は弱い発現が少数見られ、CD34+芽球の増加はない。CD42+巨核球は観察されない。

本例は、爪の変形、歯牙形成不全、白皮症などから、テロメア長の検討でその短縮が見られ、DC と診断された症例である。骨髄病理組織像は、再生不良性貧血よりは MDS を考えるパターンで、dyskeratosis congenita の骨髄病変が考えられた。

2）2010 年 1 月末までの 1 年間に中央診断に送付されてきた検体数は 179 例(後天性再生不良性貧血；69 例、先天性骨髄不全症候群；11 例、骨髄異形性症候群；45 例、その他；54 例)で、病理組織学的に検討が可能であった症例は 151 例(13 例は検体不良）であった。病理組織学的に、47 例は MDS, 40 例は再生不良性貧血と診断され、7 例は組織学的に先天性造血障害症候群のいずれかと考えられた。25 例のさまざまな類型化できない造血障害症例が含まれた。一方、臨床病態やテロメア長の検索、遺伝子検索から、2 例が DC と診断され、身体的特徴がみられず、特発性再生不良性貧血と考えられていた 2 例が、テロメア長の短縮の発見を契機に遺伝子検索をおこないDC と診断された。これら DC と診断された 4 例が病理組織学的に検討できた。いずれの症例も、再生不良性貧血としては非定型的な像で、巨核球が観察され、少数の p53 陽性細胞の出現、弱い異形成が見られるなど、より MDS 的な所見が見られるが、いずれも細胞学的には異形成が弱い特徴が見られた。

D．考察

小児期骨髄不全症候群は診断困難な症例も少なくはない。また、造血障害の場である骨髄の病理組織学的検討は十分には行われていない。WHO の造血器腫瘍分類では、小児期骨髄異形成症候群（RCC）は成人の MDS とは多くの点で異なり、その独立性が暫定的な診断項目として採用されている。この診断には、病理組織学的検討が重要とされている。小児造血不全症候群の集積事業は、本邦では継続的に行われ、昨年からは MDS、再生不良性貧血およびその境界病変群も包括して、セントラルレビューによる細胞学的および病理組織学的検討が行われるようになった。骨髄塗抹標本や骨髄病理標本の中央診断、さらに血球テロメア長の測定や遺伝子診断を含む中央検査システムにより総合的に評価することが重要であるが、病理組織学的特徴から造血不全症候群の層別化ができれば、よりスクリーニングとしての意義が高まると考えられる。実際、DC と診断された症例では、先天性造血不全症候群がより積極的に疑われていた症例が含まれていた。

E．結論

本研究により、小児造血不全症候群における先天性造血障害症候群の病理組織学的意義と、その登録事業、中央診断の重要性が明らかとなった。今後さらなる精密な病理組織学的検討により、病理像と病態との関連が提示できる可能性がある。

F. 研究発表

1. 論文発表

(1) Hiraga J, Tomita A, Sugimoto T, Shimada K, Ito M, Nakamura S, Kiyoi H, Kinoshita T, Naoe T. Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination

chemotherapies: its prevalence and clinical significance. Blood 113(20):4885-93, 2009
(2) Yamamoto S, Nakase H, Chiba T, Yamashita K, Ito M. Clinical challenges and images in GI. Image 4. Transplant-associated thrombotic microangiopathy of the colon accompanied by graft-versus-host disease. Gastroenterology 136(7):2067, 2418, 2009
(3) Nishiwaki S, Terakura S, Ito M, Goto T, Seto A, Watanabe K, Yanagisawa M, Imahashi N, Tsukamoto S, Shimba M, Ozawa Y, Miyamura K. Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease. Blood 114(14):3113-6, 2009
(4) Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, Fujiwara Y, Ito M, Groudine M, Bender MA, Tucker PW, Orkin SH. Developmental and species-divergent globin switching are driven by BCL11A. Nature 460(7259):1093-7, 2009

2. 学会発表

(1) 磯田健志、遠藤明史、満生紀子、小野敏明、青木由貴、富澤大輔、高木正稔、梶原道子、長澤正之、森尾友宏、西尾信博、濱　麻人、在家祐司、真部　淳、伊藤雅文、小島勢二、水谷修紀：Low grade MDS の骨髄所見を示した先天性角化不全症 TINF2 変異の一例。第 51 回日本小児血液学会総会、千葉、2009.11.29
(2) 伊藤雅文：MDS 診断の病理組織学的診断による新たな展開。第 51 回日本小児血液学会、千葉、2009.11.27

G. 知的財産権の出願･登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

先天性角化不全症の効果的診断法の確立と治療ガイドラインの作成に関する研究

治療プロトコールの立案

研究分担者　伊藤悦朗　弘前大学大学院医学研究科小児科

研究要旨：　典型的な先天性角化不全症（Dyskeratosis congenita,DC）は皮膚、爪、口腔粘膜に特徴的な所見がみられる先天性骨髄不全症候群であるが、特発性骨髄不全症候群や肺線維症の一部に身体的特徴はみられないものの、やはり同一の遺伝子変異がみられることから、本症の不全型が潜んでいることが判明した。不全型の多くは、診断に至らず特発性骨髄不全症候群や線維症として治療されているものと思われる。本症には特発性骨髄不全症候群の第1選択薬である免疫抑制療法の効果はみられない。本年度の一次調査により34例のDCが把握された。至適な造血幹細胞移植方法の確立をめざし、移植プロトコールを立案し、治療ガイドラインの作成の準備を進めている。

A．研究目的

DCの至適な造血幹細胞移植方法の確立をめざし、移植プロトコールを立案し、治療ガイドラインの作成を目指す。

B．研究方法

本年度は880施設を対象に一次アンケート調査をおこない、過去10年間のDCの症例数の把握を行った。

（倫理面への配慮）

一次アンケート調査は、症例数のみの調査であるため、倫理審査は必要としない。

C．研究結果

一次アンケート調査の結果、69%の回答が得られた、その結果34例のDCが把握された。今後、病像、治療法、予後を含む二次調査を予定している。

D．考察

典型的なDCは皮膚、爪、口腔粘膜に特徴的な所見がみられる先天性骨髄不全症候群である。今回一次調査により、皮膚科領域からの症例が初めて把握された。わが国では、これまで臨床診断された本症の報告例は10例に満たなかったが、本調査により34例もの症例が把握された。

特発性骨髄不全症候群や肺線維症の一部に身体的特徴はみられないものの、やはり同一の遺伝子変異がみられることから、本症の不全型が潜んでいることが判明した。不全型の多くは、診断に至らず特発性骨髄不全症候群や線維症として治療されているものと思われる。本症には特発性骨髄不全症候群の第一選択薬である免疫抑制療法の効果はみられない。不全型の患者が不必要な治療を受け健康被害を受けないようにするためにも、治療ガイド

ラインの作成が必要であると考えられる。

E．結論

今回の疾患登録調査により、本疾患の本邦における実態がより明らかになってきた。しかしながら、診断法の開発により潜在する症例がさらに見いだされる可能性は高いと考える。今後、病像、治療法、予後を含む2次調査を施行し、治療ガイドラインの作成が望まれる。

F. 研究発表

1. 論文発表

(1) Monzen S, Takahashi K, Toki T, Ito E, Sakurai T, Miyakoshi J, Kashiwakura I. Exposure to a MRI-type high-strength static magnetic field stimulates megakaryocytic/erythroid hematopoiesis in CD34+ cells from human placental and umbilical cord blood. Bioelectromagnetics 30(4):280-5, 2009

(2) Iida A, Inagaki K, Miyazaki A, Yonemori F, Ito E, Igarashi K. Bach1 deficiency ameliorates hepatic injury in a mouse model. Tohoku J Exp Med 217(3):223-9, 2009

(3) Hirono K, Miura M, Kanegane H, Miyamoto M, Yoshimura N, Ichida F, Ito E, Miyawaki T. Hepatocyte growth factor in transient myeloproliferative disorder of Down syndrome. Pediatr Int 51(5):754-5, 2009

2. 学会発表

(1) 今野友貴、丹代　諭、徐　剛、土岐　力、工藤　耕、照井君典、大賀正一、小島勢二、長谷川大一郎、青木由貴、金井理恵、今井　剛、本郷輝明、朴　明子、柳沢　龍、伊藤　悦朗：本邦のDiamond-Blackfan貧血患者におけるリボゾームタンパク遺伝子の変異。第71回日本血液学会総会、京都、2009.10.24

G. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

先天性角化不全症の効果的診断法の確立と治療ガイドラインの作成に関する研究

小児期造血障害疾患登録による先天性角化不全症疫学データベース構築

研究分担者　小原　明（東邦大学医療センター大森病院　輸血部教授）

研究要旨：　稀少な先天性角化不全症(DC)研究の基礎となる疫学データベースの構築を実施した。2007年に実施したDCに関する予備調査では6例の症例が17年間に診療されていることが明らかにされている。これを元にして小児血液学会疾患登録事業を実施し、DCの症例把握に努めた結果、2006から2008年に診断されて登録された222例の造血障害症例からは、新規診断DC症例は見いだせなかった。この結果は対象登録症例が少なかったことに起因するが、診断法の開発により潜在する症例が見いだされる可能性は高いと考える。

A．研究目的

【背景】

先行研究（2007年小児血液学会実施（責任者）日本医大前田美穂教授））により、調査対象期間（1988年から2004年）に1,337例の小児造血障害疾患があり、DC症例は6例(0.45%)であった.さらにこの期間外に8例の症例が登録され、合計14例の臨床像について調査された。その結果診断時の身体所見、血液所見など表現型様々であり、この調査時点で遺伝子診断された症例は8例であった。5例は既に死亡していた。

【目的】

本邦小児の先天性角化不全症症例の疫学データベース構築を目的に、小児血液学会疾患登録事業（全数把握）を一次調査とした疫学観察研究（小児血液再不貧2005研究・MDS2006研究）を実施した。質の高いデータベース構築により、これを基盤としたDCの診断法・治療法開発を目指す。

B．研究方法

本研究班の研究では治療介入を行わない、疫学観察研究として実施する。小児血液学会会員 235 施設を対象にした全例登録（疾患登録事業）は、前年診断症例を対象に Web 登録にて実施され、およそ診断から 1 年経過した段階で二次調査（再不貧 2005 研究・MDS2006 研究）が実施した。構築されるデータベースには小児期発症の造血障害全般を網羅し、DC 症例に限定はしない。

（倫理面への配慮）

研究計画は、疾患登録事業、小児血液再不貧 2005 研究・MDS2006 研究により構成され、いずれも小児血液学会臨床研究審査委員会の科学倫理審査承認を得た。

C．研究結果

現在研究は進行中であり、2006, 2007, 2008年診断症例を対象にすると、疾患登録(一次調査)は、2006年、2007、2008年順に163、171、170施設から登録された。この期間にDCと診断された症例は登録されなかった。同じ期間に特発性再生不良性貧血は53, 54, 45例、先天性造血障害であるFanconi貧血5, 3, 1例、Diamond-Blackfan貧血11, 5, 6例であった。同じ時期のMDS（RA, RCMD, MDS unclassified)症例数は17, 14, 6例。AML 164, 158, 131例、ALL 443, 477,356例であった。

D．考察

小児血液学会疾患登録事業は2006年に開始され、会員施設において診断された全ての血液疾患を対象にした、全数把握疫学研究事業である。2007年に実施した先行研究（1988-2004年症例）はほぼ同じ施設を対象にしており、その17年間1337例の造血障害から6例(0.45%)のDC症例が登録されている事実から外挿すると、今回の調査期間総症例数222例では0-1例ほどの症例登録が予想された。実際は一次登録でDC症例はなく、これは総症例数が少ないことに起因するものと考えた。しかしながら表現型が不全なDC症例が少なからず存在している可能性があり、遺伝子診断の開発により早期の診断、そして適切な治療介入が必要である。

E．結論

今回の疾患登録調査では新規診断DC症例は見いだせなかった。この結果は対象登録症例が少なかったことに起因するが、診断法の開発により潜在する症例が見いだされる可能性は高いと考える。

F．研究発表

1．論文発表

(1) Motoyama O, Uchino Y, Tokuyama M, Iitaka K, Ohara A. A boy with membranous nephropathy after allogeneic bone marrow transplantation. Clin Exp Nephrol 13(5):508-11, 2009

(2) Hasegawa D, Manabe A, Yagasaki H, Ohtsuka Y, Inoue M, Kikuchi A, Ohara A, Tsuchida M, Kojima S, Nakahata T; Japanese Childhood MDS Study Group. Treatment of children with refractory anemia: the Japanese Childhood MDS Study Group trial (MDS99). Pediatr Blood Cancer 53(6):1011-5, 2009

(3) Motoyama O, Takasu J, Kawamura T, Aikawa A, Shigetomi Y, Nara C, Ohara A, Iitaka K, Hasegawa A. A male patient with malignant lymphoma and thyroid papillary carcinoma after pediatric renal transplantation. Clin Exp Nephrol 14(1):94-6, 2010

(4) Watanabe S, Miyake K, Ogawa C, Matsumoto H, Yoshida K, Hirabayashi S, Hasegawa D, Inoue T, Kizu J, Machida R, Ohara A, Hosoya R, Manabe A. The ex vivo production of ammonia predicts L: -asparaginase biological activity in children with acute lymphoblastic leukemia.Int J Hematol 91(2):201-8, 2010

(5) Ohsaka A, Kikuta A, Ohto H, Ohara A, Ishida A, Osada K, Tasaki T, Kamitamari A, Iwai A, Kai S, Maekawa T, Hoshi Y. Guidelines for safety management of granulocyte

transfusion in Japan. Int J Hematol 91(2):201-8, 2010

2. 学会発表

(1) 濱　麻人、吉見礼美、村松秀城、西尾信博、永田俊人、高橋義行、平林真介、真部　淳、伊藤雅文、小原　明、土田昌宏、小島勢二：小児再生不良性貧血の骨髄像-140例のセントラルレビューによる検討。第71回日本血液学会総会、京都、2009.10.24

G. 知的財産権の出願・登録状況

なし

Ⅲ. 研究成果の刊行に関する一覧

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Yoshida N, Yagasaki H, Xu Y, Matsuda K, Yoshimi A, Takahashi Y, Hama A, Nishio N, Muramatsu H, Watanabe N, Matsumoto K, Kato K, Ueyama J, Inada H, Goto H, Yabe M, Kudo K, Mimaya J, Kikuchi A, Manabe A, Koike K, Kojima S.	Correlation of clinical features with the mutational status of GM-CSF signaling pathway-related genes in juvenile myelomonocytic leukemia.	Pediatr Res.	65(3)	334-40	2009
Narui R, Yagasaki H, Takahashi Y, Hama A, Nishio N, Muramatsu H, Shimoyama Y, Kojima S.	Concurrent Langerhans cell histiocytosis and nephroblastoma.	Pediatr Blood Cancer.	52(5)	662-4	2009
Nishio N, Yagasaki H, Takahashi Y, Muramatsu H, Hama A, Yoshida N, Kudo K, Kojima S.	Natural history of transfusion-independent non-severe aplastic anemia in children.	Int J Hematol.	89(4)	409-13	2009
Hama A, Kudo K, Itzel BV, Muramatsu H, Nishio N, Yoshida N, Takahashi Y, Yagasaki H, Ito M, Kojima S.	Plasmacytoid dendritic cell leukemia in children.	J Pediatr Hematol Oncol.	31(5)	339-43	2009
Hasegawa D, Manabe A, Yagasaki H, Ohtsuka Y, Inoue M, Kikuchi A, Ohara A, Tsuchida M, Kojima S, Nakahata T; Japanese Childhood MDS Study Group.	Treatment of children with refractory anemia: the Japanese Childhood MDS Study Group trial (MDS99).	Pediatr Blood Cancer.	53(6)	1011-5	2009
Torii Y, Yagasaki H, Tanaka H, Mizuno S, Nishio N, Muramatsu H, Hama A, Takahashi Y, Kojima S.	Successful treatment with rituximab of refractory idiopathic thrombocytopenic purpura in a patient with Kabuki syndrome.	Int J Hematol.	90(2)	174-6	2009
Kimura H, Miyake K, Yamauchi Y, Nishiyama K, Iwata S, Iwatsuki K, Gotoh K, Kojima S, Ito Y, Nishiyama Y.	Identification of Epstein-Barr Virus (EBV)-Infected Lymphocyte Subtypes by Flow Cytometric In Situ Hybridization in EBV-Associated Lymphoproliferative Diseases.	J Infect Dis.	200 (7)	1078-87	2009
Xu Y, Takahashi Y, Wang Y, Hama A, Nishio N, Muramatsu H, Tanaka M, Yoshida N, Villalobos IB, Yagasaki H, Kojima S.	Downregulation of GATA-2 and Overexpression of Adipogenic Gene-PPARgamma in Mesenchymal Stem Cells from Patients with Aplastic Anaemia.	Exp Hematol.	37 (12)	1393-9	2009
Makishima H, Cazzolli H, Szpurka H, Dunbar A, Tiu R, Huh J, Muramatsu H, O'Keefe C, Hsi E, Paquette RL, Kojima S, List AF, Sekeres MA, McDevitt MA, Maciejewski JP.	Mutations of E3 Ubiquitin Ligase Cbl Family Members Constitute a Novel Common Pathogenic Lesion in Myeloid Malignancies.	J Clin Oncol.	27 (26)	6109-16	2009
Yagasaki H, Kojima S, Yabe H, Kato K, Kigasawa H, Sakamaki H, Tsuchida M, Kato S, Kawase T, Muramatsu H, Morishima Y, Kodera Y.	Tacrolimus/Methotrexate versus cyclosporine/methotrexate as graft-versus-host disease prophylaxis in patients with severe aplastic anemia who received bone marrow transplantation from unrelated donors: results of matched pair analysis.	Biol Blood Marrow Transplant.	15 (12)	1603-8	2009
Muramatsu H, Kojima S, Yoshimi A, Atsuta Y, Kato K, Nagatoshi Y, Inoue M, Koike K, Kawase T, Ito M, Kurosawa H, Tanizawa A, Tono C, Hamamoto K, Hotta N, Watanabe A, Morishima Y, Kawa K, Shimada H.	Outcome of 125 children with CML who received transplants from unrelated donors: the Japan Marrow Donor Program (JMDP).	Biol Blood Marrow Transplant.	16(2)	231-8	2010

Muramatsu H, Makishima H, 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 (10)	1969-75	2010
Villalobos IB, Takahashi Y, Akatsuka Y, Muramatsu H, Nishio N, Hama A, Yagasaki H, Saji H, Kato M, Ogawa S, Kojima S.	Relapse of leukemia with loss of mismatched HLA due to uniparental disomy following haploidentical hematopoietic stem cell transplantation.	Blood		[in press]	2010
Hiraga J, Tomita A, Sugimoto T, Shimada K, Ito M, Nakamura S, Kiyoi H, Kinoshita T, Naoe T.	Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance.	Blood	113 (20)	4885-93	2009
Yamamoto S, Nakase H, Chiba T, Yamashita K, Ito M.	Clinical challenges and images in GI. Image 4. Transplant-associated thrombotic microangiopathy of the colon accompanied by graft-versus-host disease.	Gastroenterology	136 (7)	2067, 2418	2009
Nishiwaki S, Terakura S, Ito M, Goto T, Seto A, Watanabe K, Yanagisawa M, Imahashi N, Tsukamoto S, Shimba M, Ozawa Y, Miyamura K.	Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease.	Blood	114 (14)	3113-6	2009
Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, Fujiwara Y, Ito M, Groudine M, Bender MA, Tucker PW, Orkin SH.	Developmental and species-divergent globin switching are driven by BCL11A.	Nature	460 (7259)	1093-7	2009
Monzen S, Takahashi K, Toki T, Ito E, Sakurai T, Miyakoshi J, Kashiwakura I.	Exposure to a MRI-type high-strength static magnetic field stimulates megakaryocytic/erythroid hematopoiesis in CD34+ cells from human placental and umbilical cord blood.	Bioelectromagnetics	30(4)	280-5	2009
Iida A, Inagaki K, Miyazaki A, Yonemori F, Ito E, Igarashi K.	Bach1 deficiency ameliorates hepatic injury in a mouse model.	Tohoku J Exp Med	217 (3)	223-9	2009
Hirono K, Miura M, Kanegane H, Miyamoto M, Yoshimura N, Ichida F, Ito E, Miyawaki T.	Hepatocyte growth factor in transient myeloproliferative disorder of Down syndrome.	Pediatr Int	51(5)	754-5	2009
Motoyama O, Uchino Y, Tokuyama M, Iitaka K, Ohara A.	A boy with membranous nephropathy after allogeneic bone marrow transplantation.	Clin Exp Nephrol	13(5)	508-11	2009
Hasegawa D, Manabe A, Yagasaki H, Ohtsuka Y, Inoue M, Kikuchi A, Ohara A, Tsuchida M, Kojima S, Nakahata T; Japanese Childhood MDS Study Group.	Treatment of children with refractory anemia: the Japanese Childhood MDS Study Group trial (MDS99).	Pediatr Blood Cancer	53(6)	1011-5	2009
Motoyama O, Takasu J, Kawamura T, Aikawa A, Shigetomi Y, Nara C, Ohara A, Iitaka K, Hasegawa A.	A male patient with malignant lymphoma and thyroid papillary carcinoma after pediatric renal transplantation.	Clin Exp Nephrol	14(1)	94-6	2010
Watanabe S, Miyake K, Ogawa C, Matsumoto H, Yoshida K, Hirabayashi S, Hasegawa D, Inoue T, Kizu J, Machida R, Ohara A, Hosoya R, Manabe A.	The ex vivo production of ammonia predicts L-asparaginase biological activity in children with acute lymphoblastic leukemia.	Int J Hematol	91(2)	201-8	2010
Ohsaka A, Kikuta A, Ohto H, Ohara A, Ishida A, Osada K, Tasaki T, Kamitamari A, Iwai A, Kai S, Maekawa T, Hoshi Y.	Guidelines for safety management of granulocyte transfusion in Japan.	Int J Hematol	91(2)	201-8	2010

Ⅳ. 研究成果の刊行物・別刷り

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Correlation of Clinical Features With the Mutational Status of GM-CSF Signaling Pathway-Related Genes in Juvenile Myelomonocytic Leukemia

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ABSTRACT: Mutations in *RAS*, neurofibromatosis type 1 (*NF1*), and *PTPN11*, constituents of the granulocyte-macrophage colony-stimulating factor signaling pathway, have been recognized in patients with juvenile myelomonocytic leukemia (JMML). We assessed 71 children with JMML for *NRAS*, *KRAS*, and *PTPN11* mutations and evaluated their clinical significance. Of the 71 patients, three had been clinically diagnosed with neurofibromatosis type 1, and *PTPN11* and *NRAS/KRAS* mutations were found in 32 (45%) and 13 (18%) patients, respectively. No simultaneous aberrations were found. Compared with patients with *RAS* mutation or without any aberrations, patients with *PTPN11* mutation were significantly older at diagnosis and had higher fetal Hb levels, both of which have been recognized as poor prognostic factors. As was expected, overall survival was lower for patients with the *PTPN11* mutation than for those without (25 *versus* 64%; $p = 0.0029$). In an analysis of 48 patients who received hematopoietic stem cell transplantation, *PTPN11* mutations were also associated with poor prognosis for survival. Mutation in *PTPN11* was the only unfavorable factor for relapse after hematopoietic stem cell transplantation ($p = 0.001$). All patients who died after relapse had *PTPN11* mutation. These results suggest that JMML with *PTPN11* mutation might be a distinct subgroup with specific clinical characteristics and poor outcome. ***(Pediatr Res* 65: 334–340, 2009)**

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myelodysplastic/myeloproliferative disorder that affects young children. It is characterized by specific hypersensitivity of JMML cells to granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro*, but is thought to be a genetically and phenotypically heterogeneous disease (1–4). JMML seems to have its genesis in dysregulation of GM-CSF signal transduction, and gene mutations interfering with downstream components of the GM-CSF signaling pathway can be identified in approximately 70% of children with this disorder (2,5–11). Constitutional mutations of *NF1* occur in approximately 10% of patients with JMML (2,5,12). *NF1* is known to be the causative gene of neurofibromatosis type 1 (NF1), an autosomal dominant cancer predisposition syndrome. *NF1* codes for neurofibromin, a GTPase activating protein for Ras, and acts as a tumor suppressor (13). Similarly, oncogenic *RAS* mutations at codons 12, 13, and 61 have been identified in approximately 20–25% of patients with JMML (2,6–8). These mutations lead to elevated levels of Ras-GTP, the active form of Ras, resulting in constitutive activation of the signal transduction pathway (14).

Somatic mutations in *PTPN11*, which encodes the protein tyrosine phosphatase SHP-2, a molecule that also relays the signal from the GM-CSF receptor to Ras, have been reported in approximately 35% of patients with JMML (9–11). Germline mutations in *PTPN11* were first observed in Noonan syndrome (NS) (15), and somatic mutations have also been identified in hematological malignancies (9–11,16,17). SHP-2 is a positive regulator in this signal transduction pathway and *PTPN11* mutations cause gain of function in SHP-2, resulting in inappropriate activation of the GM-CSF pathway (10). *PTPN11* mutations have been found without coexisting *NRAS*, *KRAS*, or *NF1* mutations (9,11,16). These alterations are thought to be responsible for GM-CSF hypersensitivity and the clinical features associated with this condition. Given this information, mutational analysis of *PTPN11* and *RAS*, or

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Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; **HbF,** fetal hemoglobin; **HSCT,** hematopoietic stem cell transplantation; **JMML,** juvenile myelomonocytic leukemia; **NF1,** neurofibromatosis type 1; **NS,** Noonan syndrome; **OS,** Overall survival

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either identification of an *NF1* mutation or a clinical diagnosis of NF1, are currently key diagnostic procedures for JMML. However, we have a poor understanding of the relationship between mutational status and the clinical features of JMML.

Most patients with JMML usually experience an aggressive clinical course and die from progressive disease unless treated with hematopoietic stem cell transplantation (HSCT) (1,4,18,19). Recent studies have shown that children with JMML have better outcomes when they undergo HSCT early in the course of the disease (20,21). In contrast, there is a certain proportion of patients who have a stable clinical course for a considerable period of time, and disease that sometimes spontaneously resolves without any treatment (1,22,23). Therefore, information on prognostic factors that can be used to identify patients requiring early HSCT is important in developing a treatment plan.

Although several clinical characteristics have been reported as prognostic factors for JMML, including age at diagnosis, sex, fetal Hb (HbF) level, platelet count, and cytogenetic abnormality (1,19–21,23–26), the relationship between prognosis and particular genetic aberrations is unclear and needs to be clarified. Thus, in the current study, we assessed 71 children with JMML for *NRAS*, *KRAS*, and *PTPN11* mutations and analyzed the association between mutational status and previously recognized prognostic factors for JMML, then evaluated the clinical significance of these mutations to clarify whether genotype-phenotype correlations exist.

MATERIALS AND METHODS

Patients. A total of 71 children with JMML diagnosed between 1987 and 2006 in 30 institutions throughout Japan were studied retrospectively. The diagnosis of JMML was based on the internationally accepted criteria previously published (27). We excluded patients with NS, a JMML-like myeloproliferative disease characterized by spontaneous regression of the disease. The clinical and hematological characteristics of the 71 patients are summarized in Table 1. The median age at diagnosis was 24 mo (range, 1–69 mo).

Table 1. *Patients characteristics*

No. of patients	71
Median age at diagnosis, mo (range)	24 (1–69)
Male/female	43/28
Peripheral blood	
Median Hb at diagnosis, g/dL (range)	9.3 (4.9–13.0)
Percentage of HbF at diagnosis (range)	19.0 (1.0–78.0)
Median WBC count at diagnosis, $\times 10^9$/L (range)	31.8 (7.6–563.0)
Median monocyte count at diagnosis, $\times 10^9$/L (range)	4.2 (1.0–84.5)
Median platelets count at diagnosis, $\times 10^9$/L (range)	42.0 (1.4–320.0)
Hepatomegaly (yes/no)	67/4
Splenomegaly (yes/no)	68/3
Cytogenetic study, no.of patients	
Normal	55
Monosomy 7	9
Other abnormalities	2
+8	1
−Y	1
+X,+13	1
Inv(4)(p14p16)	1
t(3;18)(q25;q21)	1
Del(6)(q?),−20	
No. of patients with clinical evidence of NF1	3
No. of patients received HSCT	48

WBC, white blood cell.

Karyotypic abnormalities were detected in 16 patients, including nine patients with monosomy 7. Three children had clinical evidence of NF1. Treatment was planned in the institute responsible for each child and 48 of 71 patients had been treated with HSCT. The source of grafts was bone marrow from a related donor for 15 patients, bone marrow from an unrelated donor for 20, unrelated cord blood for 11, and related peripheral blood for two. Total body irradiation, TBI, was used in half of the patients and the remainder underwent a non-TBI regimen in which the drug dosage varied widely. Approval for this study was obtained from the Ethics Committee of Nagoya University Graduate School of Medicine.

Screening for mutations of the PTPN11, NRAS, and KRAS genes. Written informed consent was obtained from the parents of each patient, and bone marrow or peripheral blood samples were obtained at initial diagnosis. Mononuclear cells were isolated using Ficoll-Hypaque density gradient centrifugation and they were cryopreserved until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Chatsworth, CA). To screen for *PTPN11* mutations, we amplified genomic DNA corresponding to exons 2, 3, 4, 7, 8, 12, and 13 of *PTPN11* using the PCR with cycling parameters and primers as previously reported (28,29). The PCR products were purified and directly sequenced on an ABI Prism 3100 DNA Analyzer (Applied Biosystems, Foster City, CA) using a BigDye terminator cycle sequencing kit (Applied Biosystems).

NRAS and *KRAS* mutations of codons 12, 13, and 61 were identified as previously described (11,30) and were confirmed by sequencing.

Analysis of correlations between clinical characteristics and mutational status. To assess the correlations between clinical characteristics and mutational status, patients were subdivided into four groups: those with a *PTPN11* mutation, a *RAS* mutation, a clinical diagnosis of NF1, or none of these. However, the number of patients with NF1 was too small to allow subgroup analysis, so these patients were excluded from this analysis. Then, for the three remaining groups, we performed a comparison of clinical and laboratory findings.

Statistical analysis. The date of analysis was January 30, 2008. Survival probabilities were estimated by the Kaplan-Meier method and comparisons between probabilities for different patient groups were performed using the two-sided log-rank test. All results are expressed as 5-y probabilities with a 95% confidence interval (CI). Overall survival (OS) for all patients was defined as the time from diagnosis to death or last follow-up. OS in patients who received HSCT was defined as the time from transplantation to death or last follow-up. Relapse incidence was defined as the probability of experiencing a relapse and death without relapse was considered a competing event. The parameters for univariate analyses of OS and relapse incidence included age at diagnosis, sex, platelet count, percentage of HbF, karyotype, and mutational status. For multivariate analyses, the Cox proportional hazard regression model was used. To evaluate correlations between clinical characteristics and mutational status, differences in continuous variables were analyzed using the Mann-Whitney U test and differences in frequencies were tested using the χ^2 test. When appropriate (because of small sample size), Fisher's exact test was used. p values less than 0.05 were considered statistically significant. These statistical analyses were performed with StatView-J 5.0 software (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Mutation analysis. The results of the *PTPN11* and *NRAS/KRAS* mutational screening for the 71 Japanese children with JMML are listed in Table 2. We found *PTPN11* mutations in 32 of 71 (45%) patients. All mutations were missense changes, 30 of which were in exon 3 and two of which were in exon 13. Thirteen of 71 (18%) patients had *RAS* mutations, 11 of which were in *NRAS* and two of which were in *KRAS*. Ten of 13 patients with *RAS* mutations had been reported in a previous study (22). Three (4%) patients were clinically diagnosed with NF1. No patient with NF1 was found to have mutations in *PTPN11* or *RAS*, and 23 (32%) patients had neither a *PTPN11* mutation nor a *RAS* mutation, nor a clinical diagnosis of NF1.

Comparison of clinical characteristics of patients according to mutational status. We compared the laboratory and clinical parameters for patients with a *PTPN11* mutation, with a *RAS* mutation, and without any aberration (Table 3). In the

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group corresponding to individuals with a *PTPN11* mutation, quite distinct characteristic features were evident, whereas there was no difference in the clinical characteristics displayed by individuals in the groups with a *RAS* mutation and no aberration. Patients with *PTPN11* mutations were significantly older at diagnosis (median: 35 mo) than those with *RAS* mutations (median: 10 mo; $p < 0.0001$) or those without any aberrations (median: 10 mo; $p = 0.0037$), and the presence of the *PTPN11* mutation in infants was rare (only two of 32 patients). In addition, the HbF level was significantly higher in the *PTPN11* mutation group than in the *RAS* mutation group or the group with no aberration (25.6 versus 8.6%; $p = 0.0026$ or versus 9.8%; $p = 0.0014$). The patients with monosomy 7 are known to have normal or only slightly elevated HbF levels (1). When the patients with monosomy 7 were excluded from the analysis, strong correlations of higher HbF level with *PTPN11* mutation compared with *RAS* mutation or no aberration were still observed ($p = 0.0004$ or $p = 0.0014$, respectively). Even after the groups other than the *PTPN11* group were combined (including the patients with NF1), the factors of older age at diagnosis and higher HbF level were still significantly different between the *PTPN11* mutation group and the other group. Karyotypic aberrations other than monosomy 7 occurred only in patients with the *PTPN11* mutation. Patients with a *PTPN11* mutation were more likely to receive HSCT than those with a *RAS* mutation or without any aberrations. As shown in Table 3, no correlation was observed between mutational subgroup and sex ratio, white blood cell count, or platelet count.

Because of the small number of patients in the NF1 group, we excluded these three patients from subgroup analysis. However, consistent with previous findings (1), children with NF1 had been given a diagnosis at an older age except for one patient with a family history (JMML was diagnosed in two girls with NF1 at 7 and 47 mo and in one boy with NF1 at 69 mo) but no other clinical parameters differed from those of the other mutational subgroups.

Prognostic impact of the GM-CSF signaling pathway-related genes. For all 71 children, the OS probability at 5 y was 43% (95% CI: 35–51), and the median follow-up time for all living patients was 59 mo (range, 13–240 mo). Given the quite distinct clinical characteristics of the *PTPN11* mutation group, which associated with recognized poor prognostic factors, we compared the clinical outcomes for patients with or without a *PTPN11* mutation. The survival of patients with a *PTPN11* mutation was significantly inferior to survival of patients without (25 *versus* 64%; $p = 0.0029$) as shown in Figure 1. Of the patients without *PTPN11* mutation, survival

Table 2. *PTPN11, NRAS, and KRAS mutations in 71 children with JMML*

Gene	No. of patients	Nucleotide substitution	Amino acid substitution
PTPN11	2	179G>T	Gly60Val
	4	181G>T	Asp61Tyr
	3	182A>T	Asp61Val
	1	214G>A	Ala72Thr
	5	215C>T	Ala72Val
	11	226G>A	Glu76Lys
	1	226G>C	Glu76Gln
	3	227A>G	Glu76Gly
	2	1508G>C	Gly503Ala
NRAS	3	34G>A	Gly12Ser
	2	34G>T	Gly12Cys
	1	35G>A	Gly12Asp
	3	38G>A	Gly13Asp
	1	181C>A	Gln61Lys
	1	182A>T	Gln61Leu
KRAS	1	35G>A	Gly12Asp
	1	35G>T	Gly12Val

Table 3. *Correlation between mutational status and clinical characteristics in JMML*

	Mutational group				
	PTPN11, $n = 32$ (45%)	*NRAS/KRAS*, $n = 13$ (18%)	p*	No aberrations, $n = 23$ (32%)	p*
Median age at diagnosis, mo	35	10	<0.0001	10	0.0037
Older than 24 mo, no.	24	1	<0.0001	8	0.0067
24 mo or younger, no.	8	12		15	
Gender, male/female					
Male, no.	22	8	NS	12	NS
Female, no.	10	5		11	
Median HbF level, %	25.6	8.6	0.0026	9.8	0.0014
More than 10%, no.	29	5	0.0008	11	0.0023
10% or less, no.	3	8		12	
Median WBC count, $\times 10^9$/L	27.7	29.4	NS	36.0	NS
Median platelets count, $\times 10^9$/L	38.5	55.0	NS	45.0	NS
Less than 40 $\times 10^9$/L, no.	17	5	NS	10	NS
40 $\times 10^9$/L or more, no.	15	8		13	
Cytogenetics					
Abnormal karyotype, no.	11	2	NS	2	NS
Monosomy 7, no.	4	2	NS	2	NS
Other abnormalities, no.	7	0		0	
Normal karyotype, no.	21	11		21	
No. of patients received HSCT	28	6	0.0107	11	0.0023

* These were compared with those of *PTPN11* group.
WBC, white blood cell; NS, not significant.

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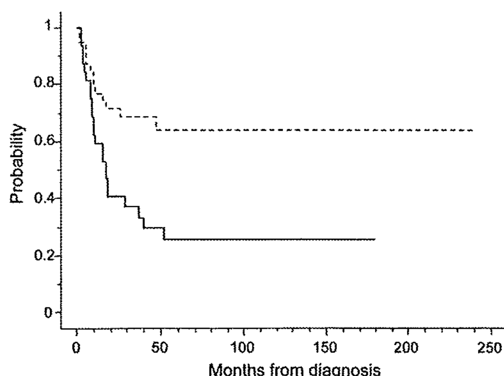

Figure 1. Kaplan-Meier estimate of overall survival in all patients according to *PTPN11* mutation status. *PTPN11*-mutation group (n = 32): *solid lines* *PTPN11* wild-type group (n = 39): *broken line*. The survival of *PTPN11*-mutation group was significantly inferior to survival of *PTPN11* wild-type group: 25% (95% CI: 17–33) *vs* 64% (95% CI: 56–72); p = 0.0029.

Table 4. *Probability of 5-y overall survival (OS) in 71 patients with JMML*

Variable	No. of patients	Probability (%)	95% CI	p
Mutational status				
PTPN11 mutation	32	25	17–33	0.0029
PTPN11 wild type	39	64	56–72	
RAS mutation	13	61	45–78	
No aberration	23	65	55–75	
Age at diagnosis				
Older than 24 mo	35	33	25–42	0.0030
24 mo or younger	36	58	48–67	
Cytogenetics				
Abnormal karyotype	16	22	11–34	0.0125
Normal karyotype	55	53	46–61	
Platelets count				
Less than 40×10^9/L	34	43	34–52	NS
40×10^9/L or more	37	49	40–58	
HbF level				
More than 10%	47	41	33–49	NS
10% or less	24	57	45–69	
Gender				
Male	43	43	35–51	NS
Female	28	49	38–60	

NS, not significant.

values for the *RAS* mutation group and the no aberration group were 61 and 65%, respectively. The three patients with NF1 all received HSCT. One patient died because of transplantation-related toxicity and the others survived without the disease. The prognostic significance of the initial clinical and laboratory parameters, together with mutational status, is shown in Table 4. In the univariate analysis, age greater than 24 mo (p = 0.0030) and presence of cytogenetic abnormality (p = 0.0125) were associated with poor prognosis, as was the presence of *PTPN11* mutation. Of particular interest cytogenetically is the fact that patients with monosomy 7 had a comparable outcome to that of children with a normal karyotype. However, all seven patients with an abnormal karyotype other than monosomy 7 died, and all had a *PTPN11* mutation. Multivariate analysis showed that none of the variables influenced survival (Table 6).

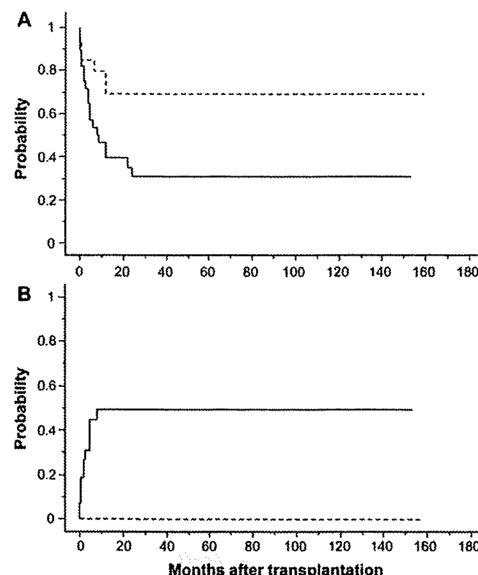

Figure 2. Kaplan-Meier estimate of overall survival and probability of relapse after HSCT in 48 patients according to *PTPN11* mutation status. *PTPN11*-mutation group (n = 28): *solid lines*. *PTPN11* wild-type group (n = 20): *broken lines*. (*A*) Overall survival. *PTPN11*-mutation group had significantly lower survival than *PTPN11* wild-type group: 30% (95% CI: 21–39) *vs* 69% (95% CI: 59–80); p = 0.018. (*B*) Relapse incidence. Whereas no relapse was observed in *PTPN11* wild-type group, the relapse incidence of *PTPN11*-mutation group was 49% (95% CI: 39–60); p = 0.001.

We then analyzed the prognostic value of *PTPN11* mutations in the 48 of 71 patients who received HSCT. *PTPN11* mutations were found in 28 of 48 (58%) patients, and of the 48 patients, 25 patients died after HSCT. As shown in Figure 2*A*, patients with a *PTPN11* mutation had significantly lower survival than patients without also in this cohort. (30 *versus* 69%; p = 0.018). We found that the presence of a *PTPN11* mutation was the most significantly associated factor with OS after HSCT, and followed by age greater than 24 mo and presence of cytogenetic abnormality (Fig. 3*A* and Table 5). No variables significantly associated with inferior survival after HSCT in a multivariate model (Table 6). In addition, we compared the probability of relapse after HSCT between patients with and without *PTPN11* mutations and found that the patients with a *PTPN11* mutation had significantly higher risk for relapse (p = 0.001) (Fig. 2*B*). No other variables including older age and cytogenetic abnormality arose statistically significant difference with the probability of relapse after HSCT (Fig. 3B and Table 5). Twelve patients died of relapse after transplantation and 13 died of transplantation-related toxicity. Notably, all 12 patients who died after relapse had a *PTPN11* mutation.

All four patients with a *PTPN11* mutation who did not receive HSCT died (at 3, 4, 19, and 29 mo after diagnosis), whereas 12 of 19 patients without a *PTPN11* mutation who did not receive HSCT remain alive, with a median follow-up of 80 mo (range, 21–240 mo) from diagnosis.

DISCUSSION

Since the discovery of *PTPN11* mutations in JMML (9), biomedical and molecular research on this disease has pro-

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gressed rapidly, and data on molecular aberrations are now of great importance in the diagnosis of JMML. In the current study, we confirmed that *PTPN11* mutations are the most frequent molecular aberrations (45%) in Japanese children with JMML. If a *PTPN11* mutation is present, it is important to rule out the possibility of NS, especially in infants, because the JMML-like disorder in these patients may spontaneously disappear without therapy, so it is considered distinct from common JMML (31). All mutations detected in our cohort were located in exons 3 and 13 of the *PTPN11* gene, which accords with previous findings that mutations associated with JMML exist only in these two exons (9,16). In contrast, mutations in NS are located in a much broader range of locations, in exons 2, 3, 4, 7, 8, and 13 (29). Kratz *et al.* (32) clearly demonstrated that a different spectrum of *PTPN11* mutations between JMML and JMML-like disorder with NS. According to Kratz *et al.*, all mutations in the present study were those associated with JMML, not a JMML-like disorder. These findings suggest that our study population included only patients with common JMML and that the observed mutations are somatic changes.

The prevalence of *PTPN11* mutations in our cohort was slightly higher than that reported previously (9–11), and the prevalence of *RAS* mutations was comparable with that found in previous studies (2,6–8). In other studies, the proportion of patients with clinically diagnosed NF1 has been found to be 9 and 14% (1,33), but in our cohort, the proportion was smaller, only 3 of 71 (4%) patients. A similar NF1 prevalence (4 of 83 patients; 5%) was observed in an ongoing prospective study conducted by the MDS Committee of the Japanese Society of Pediatric Hematology, so NF1 might be less prevalent in the Japanese population. Another possibility is that NF1 was under diagnosed because of the paucity of signs and symp-

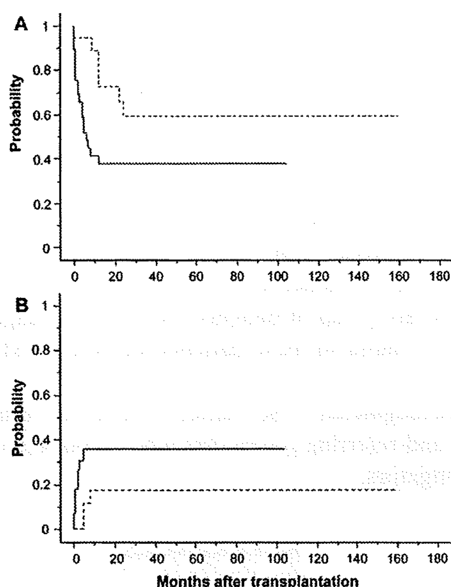

Figure 3. Kaplan-Meier estimate of overall survival and probability of relapse after HSCT in 48 patients according to age at diagnosis. Age >24 mo (n = 29): *solid lines.* Age ≦24 mo (n = 19): *broken lines.* (*A*) Overall survival. Age ≦24 mo: 59% (95% CI: 47–71) *vs* Age >24 mo: 38% (95% CI: 29–47); p = 0.033. (*B*) Relapse incidence. Age ≦24 mo: 19% (95% CI: 9–29) *vs* Age >24 mo: 35% (95% CI: 26–45); p = NS.

Table 6. *Multivariate analysis of survival for all 71 patients and 48 patients who received HSCT*

	Relative risk	95% CI	p
All patients (n = 71)			
PTPN11 mutation	1.854	0.852–5.139	NS
Older than 24 mo	2.011	0.925–4.790	NS
Abnormal karyotype	1.793	0.800–5.401	NS
Patients received HSCT (n = 48)			
PTPN11 mutation	2.226	0.852–5.814	NS
Older than 24 mo	1.707	0.742–3.925	NS
Abnormal karyotype	1.863	0.764–4.542	NS

NS, not significant.

Table 5. *Univariate analysis of 5-y overall survival (OS) and relapse incidence (RI) after HSCT in 48 patients with JMML*

		OS			RI		
Variable	No. of patients	Probability (%)	95% CI	p	Probability (%)	95% CI	p
Mutational status							
PTPN11 mutation	28	30	21–39	0.0181	49	39–60	0.0012
PTPN11 wild type	20	69	59–80		0	0–0	
RAS mutation	6	63	41–83		0	0–0	
No aberration	11	72	59–86		0	0–0	
Age at diagnosis							
Older than 24 mo	29	38	29–47	0.0331	35	26–45	NS
24 mo or younger	19	59	47–71		19	9–29	
Cytogenetics							
Abnormal karyotype	14	17	4–30	0.0474	48	32–64	NS
Normal karyotype	34	56	47–64		23	15–30	
Platelets count							
Less than 40×10^9/L	27	50	41–60	NS	22	13–30	NS
40×10^9/L or more	21	40	29–51		41	29–53	
HbF level							
More than 10%	37	45	37–54	NS	33	24–42	NS
10% or less	11	56	40–72		26	10–42	
Gender							
Male	29	40	30–49	NS	33	23–42	NS
Female	19	56	44–68		24	13–34	

NS, not significant.

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toms in young children. Niemeyer *et al.* (1) found that patients with NF1 were more likely to have higher platelet counts and normal karyotypes. In our patients with NF1, no clinical parameters except for age seemed to differ from the other groups, although the number of patients was too small to draw any conclusions. The outcome of patients with NF1 remains unclear; therefore, further accumulations of prognostic data in this condition are needed.

Our analysis showed a striking correlation between mutational status and clinical and laboratory findings of known prognostic factors. Compared with the *RAS* mutation group and the no aberration group, age and HbF level at diagnosis were significantly higher in the *PTPN11* mutation group. Given that in previous reports older age at diagnosis and elevated HbF level have been repeatedly described as risk factors for survival (1,19–21,23–26), these results suggest that JMML with *PTPN11* mutation is a distinct subgroup and that the outcome for patients with this condition might be poorer. In this study, both *PTPN11* mutation and age were the strongest predictors of the probability of survival in univariate analyses. The poor survival of the *PTPN11* mutation group was also observed when only the patients who had been treated with HSCT were included in the analysis. Because multivariate analysis did not discriminate between age and *PTPN11* mutation, it remains unclear whether mutation in *PTPN11* is an independent predictor for poor survival. However, this could possibly be ascribed to the strong relationship between the *PTPN11* mutation group and older age. Poor outcome in patients with a *PTPN11* mutation may be due to the presence of several unfavorable factors, suggesting that previously recognized prognostic factors might reflect the genetic status.

Presently, HSCT is the only curative treatment for JMML; however, disease recurrence remains the major cause of treatment failure. Notably, mutation in *PTPN11* was the only risk factor for relapse after HSCT in our study. Previously published studies have found that older age, elevated HbF level, and abnormal karyotype are patient-specific risk factors for relapse after HSCT (20,21). The finding that our patients with a *PTPN11* mutation had an association with all these factors and our results on risk factors for relapse also support the idea that the genetic status may be an explanation of previous prognostic factors.

In our study, all 12 patients who relapsed after HSCT had a *PTPN11* mutation, suggesting that patients with *PTPN11* mutation may experience an aggressive clinical course. In addition, patients with *PTPN11* mutation were more likely to receive HSCT, also suggesting that there was a bias attributable to the aggressive clinical course in these patients. Indeed, all patients in the *PTPN11* mutation group who did not receive HSCT died, whereas five of seven patients in the *RAS* mutation group and seven of 12 patients in the no aberration group were alive without HSCT. Moreover, all patients with an abnormal karyotype other than monosomy 7 had a *PTPN11* mutation, and all died, suggesting that clones with a *PTPN11* mutation might be more likely to acquire additional chromosomal alterations.

To the best of our knowledge, this is the first report to investigate the prognostic relevance of the GM-CSF signaling pathway-related genes in patients with JMML and demonstrate the correlation between mutational status and recognized prognostic factors. The finding that mutations in *PTPN11* or *RAS* and a clinical diagnosis of NF1 were mutually exclusive is consistent with the idea that these molecules act in the same pathway. Nonetheless, the clinical features were quite different in these groups. This difference might be caused by distinct gain-of-function effects of each gene on the GM-CSF pathway and unknown additional genetic alterations may cooperate with these mutations. Furthermore, considering the present and previous findings together, the previously recognized prognostic factors might reflect the genetic status of this pathway. Further biologic studies are necessary to clarify what kind of genetic alterations cooperate with altered GM-CSF pathway-related genes during the development of JMML.

In conclusion, JMML with mutation in *PTPN11* seems to be a distinct subgroup with specific clinical characteristics and poor outcome. Consideration should be given to early HSCT therapy in this group of patients and better strategies to lower the risk of relapse in these patients are warranted.

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REFERENCES

1. Niemeyer CM, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, Haas O, Harbott J, Hasle H, Kerndrup G, Locatelli F, Mann G, Stollmann-Gibbels B, van't Veer-Korthof ET, van Wering E, Zimmermann M 1997 Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). Blood 89:3534–3543
2. Lauchle JO, Braun BS, Loh ML, Shannon K 2006 Inherited predispositions and hyperactive Ras in myeloid leukemogenesis. Pediatr Blood Cancer 46:579–585
3. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS 1991 Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 77:925–929
4. Emanuel PD 2004 Juvenile myelomonocytic leukemia. Curr Hematol Rep 3:203–209
5. Side LE, Emanuel PD, Taylor B, Franklin J, Thompson P, Castleberry RP, Shannon KM 1998 Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. Blood 92:267–272
6. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J, Hasle H, Arico M, Biondi A, Niemeyer CM 1999 RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia 13:32–37
7. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, Talpaz M, Cork A, Freireich EJ, Trujillo JM, Lee MS, Stass SA 1990 RAS mutations are rare events in Philadelphia chromosome-negative/bcr gene rearrangement-negative chronic myelogenous leukemia, but are prevalent in chronic myelomonocytic leukemia. Blood 76:1214–1219
8. Kalra R, Paderanga DC, Olson K, Shannon KM 1994 Genetic analysis is consistent with the hypothesis that NF1 limits myeloid cell growth through p21ras. Blood 84:3435–3439
9. Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, Hahlen K, Hasle H, Licht JD, Gelb BD 2003 Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 34:148–150
10. Neel BG, Gu H, Pao L 2003 The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci 28:284–293
11. Tartaglia M, Martinelli S, Cazzaniga G, Cordeddu V, Iavarone I, Spinelli M, Palmi C, Carta C, Pession A, Arico M, Masera G, Basso G, Sorcini M, Gelb BD, Biondi A 2004 Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. Blood 104:307–313
12. Flotho C, Steinemann D, Mullighan CG, Neale G, Mayer K, Kratz CP, Schlegelberger B, Downing JR, Niemeyer CM 2007 Genome-wide single-nucleotide polymorphism analysis in juvenile myelomonocytic leukemia identifies uniparental disomy surrounding the NF1 locus in cases associated with neurofibromatosis but not in cases with mutant RAS or PTPN11. Oncogene 26:5816–5821

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13. Largaespada DA, Brannan CI, Jenkins NA, Copeland NG 1996 Nf1 deficiency causes Ras-mediated granulocyte/macrophage colony stimulating factor hypersensitivity and chronic myeloid leukaemia. Nat Genet 12:137–143
14. Lanfrancone L, Pelicci G, Brizzi MF, Aronica MG, Casciari C, Giuli S, Pegoraro L, Pawson T, Pelicci PG, Arouica MG 1995 Overexpression of Shc proteins potentiates the proliferative response to the granulocyte-macrophage colony-stimulating factor and recruitment of Grb2/SoS and Grb2/p140 complexes to the beta receptor subunit. Oncogene 10:907–917
15. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, Kalidas K, Patton MA, Kucherlapati RS, Gelb BD 2001 Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 29:465–468
16. Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, Cheng JW, Lee CM, Stokoe D, Bonifas JM, Curtiss NP, Gotlib J, Meshinchi S, Le Beau MM, Emanuel PD, Shannon KM 2004 Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 103:2325–2331
17. Loh ML, Reynolds MG, Vattikuti S, Gerbing RB, Alonzo TA, Carlson E, Cheng JW, Lee CM, Lange BJ, Meshinchi S 2004 PTPN11 mutations in pediatric patients with acute myeloid leukemia: results from the Children's Cancer Group. Leukemia 18:1831–1834
18. Luna-Fineman S, Shannon KM, Atwater SK, Davis J, Masterson M, Ortega J, Sanders J, Steinherz P, Weinberg V, Lange BJ 1999 Myelodysplastic and myeloproliferative disorders of childhood: a study of 167 patients. Blood 93:459–466
19. Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B, Reeves BR, Chessells JM 1995 Pediatric myelodysplasia: a study of 68 children and a new prognostic scoring system. Blood 85:1742–1750
20. Manabe A, Okamura J, Yumura-Yagi K, Akiyama Y, Sako M, Uchiyama H, Kojima S, Koike K, Saito T, Nakahata T 2002 Allogeneic hematopoietic stem cell transplantation for 27 children with juvenile myelomonocytic leukemia diagnosed based on the criteria of the International JMML Working Group. Leukemia 16:645–649
21. Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, Pession A, Kabisch H, Uderzo C, Bonfim CS, Bader P, Dilloo D, Stary J, Fischer A, Revesz T, Fuhrer M, Hasle H, Trebo M, van den Heuvel-Eibrink MM, Fenu S, Strahm B, Giorgiani G, Bonora MR, Duffner U, Niemeyer CM 2005 Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 105:410–419
22. Matsuda K, Shimada A, Yoshida N, Ogawa A, Watanabe A, Yajima S, Iizuka S, Koike K, Yanai F, Kawasaki K, Yanagimachi M, Kikuchi A, Ohtsuka Y, Hidaka E, Yamauchi K, Tanaka M, Yanagisawa R, Nakazawa Y, Shiohara M, Manabe A, Kojima S 2007 Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. Blood 109:5477–5480
23. Castro-Malaspina H, Schaison G, Passe S, Pasquier A, Berger R, Bayle-Weisgerber C, Miller D, Seligmann M, Bernard J 1984 Subacute and chronic myelomonocytic leukemia in children (juvenile CML). Clinical and hematologic observations, and identification of prognostic factors. Cancer 54:675–686
24. Hasle H, Baumann I, Bergstrasser E, Fenu S, Fischer A, Kardos G, Kerndrup G, Locatelli F, Rogge T, Schultz KR, Stary J, Trebo M, van den Heuvel-Eibrink MM, Harbott J, Nollke P, Niemeyer CM 2004 The International Prognostic Scoring System (IPSS) for childhood myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML). Leukemia 18:2008–2014
25. Arico M, Bossi G, Schiro R, Galimberti M, Longoni D, Macchia P, Miniero R, Natale D, Pession A, Pillon M 1993 Juvenile chronic myelogenous leukemia: report of the Italian Registry. Associazione Italiana di Ematologia Oncologia Pediatrica (AIEOP). Haematologica 78:264–269
26. Owen G, Lewis IJ, Morgan M, Robinson A, Stevens RF 1992 Prognostic factors in juvenile chronic granulocytic leukaemia. Br J Cancer Suppl 18:S68–S71
27. Niemeyer CM, Fenu S, Hasle H, Mann G, Stary J, Wering E 1998 Differentiating juvenile myelomonocytic leukemia from infectious disease. Blood 91:365–367
28. Yamamoto T, Isomura M, Xu Y, Liang J, Yagasaki H, Kamachi Y, Kudo K, Kiyoi H, Naoe T, Kojma S 2006 PTPN11, RAS and FLT3 mutations in childhood acute lymphoblastic leukemia. Leuk Res 30:1085–1089
29. Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, Brunner HG, Bertola DR, Crosby A, Ion A, Kucherlapati RS, Jeffery S, Patton MA, Gelb BD 2002 PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. Am J Hum Genet 70:1555–1563
30. Mitani K, Hangaishi A, Imamura N, Miyagawa K, Ogawa S, Kanda Y, Yazaki Y, Hirai H 1997 No concomitant occurrence of the N-ras and p53 gene mutations in myelodysplastic syndromes. Leukemia 11:863–865
31. Flotho C, Kratz CP, Niemeyer CM 2007 How a rare pediatric neoplasia can give important insights into biological concepts: a perspective on juvenile myelomonocytic leukemia. Haematologica 92:1441–1446
32. Kratz CP, Niemeyer CM, Castleberry RP, Cetin M, Bergstrasser E, Emanuel PD, Hasle H, Kardos G, Klein C, Kojima S, Stary J, Trebo M, Zecca M, Gelb BD, Tartaglia M, Loh ML 2005 The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. Blood 106:2183–2185
33. Stiller CA, Chessells JM, Fitchett M 1994 Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. Br J Cancer 70:969–972

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Concurrent Langerhans Cell Histiocytosis and Nephroblastoma

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Both Langerhans cell histiocytosis (LCH) and nephroblastoma are rare in children. We report herein the first case of a patient with both diseases concurrently. A 2-year-old female presented with bone pain and swelling of the right humerus. As a result of the local incision biopsy, she was diagnosed as LCH. A nephroblastoma of the left kidney was discovered during her staging work-up. After complete resection of the nephroblastoma, she received standard chemoradiotherapy for nephroblastoma. She is alive without relapse 14 months after initial presentation. Pediatr Blood Cancer 2009;52: 662–664. © 2009 Wiley-Liss, Inc.

Key words: children; langerhans cell histiocytosis; nephroblastoma

INTRODUCTION

Langerhans cell histiocytosis (LCH) is characterized by a clonal proliferation of Langerhans cells, resulting in a wide range of clinical manifestations. The clinical heterogeneity of the disease ranges from a solitary lytic bone lesion with a favorable course to a disseminated disorder, occurring in young children, with fatal outcome, mainly from progressive organ failure. However, the pathogenesis of the disease is still poorly understood. An overall incidence rate of 2.6 to 8.9 cases in 1,000,000 children per year was recently reported from Northwest England, France, and Sweden [1–3].

Nephroblastoma, also called Wilms tumor, is one of the most common solid tumors of childhood, originating from the kidney. It occurs in 10 out of 1,000,000 children less than 15 years of age [4]. The risk of acquiring the disease increases in association with several recognizable congenital anomalies such as aniridia, hemihypertrophy, Beckwith-Wiedemann syndrome (BWS), genitourinary tract anomalies including WAGR syndrome and Denys-Drash syndrome. The following genes and chromosomal area are associated with the development of nephroblastoma: WT1 at 11p13, WT2 at 11p15.5, WT3 at 16q, WT4 at 17q12-q21, and WT5 at 7p15-p11.2 [5].

There have been reports of LCH associated with various malignancies [6–13]. Among them, a large group of patients had hematological diseases including leukemia and malignant lymphoma. Childhood solid tumors were very few compared with adult malignancies. We report a pediatric patient with both LCH of bone and nephroblastoma.

CASE REPORT

A 2-year-old female presented with a 2-week history of swelling and pain of the right shoulder. She had been previously well, and her past and family histories were unremarkable. Physical examination was normal except swelling and limited range of motion of the right shoulder. Imaging studies revealed a lesion at the proximal right humerus; the lesion was positive for periosteal reaction with destruction of the normal trabecular bone pattern on the X-ray (Fig. 1a). The diagnosis of eosinophilic granuloma was made on the finding of incision biopsy specimens immunostained for CD1a, langerin and S100. Methylpredonisolone was injected into the bone lesion. A radionuclide bone scan did not show involvement in other sites.

We performed a computed tomography scan of the chest and abdomen in addition to an X-ray and scintigraphy of the whole body for the staging work-up. A mass (80 mm × 73 mm × 66 mm) was found at the inferior pole of the left kidney (Fig. 2). Laboratory findings were normal, including urinary homovanillic acid, vanillylmandelic acid, and serum neuron-specific enolase levels. The mass was completely resected, revealing a nephroblastoma focal nephroblastic subtype. The patient did not have features of BWS, aniridia, hemihypertrophy or genitourinary tract anomalies. Thereafter, she received chemoradiotherapy according to National Wilms Tumor Study protocol for nephroblastoma (Stage II) for 4 months; a combination therapy with actinomycin D, vincristine and doxorubicin after local radiation to the tumor bed in the left abdomen (10.8 Gy). She did not receive any LCH-oriented therapy except for a local injection of steroid into the bone lesion. At the time of her discharge from the hospital, the bone defect in the right humerus did not show any change on the plain X-ray. Currently, the patient is alive and well 14 months after the initial presentation. The osteolytic site of LCH involvement of the right humerus resolved completely (Fig. 1b).

DISCUSSION

Association between malignant neoplasms and LCH has been recognized for the past 2 decades. Both diseases occur sequentially or concurrently, and the incidence seems to be greater than that expected by chance. Among neoplasms, the incidence of hematological malignancies, especially acute leukemia and lymphoma, is relatively common. Acute myeloid leukemia (AML) usually develops after the completion of chemotherapy for LCH, whereas lymphoma precedes LCH or occurs concurrently. The interval between the diagnoses of LCH and AML averaged 5.5 years [7]. In one case of lymphoma, LCH may represent a reaction to lymphoma

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

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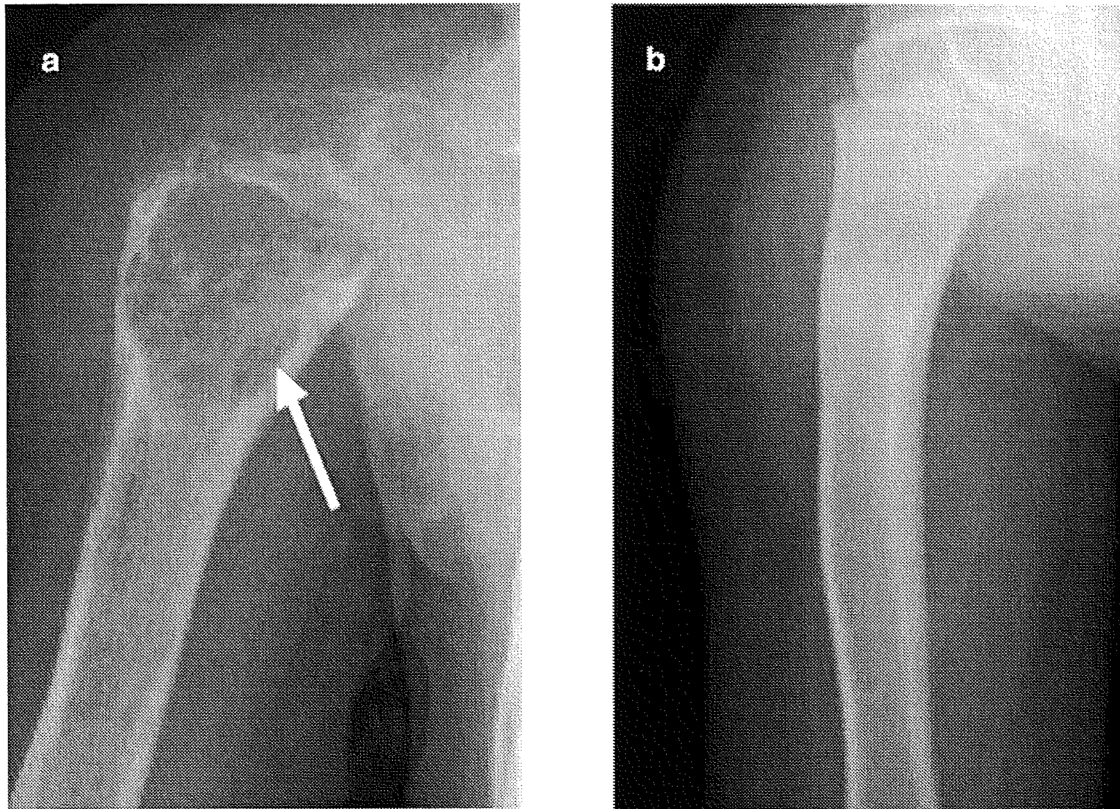

Fig. 1. X-ray finding at the proximal right humerus. **a**: A periosteal reaction with destruction of the normal trabecular bone was found at diagnosis. **b**: The osteolytic sign resolved completely at 1 year after the treatment.

cells because focal microscopic lesions of LCH are usually present in the draining lymph nodes without any evidence of systemic spread of LCH [6]. In another case, the same T cell receptor arrangement was identified in both the LCH and T cell acute lymphoblastic leukemia (T-ALL) [14]. Only a few cases of concurrent LCH and solid tumors other than malignant lymphoma have been reported: 9 lung cancers and 1 stomach cancer in adults [8–11] and 3 retinoblastomas and 1 neuroblastoma in children [7,12,13]. The current standard for the initial treatment of patients with LCH is to use the least toxic therapy. Systemic chemotherapy for patients with solitary LCH is not indicated. In this case, the patient did not receive an adjuvant therapy for LCH except for local injection of steroid.

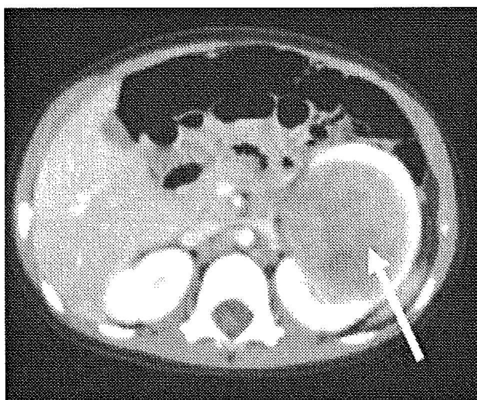

Fig. 2. An enhanced computed tomography scan of the abdomen showed tumor (80 mm × 73 mm × 66 mm) at the inferior pole of the left kidney.

REFERENCES

1. Alston RD, Tatevossian RG, McNally RJ, et al. Incidence and survival of childhood Langerhans cell histiocytosis in Northwest England from 1954 to 1998. Pediatr Blood Cancer 2007;48:555–560.
2. Guyot-Goubin A, Donadieu J, Barkaoui M, et al. Descriptive epidemiology of childhood Langerhans cell histiocytosis in France, 2000-2004. Pediatr Blood Cancer 2008;51:71–75.
3. Stalemark H, Laurencikas E, Karis J, et al. Incidence of Langerhans cell histiocytosis in children: A population-based study. Pediatr Blood Cancer 2008;51:76–81.
4. Stiller CA, Parkin DM. International variations in the incidence of childhood renal tumours. Br J Cancer 1990;62:1026–1030.
5. Scott RH, Stiller CA, Walker L, et al. Syndromes and constitutional chromosomal abnormalities associated with Wilms tumour. J Med Genet 2006;43:705–715.
6. Egeler RM, Neglia JP, Puccetti DM, et al. Association of Langerhans cell histiocytosis with malignant neoplasms. Cancer 1993;71:865–873.
7. Egeler RM, Neglia JP, Arico M, et al. The relation of Langerhans cell histiocytosis to acute leukemia, lymphomas, and other solid tumors. The LCH-Malignancy Study Group of the Histiocyte Society. Hematol Oncol Clin North Am 1998;12:369–378.
8. Lombard CM, Medeiros LJ, Colby TV. Pulmonary histiocytosis X and carcinoma. Arch Pathol Lab Med 1987;111:339–341.