequencing for all coding exons (exons 2–6) using an ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Branchburg, NJ, USA) (Table S2). For AML patients, *CEBPA* and *NPM1* mutations were also examined. Mutational analyses of *FLT3*, *KIT*, *WT1* and *RAS* genes in our AML patients was performed as described previously (Shimada *et al*, 2006). Informed consent was obtained from the patients or the patients' parents according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review boards of Gunma Children's Medical Centre approved this project.

GATA2 mutations were found in eight out of 157 AML patients (5·1%), including three APL patients (Fig 1A,B), but were absent in 18 patients with acute megakaryocytic leukaemia (FAB-M7; Table S3). Furthermore, there were no *GATA2* mutations in patients with other leukaemias, in the cell lines, or in the 60 healthy subjects, suggesting that *GATA2* mutations were indeed associated with leukaemogenesis in a subset of patients with *de novo* AML.

Germline *GATA2* mutations were also examined in five AML patients whose complete remission (CR) samples were available, and a germline mutation was identified in one patient. Furthermore, we performed *GATA2* mutation analyses of the patient's parents and two siblings, and identified the same *GATA2* mutations in her father (II-4) and brother (III-1) but not in her mother (II-5) or sister (III-2) (Fig 1C). Her father and brother lacked abnormalities in their full blood cell counts, lymphocyte subsets, or episodes of opportunistic infections. The proband experienced severe mycotic pneumonia during induction chemotherapy. Remarkably, she has been in CR for more than 11 years, despite discontinuation of chemotherapy. Three patients, for whom CR samples were not available, had no history of MonoMAC syndrome.

In addition, 16 *CEBPA* mutations (10·2%) and three *NPM1* mutations (1·9%) were found in 157 paediatric AML patients. Thirteen (81·3%) of 16 patients with *CEBPA* mutations had been in CR for more than 4 years, suggesting that *CEBPA* mutations may be associated with favourable outcomes. Although most *GATA2* mutations were found in patients with biallelic *CEBPA* mutations in adult AML (Greif *et al*, 2012; Green *et al*, 2013), only two of eight *GATA2* mutation-positive patients had monoallelic *CEBPA* mutations in this study (Table I).

We compared the clinical and molecular features between patients with and without *GATA2* mutations. However, there were no significant differences in terms of age, initial white blood cell count, gender, and cytogenetics (Table S3). Of the eight patients with *GATA2* mutations, one had a *WT1* mutation, one had a *KIT* mutation, and two patients had *RAS* mutations (Table I). *FLT3*-internal tandem duplication, *MLL*-partial tandem duplication, and *NPM1* mutations were not found in any patients with *GATA2* mutations (Table S3). All of the *GATA2* mutations were found in the intermediate risk subgroup or APL patients with t(15;17), whereas none were found in those with core-binding factor AML [i.e. t(8;21) and inv(16)]. *GATA2* mutations were found in two patients with 11q23 translocations, including t(11;19) and t(7;11), and three patients with complex chromosomal abnormalities, whereas most *GATA2* mutations were found in cytogenetically normal AML patients in previous reports (Table I) (Greif *et al*, 2012; Luesink *et al*, 2012).

GATA2 mutations were previously reported in patients with M1, M2, and M4 subtypes of AML (Greif *et al*, 2012; Luesink *et al*, 2012), which is in accordance with our results. *GATA2* mutations have not been previously reported in APL, but our study found these mutations in three APL patients. Of note, promyelocytic leukaemia protein has been shown to interact with GATA2 and potentiate its transactivation capacity (Tsuzuki *et al*, 2000).

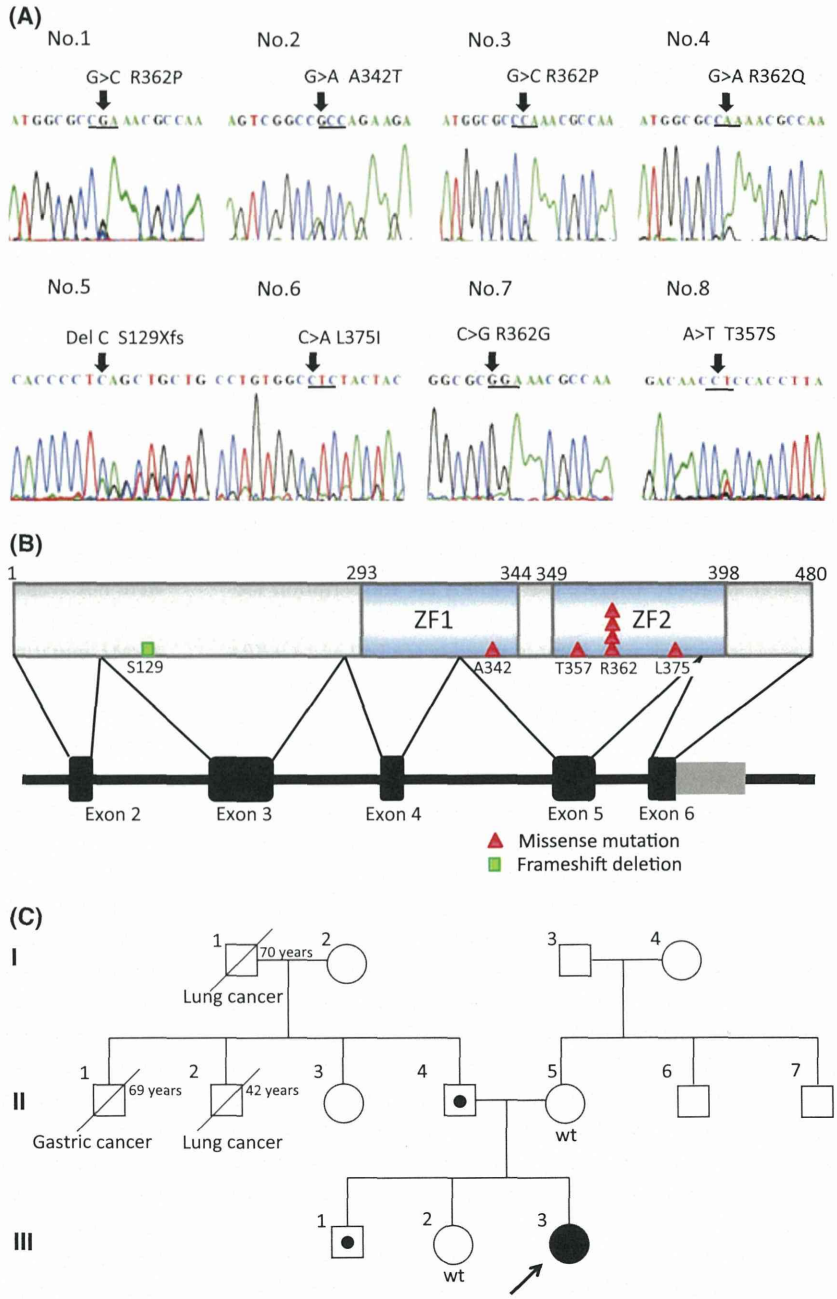

Fig 1. Identification of *GATA2* mutations by direct sequencing. (A) Eight *GATA2* mutations were identified in 157 Japanese paediatric *de novo* acute myeloid leukaemia (AML) patients (5·1%). Major missense mutations were R362 (R362P, R362Q, and R362G). Small vertical arrows indicate the mutated nucleotides. (B) Of the eight mutations, six mutations were identified in the ZF2 domain, one mutation was identified in the ZF1, and a mutation was identified on the outside of the ZF domain. (C) The family pedigree is shown. Squares indicate males and circles indicate females. The proband (III-3) is indicated by an arrow. The proband, her father (II-4), and her brother (III-1) harboured *GATA2* mutations (shown by squares containing dots). Her uncles and grandfather died of lung cancer (I-1 and II-2) and gastric cancer (II-1). wt, wild-type.

The outcomes of our patients with *GATA2* mutations was not poor (3-year overall survival and event free survival: 87·5%), which is in agreement with previous reports on *de novo* AML (Greif *et al*, 2012; Luesink *et al*, 2012): two of eight patients received autologous-stem cell transplantation (Auto-SCT), and one died of gastrointestinal haemorrhage after Auto-SCT. The remaining six patients who did not receive Auto-SCT were still alive (Table I).

In this study, one patient with a germline *GATA2* mutation developed AML. Her paternal grandfather (I-1) and second uncle (II-2) died of lung cancer at the age of 70 and 42 years, respectively, while her first uncle (II-1) died of gastric cancer at 69 years of age (Fig 1C). Increased GATA2 protein expression has been associated with biochemical recurrence and distant metastatic progression in prostate cancer (Böhm *et al*, 2009), as loss of GATA2 reduced the viability of Non-small cell lung cancer cells with RAS-pathway mutations, whereas wild-type cells were unaffected (Kumar *et al*, 2012). These facts indicate that GATA2 upregulation is strongly associated with maintenance of cancer cells. The association between *GATA2* mutations and solid tumours remains to be elucidated.

Our results indicate that *GATA2* mutations are associated with a favourable outcome in paediatric AML. Therefore, less aggressive treatment strategies without SCT may be

Table I. Clinical and molecular characteristics of patients with *GATA2* mutations.

Pt	Sex	Age (years)	FAB	WBC ($\times 10^9$/l)	Chromosome	Risk	Tx	Relapse	Prognosis (months)	*GATA2* mutation	Germline	Additional mutations
1	M	3	M4	23·8	46, XY, t(11;19) (q23; p13·1)	IR	Auto	Yes	16	R362P	N/A	–
2	F	7	M0	3·7	45,XX,add(3)(p13),del(6)(q?), der(8) t(3;8)(p21;q24), −13	IR	Chemo	No	+141	A342T	Yes	*NRAS*
3	F	8	M1	1·8	46, XX	IR	Chemo	No	+56	R362P	No	*KRAS*
4	M	14	M1	440·0	46XY [2/8], 46, XY, del(6) (q15 q21), −7, −9, −10, +3mar[1/8], 46, XY, ?de(3) (p25)[1/8], 47, XY, −5, −8, −10, add(12)(q24·1), −16, −18, +6mar [1/8], 46, XY, −2, −6, −8, +3mer [1/8], 46, XY, −8, +mar [1/8], 46, Y, ?add(X)(p11·2) [1/8]	IR	Auto	No	+51	R362Q	No	*WT1*, *CEBPA*-SM
5	M	11	M3	16·1	46,XX,inv(9)(p11q13),t(15;17)(q22;q11-21)	M3	Chemo	No	+50	S129X	N/A	–
6	M	3	M3	11·6	46,XY,t(15;17)(q22;q11?21)	M3	Chemo	No	+45	L375I	No	*CEBPA*-SM
7	M	10	M3	13·6	47,XY, +8, t(15;17)(q22;q11-21)	M3	Chemo	No	+41	R362G	N/A	*KIT*
8	F	2	M4	12·7	48, XX, +6, +10, t(11?; 7) (q23;q25)	IR	Chemo	No	+38	T357S	No	–

Pt, Patient; FAB, French–American–British classification; WBC, white blood cell count; Tx, Treatment; M, Male; F, Female; IR, Intermediate risk; Auto, Autologous stem cell transplantation; Chemo, Chemotherapy; N/A, not available; +, alive; SM, single mutation.

appropriate for paediatric AML patients with *GATA2* mutations, although most patients with *GATA2* mutations were classified into an intermediate risk group. Furthermore, the association between germline *GATA2* mutations and solid tumours remains to be elucidated.

Acknowledgements

We are grateful to all members of the Japanese Childhood AML Cooperative Study Group. Members of the Japanese Childhood AML Cooperative Study Group who contributed data to the study include Ryoji Hanada, Department of Haematology/Oncology, Saitama Children's Medical Centre; Masahiro Tsuchida, Department of Haematology/Oncology, Ibaraki Children's Medical Centre; Akira Morimoto, Department of Paediatrics, Kyoto Prefectural University of Medicine; Ryoji Kobayashi, Department of Paediatrics, Hokkaido University School of Medicine; Hiromasa Yabe, Department of Paediatrics, Tokai University School of Medicine; Kazuko Hamamoto, Department of Paediatrics, Hiroshima Red Cross Hospital; Shigeru Tsuchiya, Department of Paediatric Oncology, Institute of Development, Aging and Cancer, Tohoku University; Yuichi Akiyama, Department of Paediatrics, National Hospital Organization Kyoto Medical Centre; Hisato Kigasawa, Department of Haematology, Kanagawa Children's Medical Centre; Akira Ohara, Department of First Paediatrics, Toho University School of Medicine; Hideki Nakayama, Department of Paediatrics, Hamanomachi Hospital; Kazuko Kudo, Department of Paediatrics, Nagoya University Graduate School of Medicine; and Masue Imaizumi, Department of Haematology/Oncology, Miyagi Prefectural Children's Hospital. The authors would like to thank Enago (www.enago.jp) for the English language review. This work was supported by a grant for Cancer Research, a grant for Research on Children and Families, and Research on Intractable Diseases, Health and Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan, by Grants-in-Aid for Scientific Research (B, C) and Exploratory Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by the Programme for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NiBio) of Japan, and by a Research grant for Gunma Prefectural Hospitals.

Author contributions

Y.H. designed the study; M.F., S.A., M.K., A.K., M.S., A.T., K.H. and I.T. collected patient samples and clinical data; N.S., K.O., M.-J.P., Y.M. and S.M. performed the laboratory research; N.S., M.-J.P. and Y.H. analysed and interpreted the data; N.S. performed the statistical analysis; N.S. and Y.H. wrote the manuscript; H.A. and Y.H. supervised the work; and all authors critically reviewed the manuscript and gave their final approval.

Conflicts of interest

The authors declare no competing financial interests.

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Keywords: *GATA2*, *CEBPA*, paediatric acute myeloid leukaemia, acute promyelocytic leukaemia, germline mutation

First published online 14 September 2013
doi: 10.1111/bjh.12559

Supporting Information

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

Table S1. Clinical and cytogenetically characteristics of 157 AML patients.

Table S2. PCR primers used for mutation screening.

Table S3. Clinical and molecular characteristics of *GATA2* mutation positive patients.

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SETBP1 mutations in juvenile myelomonocytic leukaemia and myelodysplastic syndrome but not in paediatric acute myeloid leukaemia

Juvenile myelomonocytic leukaemia (JMML) is a rare myeloproliferative disorder that is characterized by excessive myelomonocytic proliferation (Loh, 2011). Gene mutations in the components of the RAS signalling pathways are a hallmark of JMML and are considered to be central to the pathogenesis of JMML. Mutations in *NRAS, KRAS, PTPN11, NF1,* and *CBL* genes are found in approximately 75–85% of patients with JMML and are implicated in the aberrant RAS signalling (Loh, 2011). These mutations are also associated with congenital abnormalities, such as cardio–facio–cutaneous syndrome (*KRAS*), Noonan syndrome (*PTPN11*), neurofibromatosis (*NF1*), and Noonan-like syndrome (*CBL*). However, no other mutations have been identified in the remaining approximately 20% of patients with JMML.

In this regard, massively parallel sequencing technology has recently identified recurrent somatic mutations in *SETBP1* in atypical chronic myeloid leukaemia (aCML) (Piazza *et al*, 2012). Of the 70 patients with aCML that were examined, 17 (24%) were found to carry *SETBP1* mutations. These mutations clustered between codons 858 and 871, all located in the SKI-homologous region of *SETBP1*. Identical nucleotide alterations have been reported in Schinzel–Giedion syndrome (Hoischen *et al*, 2010), a rare congenital disorder that is characterized by severe mental retardation, distinctive facial features, and higher than normal prevalence of tumours, notably neuroepithelial neoplasia (Schinzel & Giedion, 1978). This report prompted us to search for possible *SETBP1* mutations in JMML or other paediatric haematological malignancies.

To assess the clinical significance of *SETBP1* mutations in paediatric leukaemias, we analysed a total of 414 patients with paediatric leukaemia/myelodysplastic syndrome (MDS) that comprised 42 patients with primary JMML, 24 with MDS, 22 with therapy-related leukaemia, 68 with infant acute lymphoblastic leukaemia (ALL), and 258 with *de novo* acute myeloid leukaemia (AML), including 10 patients with acute promyelocytic leukaemia (APL) and 22 with acute megakaryoblastic leukaemia (AMKL). The median age at diagnosis of JMML was 1 year and 10 months (range, 2 months to 8 years and 4 months), with 27 males and 15 females. MDS included 9 patients with refractory anaemia (RA), 14 with RA with an excess of blasts, and 1 with secondary MDS. The genomic region of the *SETBP1* gene, containing codons 858–871 with the mutation hotspots D868 and G870 in the SKI-homologous region, was amplified using polymerase chain reaction (PCR) with the following primer sequences: forward, 5′-ACCAAAACCCAAAAGGGAAT-3′; reverse, 5′-CGGTTTTGCAGGCTTTTC-3′. Purified PCR products were sequenced using an ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Branchburg, NJ). Mutations in *RAS*, *PTPN11*, and *CBL* have been previously reported in JMML (Shiba *et al*, 2010). The present study adhered to the principles of the Helsinki Declaration and was conducted under the regulations outlined by the Ethics Board of Gunma Children's Medical Centre.

SETBP1 mutations were found in 2 of the 42 patients with JMML (4·8%; Gly870Arg in JMML 2, Ser869Arg in JMML 24) and one of the 24 patients with MDS (4·2%; Ile871Thr in MDS 3) but not in the 22 patients with secondary AML, 68 with infant ALL, or 258 with *de novo* paediatric AML, including 10 patients with APL and 22 with AMKL (Fig 1A). The origin of the mutations was not determined due to the lack of appropriate normal tissue samples. In all 3 patients with *SETBP1* mutations, a chromatogram exclusively showed a mutated sequence, indicating that the mutations were heterozygous (Fig 1A). Although one of the 2 JMML patients with an *SETBP1* mutation survived after unrelated cord blood transplantation, the other died following relapse 4 months after undergoing related peripheral blood stem cell transplantation (Table I). In contrast, the MDS patient who had an *SETBP1* mutation was initially diagnosed with neuroblastoma at the age of 6 years. He was subsequently treated with chemotherapy and autologous bone marrow transplantation and achieved complete remission (CR). However, 3 years after the initial diagnosis, blast cells appeared in his peripheral blood and he was diagnosed with secondary MDS. Chromosomal analysis of the bone marrow cells revealed 45, XY, −15, der(7)t(7;15)(p13;q15), add(18)(q21) and add(20)(p13). He received chemotherapy with etoposide and cytarabine; however, he did not achieve CR. He died of haemorrhagic shock 18 months after being diagnosed with secondary MDS.

Mutations in *NRAS, KRAS, PTPN11* and *CBL* genes were found in 21%, 4·8%, 38% and 12% of patients with JMML respectively, in our study (Fig 1B) (Shiba *et al*, 2010). Although almost all of the *NRAS, KRAS, PTPN11* and *CBL* mutations occurred in a mutually exclusive manner, *SETBP1* mutations were found in patients with *PTPN11* or *NRAS* mutations (Table I and Fig 1B). This finding suggests that both gene mutations associated with the *RAS* pathway and *SETBP1* mutations can cooperate in the pathogenesis of JMML.

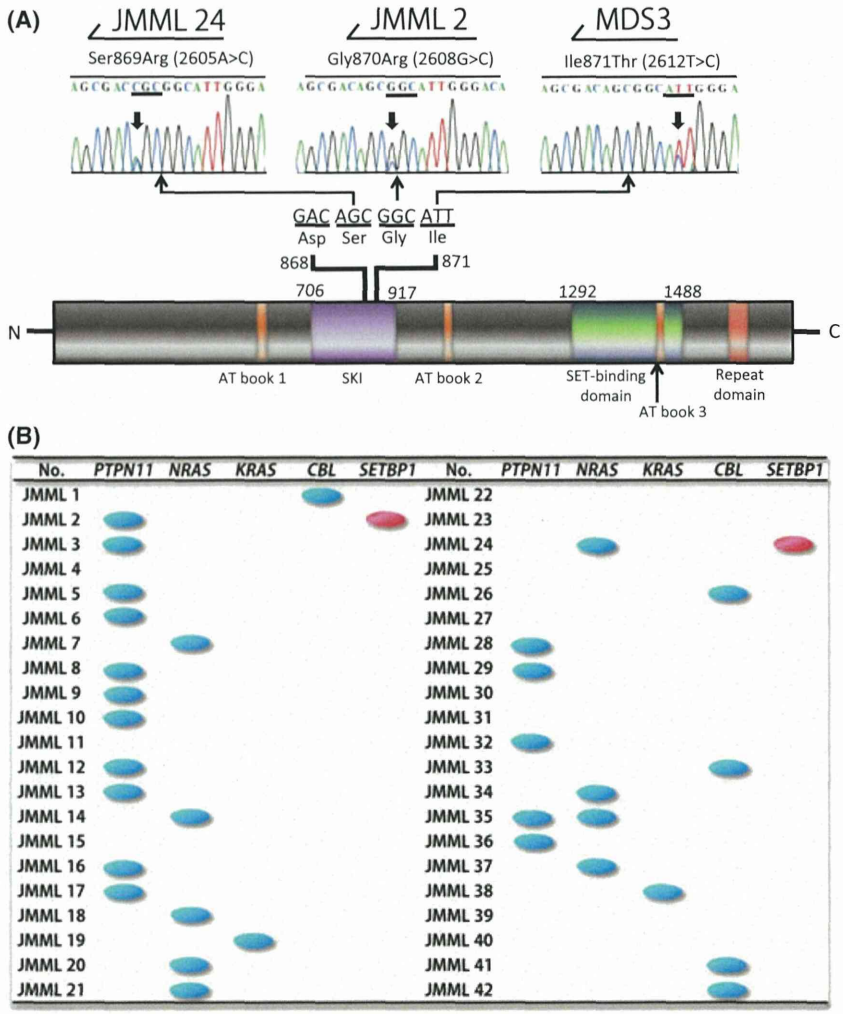

Fig 1. (A) Location and type of *SETBP1* mutations in patients with juvenile myelomonocytic leukaemia (JMML) and myelodysplastic syndrome (MDS). (B) Mutation profile of 42 JMML patients for a panel of 5 genes. Mutations in *PTPN11*, *NRAS*, *KRAS*, *CBL*, and *SETBP1* genes were found in 21%, 4·8%, 38%, 12%, and 4·8% of 42 patients with JMML, respectively.

Table I. Clinical characteristics of the patients with *SETBP1* mutations.

Patient	Sex	Age	WBC ($\times 10^9$/l)	Karyotype	Nucleotide change	Amino acid change	SCT	Relapse	Survival (months)	Other mutations
JMML-2	F	24 months	39·9	46XX	2608A > C	Gly870Arg	U-CBT	–	182+	*PTPN11*
JMML-24	M	26 months	24·5	46XY, −7	2605G > C	Ser869Arg	allo-PBSCT	+	6*	*NRAS*
MDS-3	M	12 years	6·3	45XY, −15, der(7)t(7;15)(p13;q15), add(18)(q21), add(20)(p13)	2612T > C	Ile871Thr	–	–	18†	–

F, female; M, male; Gly, glycine; Arg, arginine; Ser, serine; Ile, isoleucine; Thr, threonine; SCT, stem cell transplantation; U-CBT, unrelated cord blood transplantation; allo-PBSCT, allogeneic peripheral blood stem cell transplantation; +, alive.
*Died of relapse 6 month after initial diagnosis.
†Died of haemorrhage shock 18 months after diagnosed with secondary MDS.

High levels of *SETBP1* expression have been described in elderly patients with AML (Cristobal *et al*, 2010), and *SETBP1* has been identified in a specific paediatric T-cell ALL as a chromosomal translocation partner of *NUP98* (Panagopoulos *et al*, 2001). SETBP1 has been reported to promote the self-renewal of murine myeloid progenitors via activation of HOXA9 and HOXA10 (Oakley *et al*, 2012). The patients with an *SETBP1* mutation had a worse prognosis and presented higher white blood cell counts at diagnosis and also exhibited higher amounts of SETBP1 and SET protein, lower

protein phosphatase 2A activity, and higher proliferation rates than those expressing the wild-type protein (Piazza *et al*, 2012). Although our cohort is too small to arrive at conclusions regarding the prognosis of patients with *SETBP1* mutations, mutated *SETBP1* indeed plays an essential role in the pathogenic mechanism in haematological malignancies.

In summary, *SETBP1* mutations were found in 4·8% of patients with JMML in this study, similar to the frequency reported previously for patients with chronic myelomonocytic leukaemia [3·7% (3/82) and 6·2% (12/195)] (Piazza *et al*, 2012; Damm *et al*, 2013) and JMML [7·6% (7/92)] (Sakaguchi *et al*, 2013). Our analysis of 414 patients with JMML or other haematological malignancies suggests that mutations of *SETBP1* may have some role in the pathogenesis of JMML and MDS but not in AML or infant ALL, although further evaluations are required.

Acknowledgements

The authors would like to thank Shinji Mochizuki, M.D., Division of Haematology/Oncology, Saitama Children's Medical Centre, Junko Takita, M.D., Department of Paediatrics, Graduate School of Medicine, University of Tokyo, and Keitaro Fukushima, M.D., Department of Paediatrics, Dokkyo Medical University, for providing the JMML samples. We also thank Ms. Yuki Hoshino, for her excellent technical assistance. This work was supported by a grant for Cancer Research, and a grant for Research on Children and Families from the Ministry of Health, Labour, and Welfare of Japan, a Grant-in-Aid for Scientific Research (B, C) and Exploratory Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by a Research grant for Gunma Prefectural Hospitals.

Authors contributions

Y.H. designed the study. K.K., M.Sotomatsu, M.Sako, and E.I. provided critical reagents and samples. N.S., K.O., and M.P. performed the experiments. E.I. and H.A. supervised the work. N.S., K.O., and M.P. analysed the results. N.S. and Y.H. wrote the paper and all the authors critically reviewed and revised it.

Conflict of interest

The authors declare no conflict of interest.

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Keywords: myelodysplastic syndrome, juvenile myelomonocytic leukaemia, SET binding protein1, acute leukaemia

First published online 14 October 2013
doi: 10.1111/bjh.12595

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