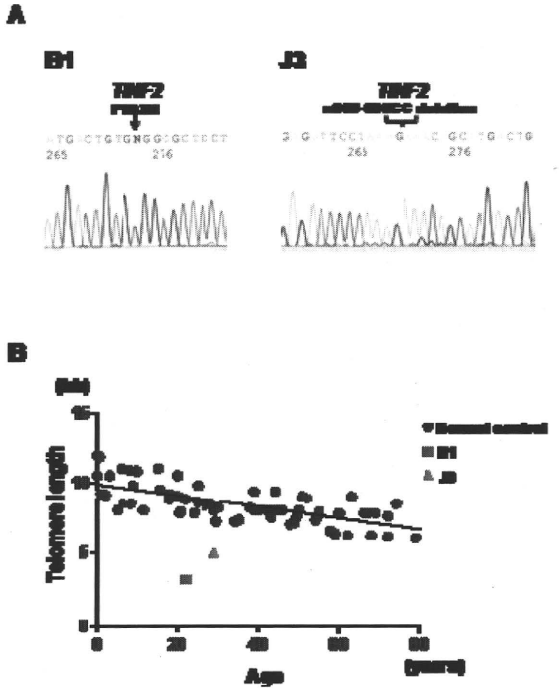
遺伝子変異が認められた(図 1A)。また TINF2 遺伝子変異を有する AA はテロメア長の短縮化が認められ、不全型の DKC であると考えられた(図 1B)。

図 1

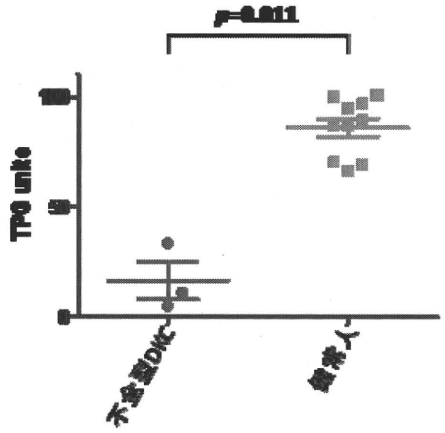


2. テロメラーゼ活性の測定の有用性の検証

不全型 DKC(n=3)は、健常人(n=10)と比較して有意差をもってテロメラーゼ活性が低下していた(不全型 DKC 16.3 TPG unite vs 健常人 86.7, p=0.011) (図 2)。

AA15 例(IST 有効 11 例、IST 無効 4 症例)において、IST 無効の AA 2 例でテロメラーゼ活性の低下は認められた。この 2 例はテロメア制御遺伝子に変異は認められず、1 例ではテロメア長の短縮化を認めたが、他の 1 例ではテロメア長は正常下限で短縮化は認められなかった。

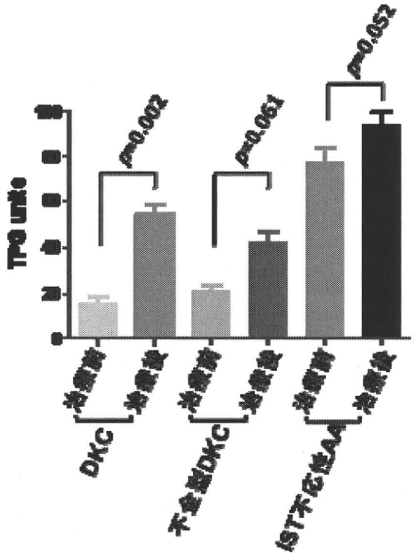
図 2



3. 性ステロイドホルモン治療によるテロメラーゼ活性の改善の検証

臨床データに関しては、不全型 DKC において治療後 3 カ月において Hb の軽度上昇(Hb 9.8→10.9g/dl)が認められたが、それ以外は有意な改善は認められていない。テロメラーゼ活性に関しては、DCK において有意に活性の亢進が認められ、不全型 DKC と IST に不応性の AA においても有意差はなかったが活性の亢進する傾向が得られた(図 3)。

図 3



D. 考察

今回の検討によって本邦の BMF においても TINF2 変異を有する不全型の DKC が存在することが明らかになった。しかしこれまでのテロメラーゼ複合体遺伝子変異による不全型 DKC と合わせても 4/142 症例(2.8%)と頻度は高くはない。しかし後述のテロメラーゼ活性の低下とテロメア長の短縮化を認める IST 不応性 AA 症例などは、既知の遺伝子変異は認められなかったが、新規のテロメア制御遺伝子変異による不全型 DKC の可能性が高い。このことは臨床的に BMF と診断された症例の中にはまだ不全型 DKC の症例が含まれていることを示している。現在原因遺伝子が同定されていない不全型 DKC 症例に対してエクソン領域や転写制御領域、疾患関連領域などをゲノム DNA から濃縮し、次世代高速シークエンサーを用いて変異解析を行い新規の原因遺伝子を同定する予定である。

テロメラーゼ活性は、テロメア長と同様に不全型 DKC のスクリーニングには有用であることが示された。特に IST 無効の AA においてテロメア長の短縮化を認めないが、テロメラーゼ活性は低下しているのは、世代促進や加齢による影響が軽度のためかもしれない。またテロメラーゼ活性の測定は治療の反応性を予想するにも有用である可能性がある。今回の検討では観察期間が短いため臨床的データの改善と関係は示せなかったが、今後の検討によって明らかにしたいと考えている。

E. 結論

本邦の BMF において TINF2 遺伝子変異を有する不全型 DKC は存在する。またテロメラーゼ活性は DKC や不全型 DKC のスクリーニングや治療効果の評価に有用かもしれない。

F. 健康危険情報

特になし。

G. 研究発表

1. 論文発表

1) Yamaguchi H, Inokuchi K, Takeuchi J, Tamai H, Mitamura Y, Kosaka F, Ly H, Dan K. Identification of TINF2 gene mutations in adult Japanese patients with acquired bone marrow failure syndromes. Br J Haematol. 2010;150(6):725-7.

2) 山口博樹, 檀 和夫. テロメア関連遺伝子異常による骨髄不全症. 臨床血液. 2010; 52: 646-653.

3) 山口博樹, 檀 和夫. 骨髄不全症に対する蛋白同化ステロイドホルモンのテロメレース活性化作用. Annual Review 血液 2011, in press.

2. 学会発表

1) Takeuchi J, Yamaguchi H, Inokuchi K, Tamai H, Mitamura Y, Kosaka F, Ly H, Dan K. Evaluation of TINF2 and SBDS gene mutations and screening telomere length in adult Japanese patients with acquired bone marrow failure syndromes. 15th. European Hematology Association. 2009 年 6 月, バルセロナ.

2) Takeuchi J, Yamaguchi H, Tamai H, Mitamura Y, Kosaka F, Inokuchi K, Dan K. Screening of telomere length among acquired aplastic anemia patients. 第 72 回日本血液学会, 2010 年 9 月, 横浜

知的財産権の出願・登録状況

特になし。

音受容に関するAdenylate Kinase-2(AK2)の内耳における役割についての研究

松 延　毅（防衛医科大学校耳鼻咽喉科学）
栗 田 昭宏（防衛医科大学校耳鼻咽喉科学）
塩 谷 彰 浩（防衛医科大学校耳鼻咽喉科学）

研究要旨

AK2 は免疫学的には骨髄細胞の分化、アポトーシスに関連していることが示唆されている。また、AK2 の発現が先天的にみられない細網異形成症の患者では免疫不全の他に難聴が高率にみられることが報告されている。そこで今回、我々は AK2 が音受容機構にどのように関与しているかを検討するために本研究を開始した。今年度はマウスにおける AK2 の存在の確認としてウエスタンブロット及び免疫組織化学を行い、内耳における AK2 の存在および局在を確認することができた。

A. 研究の目的

AK2 の発現が先天的にみられない細網異形成症の患者では免疫不全の他に難聴が高率にみられることが報告されている。難聴のタイプは内耳性難聴であることであることより AK2 が内耳内において聴覚に重要な役割を果たしていることが推察される。本研究は AK2 が音受容機構にどのように関与しているかを検討するために本研究を開始した。

B. 研究方法

マウスを麻酔後、断頭、内耳骨胞を摘出し PBS 中で内耳組織を取り出した。内耳組織をホモジナイズしてサンプルとした。抗 AK2 抗体に対するウエスタンブロットを行った。また、蝸牛を固定し抗 AK2 抗体に対する免疫組織化学を行った。

C. 研究結果

マウス内耳組織のウエスタンブロットにより、内耳組織に AK2 が確実に存在することが証明された（図 1）。また、免疫組織化学により有毛細胞およびらせん神経節細胞に存在することが示唆された（図 2-1, 2）。

図 1

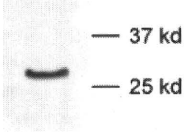
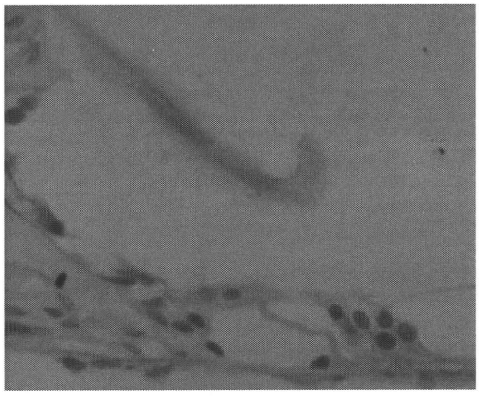
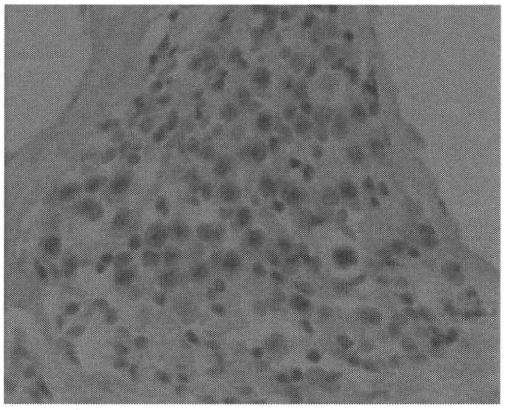


図 2-1



2-2



D. 考察

AK2 はミトコンドリアの内外膜間に存在し、好中球などにおいて細胞性免疫に大きい役割を果たしている他にいくつかの機能があることが報告されている。本研究では AK2 がマウス内耳組織などに強く発現していることが証明されたが、AK2 の機能として(1)ADP を ATP と AMP に脱リン酸化するというアデニンヌクレオチドの代謝に関与しているとされる。AK2 の欠陥が内耳障害に関与していると仮定すれば、内耳血管において ADP は内皮細胞の integrity の障害に重要な役割を果たしていることが推察される。これはこれまでにない発見であると考えられる。(2)ミトコンドリアの内外膜間に存在し、FADD (Fas-associated protein with death domain)、 caspase 10 と結合しアポトーシスを誘導するとされる。AK2 が内耳障害の起こるメカニズムにおいても深く関与していることが示唆される。今後は内耳障害モデルを用いた機能的解析を加えてゆく予定である。

E. 結論

AK2 が内耳内において聴覚受容や内耳障害の病態に重要な役割を果たしている可能性が推察された。

F. 健康危険情報

特になし。

G. 研究発表

特になし。

知的財産権の出願・登録状況

特になし。

○ 参 考 資 料

細網異形成症診断基準

平成 22 年 2 月 1 日作成

I)-V)全てを満たす場合に細網異形成症と診断する。

I)臨床所見

易感染性を認めること。

感音性難聴を認めること。

II) 免疫・血液障害

a) 末梢血で好中球減少を認めること。単球は正常であること。

b)末梢血単核球の FACS 解析で T 細胞および NK 細胞の減少を認めること。B 細胞数は正常であること。

c)骨髄検査で骨髄系細胞の減少を認めること。単球系の減少は認めないこと。

d) コロニー解析で骨髄系細胞の分化障害を認めること。単球系細胞の分化障害は認めないこと。

III) 聴力障害

聴力検査で感音性難聴があること。

IV)AK2 タンパク異常解析

Werstern blot 等で AK2 タンパク発現低下を認めること。

V) AK2 遺伝子診断

遺伝子解析で AK2 変異を認めること。

細網異形成症治療指針

平成22年2月1日作成

I)感染症治療

本症は好中球減少、T細胞減少、低γグロブリン血症があるため、重篤な感染症を起こしやすい。そのため、以下の様に感染症治療を十分に行う。また、細網異形成症を疑った場合は、無菌室入室、アイソレーター使用など無菌管理を行う。

1）細菌感染症

骨髄移植後生着までの好中球減少期の発熱に対しては血液培養後直ちにセフェム系もしくはカルバペネム系抗生剤を投与する。血液培養にてグラム陽性球菌が検出された場合は症状に応じてバンコマイシンの投与も検討する。またST合剤の投与はニューモシスチス肺炎及び肺炎連鎖球菌感染予防のため投与する。

2）真菌感染症

Candida 感染に対しては、micafungin、フルコナゾールを投与する。深在性Aspergillus症およびフルコナゾール耐性のCandida症に対してはアムホテリシンB、イトラコナゾール、リポゾーマルアンホテリシンB、ボリコナゾール、等を適宜投与する。

発熱時は常に真菌感染症を疑いβ-D グルカン、アスペルギルス抗原検査等を必要に応じて施行する。

3）ウイルス感染症

サイトメガロウイルス感染症に対しては定期的（毎週）な抗原血症もしくはウイルス血症の有無を検査し、陽性の場合はガンシクロビルの投与を行う。本剤は副作用としての好中球減少に注意し、耐性出現の場合はフォスカビルの投与も考慮する。

EBウイルス感染症に対してはrituximabの投与を考慮する。

単純ヘルペスウイルス（HSV）及び水痘帯状疱疹ウイルス（VZV）に対してはアシクロビルの予防的及び治療的投与を行う。

4）G-CSF

好中球増加を期待して使用する。

5）低ガンマグロブリン血症

静注様γグロブリン製剤の定期的投与を行う。

II）造血幹細胞移植

本症は、造血幹細胞移植により根治が期待できる一方、施行しなかった場合生後1年以内に死亡する。

造血幹細胞移植の絶対適応である。診断が付き次第、早期に造血幹細胞移植を行う。

1）ドナーの選択

移植細胞源は、血縁 HLA 一致ドナーが存在する場合を除き、非血縁臍帯血とする。この理由は緊急を要するため、移植の準備に数ヶ月を要する骨髄バンクを介しての移植は適さないことと、国内重症複合型免疫不全症の造血幹細胞移植症例の集計で非血縁臍帯血移植の粗生存率が非血縁者間骨髄移植および血縁者間 HLA 不一致骨髄移植に比べて良好であったことである。

また HLA 不一致非血縁者間臍帯血移植においては生着不全や GVHD の頻度が高まることが予想されるため、血清学的に HLA-A, B, DR が2座不一致までに限りドナーとして選定することも認められる。

移植細胞数の最低数は 2×10^5/kg とし、CD34 陽性細胞が多く含まれる臍帯血を選択する。ドナーの性別や血液型は問わない。

2）移植前処置

感染症が顕著である場合は前処置を行わない。前処置を行う場合は、骨髄非破壊的前処置として実績のある(a),(b)いずれかを推奨する。

(a) フルダラビンとメルファランによる臍帯血移植前処置

day -7、-6、-5、-4、-3　：フルダラビン　1時間点滴静注　　25 mg/m^2/日

day -4、-3　　：メルファラン　30分点滴静注　　70 mg/m^2/日

10Kg 未満では、体表面積 1m^2 あたりの投与量÷30×体重（kg）で計算する。

(b) フルダラビンとブスルファンによる臍帯血移植前処置

day -7、-6、-5、-4、-3、-2　：フルダラビン　1時間点滴静注　30 mg/m^2/日

day -3、-2　　：ブスルファン　2時間点滴静注 1mg/kg×4/日

10Kg 未満では、体表面積 $1m^2$ あたりの投与量÷30×体重（kg）で計算する。

3）GVHD 予防

day -1 から	：タクロリムス	持続点滴静注	0.02 mg/kg/日
day 1	：メトトレキサート	静注	10 mg/m^2/日
day 3、6	：メトトレキサート	静注	7 mg/m^2/日

タクロリムス血中濃度は 5-12ng/mL に維持し 15ng/mL を超えないようにする。なおタクロリムスは内服が可能となった時点で 1 日点滴量の 3-5 倍量を分 2 で内服とする。

またメトトレキサートについて、day 1 においては一回最大 10 mg/body とし、day 3、6 においては一回最大 7 mg/body とする。

III）聴力障害

補聴器による聴覚障害の治療を行う。造血幹細胞移植により難聴が改善した症例が存在するため、移植後に定期的に聴力検査を行う。

Ⅲ　研究成果の刊行に関する一覧

研究成果の刊行に関する一覧

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
Albert MH, Bittner TC, Nonoyama S, Notarangelo LD, Burns S, Imai K, Espanol T, Fasth A, Pellier I, Strauss G, Morio T, Gathmann B, Noordzij JG, Fillat C, Hoenig M, Nathrath M, Meindl A, Pagel P, Wintergerst U, Fischer A, Thrasher AJ, Belohradsky BH, Ochs HD.	X-linked thrombocytopenia (XLT) due to WAS mutations: Clinical characteristics, long-term outcome, and treatment options.	Blood			2010
Aghamohammadi A, Imai K, Moazzami K, Abolhassani H, Tabatabaeiyan M, Parvaneh N, Nasiri Kalmarzi R, Nakagawa N, Oshima K, Ohara O, Nonoyama S, Rezaei N.	Ataxia-telangiectasia in a patient presenting with hyper-immunoglobulin M syndrome.	J Investig Allergol Clin Immunol.	20 (5)	442-5	2010
Tsuge I, Kondo Y, Nakajima Y, Nakagawa N, Imai K, Nonoyama S, Oshima K, Ohara O, Hatanaka M, Kitano E, Kitamura H, Urisu A.	Hyper IgM syndrome and complement Clq deficiency in an individual with systemic lupus erythematosus-like disease.	Clin Exp Rheumatol.	28 (4)	558-60	2010
Zhao M, Kanegane H, Kobayashi C, Nakazawa Y, Ishii E, Kasai M, Terui K, Gocho Y, Imai K, Kiyasu J, Nonoyama S, Miyawaki T.	Early and rapid detection of X-linked lymphoproliferative syndrome with SH2D1A mutations by flow cytometry.	Cytometry B Clin Cytom.			2010
Hashii Y, Yoshida H, Kuroda S, Kusuki S, Sato E, Tokimasa S, Ohta H, Matsubara Y, Kinoshita S, Nakagawa N, Imai K, Nonoyama S, Oshima K, Ohara O, Ozono K.	Hemophagocytosis after bone marrow transplantation for JAK3-deficient severe combined immunodeficiency.	Pediatr Transplant.	14 (8)	E105-9	2010
Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, Okada S, Kobayashi M, Agematsu K, Takada H, Mitsuiki N, Oshima K, Ohara O, Suri D, Rawat A, Singh S, Pan-Hammarström Q, Hammarström L, Reichenbach J, Seger R, Ariga T, Hara T, Miyawaki T, Nonoyama S.	Quantification of kappa-deleting recombination excision 1 circles in Guthrie 2 cards for the identification of early B-cell maturation defects.	JACI		In press	2010
Yamaguchi H, Inokuchi K, Takeuchi J, Tamai H, Mitamura Y, Kosaka F, Ly H, Dan K.	Identification of TINF2 gene mutations in adult Japanese patients with acquired bone marrow failure syndromes.	Br J Haematol.	150 (6)	725-7	2010

Asai E, Wada T, Sakakibara Y, Toga A, Toma T, Shimizu T, Nampoothiri S, Imai K, Nonoyama S, Morio T, Muramatsu H, Kamachi Y, Ohara O, Yachie A.	Analysis of mutations and recombination activity in RAG-deficient patients.	Clin Immunol.		Epub ahead of print	2010
Hijikata A, Raju R, Keerthikumar S, Ramabadran S, Balakrishnan L, Ramadoss SK,Pandey A, Mohan S, Ohara O.	Mutation@A Glance: an integrative web application foranalysing mutations from human genetic diseases.	DNA Res.	17 (3)	197-208	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 resulting from uniparental disomy after haploidentical hematopoietic stem cell transplantation	Blood	115 (15)	3158-61	2010
Konno Y, Toki T, Tandai S, Xu G, Wang R, Terui K, Ohga S, Hara T, Hama A, Kojima S, Hasegawa D, Kosaka Y, Yanagisawa R, Koike K, Kanai R, Imai T, Hongo T, Park MJ, Sugita K, Ito E.	Mutations in the ribosomal protein genes in Japanese patients with Diamond-Blackfan anemia.	Haematologica	95 (8)	1293-9	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.	Br J Haematol.	150 (1)	83-7	2010
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
Nishio N, Kojima S.	Recent progress in dyskeratosis congenita.	Int J Hematol.	92 (3)	419-24 Epub	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, MizutaniS.	Autoimmune Lymphoproliferative Syndrome Like Disease With Somatic KRAS Mutation.	Blood	Nov 9	Epub ahead of print	2010
Seki M. Kimura H. Mori A. Shimada A. Yamada Y. Maruyama K. Hayashi Y. Agematsu K. Morio T. Yachie A. Kato M.	Prominent eosinophilia but less eosinophil activation in a patient with Omenn syndrome.	Pediatr. Int.	52	e196-9	2010

Shin MJ, Shim JH, Lee JY, Chae WJ, Lee HK, Morio T, Park JH, Chang EJ, Lee SK.	Qualitative and quantitative differences in the intensity of Fas-mediated intracellular signals determine life and death in T cells.	Int. J. Hematol.	92	262-70	2010
Okamoto K, Iwai Y, Ohhora M, Yamamoto M, Morio T, Aoki K, Ohya K, Jetten AM, Akira S, Muta T, Takayanagi H.	IkB• regulates TH17 development by cooperating with ROR nuclear receptors.	Nature	464	1381-1385	2010
Kato I., Umeda K., Awaya T., Yui Y., Niwa A., Fujino H., Matsubara H., Watanabe K., Heike T., Adachi N., Endo H., Mizukami T., Nunoi H., Nakahata T., Adachi S.	Successful treatment of refractory donor lymphocyte infusion-induced immune-mediated pancytopenia by Rituximab.	Pediatr Blood Cancer.	54	329-331	2010
Sakai H, Ito S, Nishikomori R, Takaoka Y, Kawai T, Saito M, Okafuji I, Yasumi T, Heike T, Nakahata T.	A case of early-onset sarcoidosis with a six-base deletion in the NOD2 gene.	Rheumatology (Oxford)	49	194-196	2010
Kubota M., Adachi S., Usami I., Okada M., Kitou T., Shiota M., Taniguchi Y., Tanizawa A., Nanbu M., Hamahata K., Fujino H., Matsubara K., Wakazono Y., Nakahata T.	Characterization of chronic idiopathic thrombocytopenic purpura in Japanese children: a retrospective multi-center study.	Int. J. Hematol.	91	252-257	2010
Takeuchi M., Kimura S., Kuroda J., Ashihara E., Kawatani M., Osada H., Umezawa K., Yasui E. Imoto M., Tsuruo T., Yokota A., Tanaka R., NagaoR., Nakahata T., Fujiyama Y., Maekawa T.	Glyoxalase-I is a novel target against Bcr-Abl+ leukemic cells acquiring stem-like characteristics in a hypoxic environment.	Cell Death Diff.	17	1211-1220	2010
Mizuno Y., Chang H., Umeda K., Niwa A., IwasaT., Awaya T., Fukada S., Hiroshi Yamamoto H., Yamanaka S., Nakahata T., Heike T.	Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells.	FASEB J.	24	2245-2253	2010
Kuroda Y., Kitada M., Wakao S., Nishikawa K., Tanimura Y., Makinoshima H., Goda M., Akashi H., Inutsuka A., Niwa A., Nabeshima Y., Nakahata T., Nabeshima Y., Fujiyoshi Y., Dezawa M.	Unique multipotent cells in adult human mesenchymal cell populations.	Proc. Natl. Adad. Sci. USA	107	8639-8643	2010
Matsuda K., Taira C., Sakashita K., Saito S., Yanagisawa MT., Yanagisawa R., Yozo Nakazawa Y., Shiohara M., Fukushima K., Oda M., Honda T., Nakahata T., Koike K.	Long-term survival after non-intensive chemotherapy in some juvenile myelomonocytic leukemia patients with CBL mutations, and the possible presence of normal individuals with the mutations.	Blood	115	5429-5431	2010

Matsuse D., Kitada M., Kohama M., Nishikawa K., Makinoshima H., Wakao S., Fujiyoshi Y., Heike T., Nakahata T., Akutsu H., Umezawa A., Harigae H., Kita J., Dezawa M.	Human umbilical cord-derived mesenchymal stromal cells differentiate into functional Schwann cells that sustain peripheral nerve regeneration.	J. Neuropathol. Exp. Neurol.	69	973-985	2010
Kumada T., Yamanaka Y., Kitano A., Shibata M.,Awaya T., Kato T., Okawa K.,Abe T., Oshima N. Nakahata T., Heike T.	Ttyh1, a Ca2+-binding protein localized to the endoplasmic reticulum, is required for early emblyonic development.	Develop. Dynam.	239	2233-2245	2010
Kaichi S., Hasegawa K., Takaya T., Yokoo N., Mima T., Kawamura T., Morimoto T., Baba S., Doi H., Yamanaka S., Nakahata T., Heike T.	Cell line-dependent differentiation of induced pluripotent stem cells into cardiomyocytes in mice.	Cardiovascu-lar Res.	88	314-323	2010
Kusunoki T, Morimoto T, Nishikomori R, Yasumi T, Heike T, Fujii T, Nakahata T.	The effect of past food avoidance due to allergic symptoms on the growth of children at school age.	Allergology International.	59	369-374	2010
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.		In press	
山口博樹，檀　和夫.	テロメア関連遺伝子異常による骨髄不全症.	臨床血液	52:	646-653	2010
山口博樹，檀　和夫.	骨髄不全症に対する蛋白同化ステロイドホルモンのテロメレース活性化作用.	Annual Review 血液		In press	2010
中畑龍俊	造血因子と臨床応用.	臨床検査	第54巻第6号	623-629	2010
中畑龍俊	iPS 細胞と遺伝性疾患(特集 臨床遺伝学の進歩と日常診療. 遺伝性疾患の新しい治療と今後期待される治療研究)	日本医師会雑誌	139 (3)	632-634	2010

Ⅳ　研究成果の刊行に関する一覧　別冊

From www.bloodjournal.org at IK (JPT) 031297 on February 3, 2011. For personal use only.

blood

2010 115: 3231-3238
Prepublished online Feb 19, 2010;
doi:10.1182/blood-2009-09-239087

X-linked thrombocytopenia (XLT) due to WAS mutations: clinical characteristics, long-term outcome, and treatment options

Michael H. Albert, Tanja C. Bittner, Shigeaki Nonoyama, Lucia Dora Notarangelo, Siobhan Burns, Kohsuke Imai, Teresa Espanol, Anders Fasth, Isabelle Pellier, Gabriele Strauss, Tomohiro Morio, Benjamin Gathmann, Jeroen G. Noordzij, Cristina Fillat, Manfred Hoenig, Michaela Nathrath, Alfons Meindl, Philipp Pagel, Uwe Wintergerst, Alain Fischer, Adrian J. Thrasher, Bernd H. Belohradsky and Hans D. Ochs

Updated information and services can be found at:
http://bloodjournal.hematologylibrary.org/cgi/content/full/115/16/3231

Information about reproducing this article in parts or in its entirety may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests

Information about ordering reprints may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints

Information about subscriptions and ASH membership may be found online at:
http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.

From www.bloodjournal.org at IK (JPT) 031297 on February 3, 2011. For personal use only.

X-linked thrombocytopenia (XLT) due to *WAS* mutations: clinical characteristics, long-term outcome, and treatment options

Michael H. Albert,[1] Tanja C. Bittner,[1] Shigeaki Nonoyama,[2] Lucia Dora Notarangelo,[3] Siobhan Burns,[4] Kohsuke Imai,[2] Teresa Espanol,[5] Anders Fasth,[6] Isabelle Pellier,[7] Gabriele Strauss,[8] Tomohiro Morio,[9] Benjamin Gathmann,[10] Jeroen G. Noordzij,[11] Cristina Fillat,[12] Manfred Hoenig,[13] Michaela Nathrath,[14] Alfons Meindl,[15] Philipp Pagel,[16] Uwe Wintergerst,[17] Alain Fischer,[18] Adrian J. Thrasher,[4] *Bernd H. Belohradsky,[1] and *Hans D. Ochs[19]

[1]Dr von Haunersches Kinderspital, Ludwig-Maximilians-Universität, Munich, Germany; [2]National Defense Medical College, Tokorozawa, Japan; [3]University of Brescia, Brescia, Italy; [4]University College London Institute of Child Health, London, United Kingdom; [5]Vall d'Hebron Hospital, Barcelona, Spain; [6]The Queen Silvia Children's Hospital, Göteborg, Sweden; [7]Centre Hospitalier Universitaire Angers, Angers, France; [8]Charité Campus Virchow-Klinikum, Otto-Heubner-Zentrum für Kinder- und Jugendmedizin, Berlin, Germany; [9]Tokyo Medical and Dental University, Tokyo, Japan; [10]Universitätsklinikum Freiburg, Freiburg, Germany; [11]St Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [12]Centre de Regulació Genòmica, Centro de Investigación Biomédica en Red de Enfermedades Raras, Barcelona, Spain; [13]Universitätsklinik für Kinder- und Jugendmedizin Ulm, Ulm, Germany; [14]University Children's Hospital, Technische Universität, Munich, Germany; [15]Frauenklinik am Klinikum rechts der Isar, Technische Universität, Munich, Germany; [16]Lehrstuhl für Genomorientierte Bioinformatik, Wissenschaftszentrum Weihenstephan, Technische Universität, Freising, Germany; [17]Krankenhaus St. Josef, Braunau, Austria; [18]Hôpital Necker Enfants Malades, Paris, France; and [19]University of Washington, Seattle Children's Hospital

A large proportion of patients with mutations in the Wiskott-Aldrich syndrome (WAS) protein gene exhibit the milder phenotype termed X-linked thrombocytopenia (XLT). Whereas stem cell transplantation at an early age is the treatment of choice for patients with WAS, therapeutic options for patients with XLT are controversial. In a retrospective multicenter study we defined the clinical phenotype of XLT and determined the probability of severe disease-related complications in patients older than 2 years with documented *WAS* gene mutations and mild-to-moderate eczema or mild, infrequent infections. Enrolled were 173 patients (median age, 11.5 years) from 12 countries spanning 2830 patient-years. Serious bleeding episodes occurred in 13.9%, life-threatening infections in 6.9%, autoimmunity in 12.1%, and malignancy in 5.2% of patients. Overall and event-free survival probabilities were not significantly influenced by the type of mutation or intravenous immunoglobulin or antibiotic prophylaxis. Splenectomy resulted in increased risk of severe infections. This analysis of the clinical outcome and molecular basis of patients with XLT shows excellent long-term survival but also a high probability of severe disease-related complications. These observations will allow better decision making when considering treatment options for individual patients with XLT. (*Blood*. 2010;115(16): 3231-3238)

Introduction

In 1937 Wiskott described a clinical entity characterized by thrombocytopenia, eczema, bloody diarrhea, and recurrent otitis media in male infants. After rediscovery in 1954 by Aldrich as an X-linked recessive disorder, it was designated the Wiskott-Aldrich syndrome (WAS).[1-3] X-linked thrombocytopenia (XLT), sometimes associated with mild eczema and/or infections, was recognized in the 1960s and was suspected to be a variant of WAS.[4-6] This was confirmed when patients with XLT were shown to have mutations in the Wiskott-Aldrich syndrome protein gene (*WAS*).[7-9]

WAS gene mutations result in 3 distinct clinical phenotypes: classic WAS, XLT, and X-linked neutropenia,[10,11] and a strong genotype phenotype correlation has been suggested.[12-15] Mutations completely averting WAS protein (WASP) expression typically lead to the classic phenotype. Missense mutations resulting in expression of defective WASP, often in reduced quantity, most often result in the XLT phenotype, sometimes with only intermittent thrombocytopenia.[16] X-linked neutropenia is caused by gain of function mutations resulting in constitutively activated WASP.[17-19] There are however exceptions to these rules, making it difficult to predict the clinical course of a male infant solely based on the type of *WAS* gene mutation and its effect on WASP expression.

The classic WAS phenotype with microthrombocytopenia, severe eczema, increased susceptibility to pyogenic and opportunistic infections, and increased risk of autoimmune disease and cancer usually leads to death in early childhood or adolescence if left untreated.[10,20,21] Curative treatment by allogeneic hematopoietic stem cell transplantation (HSCT) should be offered to all such patients. The outcome is excellent if performed early in life from a human leukocyte antigen–matched related or unrelated donor.[10,22-24] Hematopoietic stem cell gene therapy might in the future offer an alternative approach in patients lacking a suitable donor.[25-27]

Generally accepted treatment policies do not exist for patients exhibiting the XLT phenotype, in whom HSCT would seem like an excessively risky procedure if they have thrombocytopenia and

Submitted September 10, 2009; accepted January 25, 2010. Prepublished online as *Blood* First Edition paper, February 19, 2010; DOI 10.1182/blood-2009-09-239087.

*B.H.B. and H.D.O. contributed equally to this study.

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.

© 2010 by The American Society of Hematology

From www.bloodjournal.org at IK (JPT) 031297 on February 3, 2011. For personal use only.

eczema only. Although it has been assumed that patients with XLT have a lower risk of cancer or autoimmunity than patients with WAS, this has never been formally examined. Therefore, the risk–benefit ratio for HSCT is not known in XLT.

In this multicenter study we assessed retrospectively the spectrum of clinical phenotypes, the associated genotypes, and the long-term outcome of the largest cohort of patients with XLT studied so far.

Methods

Data accrual

Questionnaires were sent worldwide to major centers treating patients with primary immunodeficiency diseases (PIDs), asking to enroll their patients with the XLT phenotype and to provide data on the following disease parameters: infections, eczema, thrombocytopenia, bleeding, malignancy, autoimmunity, *WAS* gene mutation, WASP expression, and type and extent of therapy. An alternative possibility was documentation online with the same questionnaire in the European Society for Immunodeficiencies registry (www.esid.org). Patient information was made anonymous by the submitting physician. The study was approved by the ethics committee of the University of Munich, Germany.

Patients

All submitted patient data were evaluated, and patients were included as study patients by consensual decision of a central review board (M.H.A., T.C.B., B.H.B., H.D.O.). To be enrolled into the final study, patients had to fulfill all of the following criteria: (1) confirmed mutation within the *WAS* gene; (2) classified by their treating physician as having XLT; (3) with or without mild-to-moderate eczema or mild, infrequent infections not resulting in sequelae; (4) age older than 2 years; and (5) no severe infection, autoimmunity, or malignancy within the first 2 years of life.

Bleeding events before the age of 2 years were no reason for exclusion from the study. Older than 2 years, severe infections, the development of autoimmunity, or malignancy was recorded and included in the analysis, but it was no reason for exclusion from the study.

If patients underwent allogeneic HSCT, the transplantation was recorded as the last date of follow-up; the resulting events/outcome were not part of this analysis.

Definitions

Life-threatening infections were defined as requiring hospitalization such as sepsis, meningitis, or pneumonia needing oxygen supply or mechanical ventilation. Serious bleeding was defined as a fatal or life-threatening bleeding episode resulting in hospitalization or red blood cell transfusion. Other serious complications were a diagnosis of autoimmunity, malignancy, or death. If a patient experienced more than 1 serious event, only the first event was registered for the analysis of event-free survival. Severity of thrombocytopenia was defined as follows: less than 20.0×10^9/L (20 000/μL) was severe, 20.0 to 50.0×10^9/L (20 000 to 50 000/μL) was moderate, and greater than 50.0×10^9/L (50 000/μL) or cyclic was mild. All patients with normal or reduced levels of WASP detectable by Western blot or fluorescence-activated cell sorting were designated as WASP positive; those with truncated (by Western blot) or undetectable protein were categorized as WASP negative. Intravenous immunoglobulin (IVIG) or antibiotic (AB) prophylaxes were defined as having had IVIG or prophylactic ABs more than once for any period of time.

Mutations are reported according to the current nomenclature of the Human Genome Variation Society (www.hgvs.org).[28]

Statistical analysis

Kaplan-Meier survival estimates and cumulative incidence rates were compared with the use of the log-rank test (Prism; GraphPad Software Inc). Cumulative incidence for different events adjusting for competing risks was estimated with the use of the statistics language R[29] with the cmprsk package that used the method by Gray.[30] Other analyses used the χ^2 or Fisher exact test and were accepted as significantly different at a level of P less than .05.

Results

Study cohort

A total of 69 centers known to treat patients with PID were contacted and 50 responded (72%). Of 213 completed forms, representing 12 countries from 4 continents, 173 (171 male, 2 female) patients from 128 families and 21 centers with a median age of 11.5 years (range, 2.0-74.6 years) fulfilled the inclusion criteria, covering 2830 patient-years. The 2 female patients of our XLT cohort had been reported previously, 1 with a homozygous missense mutation and 1 with a heterozygous missense mutation and skewed X-inactivation in favor of the mutated allele.[31,32]

Mutations in patients with XLT

We identified 62 unique mutations (Table 1), including 3 mutational hotspots, defined as affecting 10 or more nonrelated families with either the identical mutation or a missense mutation affecting the same amino acid. Two hotspots were located in exon 2 affecting either a valine at position 75 (p.Val75Met or p.Val75Leu; 23 patients) or an arginine at position 86 (p.Arg86Gly, p.Arg86Cys, p.Arg86His, or p.Arg86Leu; 33 patients). The third hotspot mutation, located in intron 6 (c.559 + 5G>A) was found in 15 patients. Thus 41% of all patients had a hotspot mutation.

The majority of mutations was located in exon 1 (10% of all patients) and exon 2 (54%). Most mutations were missense (69% of all patients), followed by splice site mutations (19%), deletions (5%), insertions (3%), nonsense mutations (2%), and no-stop mutations (1%; supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). With few exceptions, patients with missense and splice site mutations expressed WASP in reduced quantity or in truncated form (Table 1).

Survival

Without curative treatment classic WAS results in premature death, often during childhood.[21,33] Patients with XLT are expected to have a better prognosis. To verify this perception, we defined the probability of survival in our cohort of patients with XLT.

Overall survival was excellent with 97% (95% confidence interval [95% CI], 95%-100%), 96% (95% CI, 91%-100%), 81% (95% CI, 66%-97%), and 81% (95% CI, 66%-97%) at 15, 30, 45, and 60 years, respectively, and only slightly reduced compared with the survival curve of the normal male German population[34] (Figure 1A). However, survival probability without having experienced a severe disease-related event was less favorable with 74% (95% CI, 65%-82%), 56% (95% CI, 43%-70%), 36% (95% CI, 20%-53%), and 27% (95% CI, 10%-44%) at 15, 30, 45, and 60 years, respectively (Figure 1B).

Thus the excellent survival in patients with XLT is associated with a high rate of severe disease-related events throughout life.

Incidence of severe disease-related events

To better define the nature and occurrence of severe disease-related events, we analyzed the cumulative incidence rate of these events separately.

From www.bloodjournal.org at IK (JPT) 031297 on February 3, 2011. For personal use only.

Table 1. *WAS* gene mutations in patients with XLT

Exon	Coding DNA mutation	Predicted protein change	Mutation type	Pt*	Fam†	Origin	WASP expression (no. of pt)	Score (no. of pt)
1	c.G5C	p.Ser2Thr	Missense	1	1	Fr	ND	2
1	c.G18A	p.Met6Ile	Missense	2	1	JPN	Reduced (2)	1, 2→5M
1	c.C71T	p.Ser24Phe	Missense	2	2	US (1), JPN (1)	Reduced (1), ND (1)	1, 2→5A
1	c.C79T	p.Leu27Phe	Missense	1	1	US	Reduced	1
1	c.88_90delCAC	p.His30del	Deletion	5	2	UK (4), Ger (1)	Reduced (3), ND (2)	1(4), 2
1	c.G91A	p.Glu31Lys	Missense	1	1	Italy	Absent	2→5A
1	c.T116C	p.Leu39Pro	Missense	6	4	US (3), Italy (2), Ger (1)	Reduced (5), absent (1)	1, 1→5A/M, 2(4)
2	c.C134T	p.Thr45Met	Missense	13	8	JPN (4), US (2), Ger (1), UK (1), Sw (5)	Reduced (6), absent (1), ND (6)	1(6), 1→5A, 2(4), 2→5A /B (2)
2	c.C140A	p.Ala47Asp	Missense	1	1	US	Reduced	2
2	c.A142G	p.Thr48Ala	Missense	1	1	JPN	Reduced	2
2	c.C143T	p.Thr48Ile	Missense	1	1	US	Reduced	1→5M
2	c.C167T	p.Ala56Val	Missense	5	4	US (3), Italy (1), JPN (1)	Reduced (4), ND (1)	1(3), 1→5A, 2
2	c.C172A	p.Pro58Thr	Missense	2	1	US	Normal (2)	1, 2
2	c.C172G	p.Asp58Ala	Missense	1	1	US	Reduced	2→5A/M
2	c.C173G	p.Pro58Arg	Missense	3	1	Italy	Reduced (2), ND (1)	1, 1→5M, 2
2	c.G199A	p.Glu67Lys	Missense	1	1	Fr	Reduced	2
2	c.G223A	p.Val75Met	Missense	22	16	Fr (6), UK (5), US (5), Ger (2), JPN (2), Sp (1), Italy (1)	Normal (1), reduced (10), absent (3), ND (8)	1(6), 1→5A, 2(14), 2→5A
2	c.G223T	p.Val75Leu	Missense	1	1	US	ND	2
2	c.A227C	p.Lys76Thr	Missense	2	2	US	Reduced (1), ND (1)	2(2)
2	c.G229C	p.Asp77His	Missense	1	1	Italy	Reduced	1
2	c.A230G	p.Asp77Gly	Missense	2	1	Italy	Reduced (2)	1, 2
2	c.A239G	p.Gln80Arg	Missense	1	1	Rus	Reduced	2
2	c.248insA	p.Tyr83X	Insertion	1	1	Fr	ND	2
2	c.C256G	p.Arg86Gly	Missense	1	1	US	Reduced	2→5A
2	c.C256T	p.Arg86Cys	Missense	24	18	US (10), Ger (6), JPN (3), UK (3), Italy (1), Sw (1)	Normal (3), reduced (9), ND (12)	1(10), 1→5M, 2(12), 2→5A
2	c.G257A	p.Arg86His	Missense	7	7	JPN (2), Fr (1), Ger (1), Isr (1), Rus (1), US (1)	Reduced (4), absent (1), ND (2)	1→5A, 2(4), 2→5A(2)
2	c.G257T	p.Arg86Leu	Missense	1	1	US	Absent	2
2	c.A263G	p.Tyr88Cys	Missense	1	1	NL	ND	2→5A
2	c.G266A	p.Gly89Asp	Missense	1	1	UK	Normal	1
3	c.A320G	p.Tyr107Cys	Missense	1	1	US	Reduced	2
3	c.326_330insC	p.Thr111HisfsX9	Insertion	1	1	US	Absent	2
3	c.G355A	p.Gly119Arg	Missense	1	1	NL	ND	1
4	c.dup355_361	p.Asp121insGD	Insertion	1	1	JPN	Absent	2
4	c.G399T	p.Glu133Asp	Missense	1	1	US	Reduced	2
5	c.G505T	p.Asn169X	Nonsense	1	1	JPN	Reduced	2→5M
6	c.G538A	p.His180Asn	Missense	1	1	Italy	Reduced	1
7	c.C707G	p.Ala236Gly	Missense	1	1	Italy	Absent	1
7	c.A724T	p.Ser242Cys	Missense	1	1	NL	ND	1
9	c.854_855insG	p.Thr286AspfsX1	Insertion	2	1	UK	Reduced and truncated (1), absent (1)	1(2)
9	c.A919G	p.Met307Val	Missense	1	1	Ger	ND	2
10	c.C961T	p.Arg321X	Nonsense	1	1	JPN	Absent	2→5M
10	c.983_984delC	Multiple products	Deletion	1	1	US	Reduced and truncated	2
10	c.991insA	p.Gly334X	Insertion	1	1	US	Absent	2
10	c.1073_1074delGA	p.Gly358AlafsX135	Deletion	1	1	US	Reduced and truncated	2
10	c.1079delC	p.Pro360HisfsX84	Deletion	2	2	Ger, JPN	Reduced (1), absent (1)	2(2)
10	c.C1090T	p.Arg363X	Nonsense	2	1	Fr	ND (2)	2(2)
11	c.G1430A	p.Arg477Lys	Missense	1	1	Sp	Reduced	2
11	c.T1442A	p.Ile481Asn	Missense	2	1	Italy	Normal (1), reduced (1)	1(2)
12	c.G1453A	p.Asp485Asn	Missense	1	1	US	Reduced	2→5A
12	c.A1454G	p.Asp485Gly	Missense	3	1	Sp	ND (3)	1(3)
12	c.G1508C	p.X503SerextX76	No-stop	2	1	US	Absent (1), ND (1)	2(2)
Int 3	c.360+1G>A	p.Ala92_Asp120del	Splice (donor site)	1	1	JPN	Reduced	2
Int 3	c.361-1G>A	p.fsX201	Splice (acceptor site)	1	1	US	Reduced	2
Int 4	c.[463+1_463+8del; 464-3_464-2insG]	p.fsX178/fsX251	Splice (donor +acceptor site)	1	1	JPN	Reduced	2
Int 6	c.559+5G>A	70% fsX190/30% normal	Splice (donor site)	15	11	US (9), Ger (2), JPN (3), UK (1)	Reduced (12), absent (1), ND (2)	1(6), 1→5M, 2(6), 2→5A(2)
Int 7	c.734+5G>A	ND	Splice (donor site)	4	1	Ger	ND (4)	2 (3), 2→5A
Int 7	c.735-25A>C	ND	Splice (acceptor site)	3	1	UK	Reduced (3)	1(3)
Int 8	c.777+1G>A	p.fsX246	Splice (donor site)	2	2	Australia, US	Absent (1), ND (1)	1, 2
Int 8	c.777+3insT	ND	Splice (donor site)	2	1	Italy	Reduced (2)	1, 2
Int 8	c.778-6G>A	ND	Splice (acceptor site)	1	1	UK	Reduced	1
Int 9	c.(931_932)ins250	ND	Splice site	1	1	JPN	Reduced	1
Int 11	c.(1484_1485)ins118	Normal and abnormal splice products	Splice site	2	1	JPN	Reduced (2)	2→5A(2)

Pt indicates number of patients with the respective mutation; Fam, number of families with the respective mutation; 1→5, WAS score progressing from 1 to 5 because of either A, autoimmunity, or M, malignancy; Fr. France; ND, not done; JPN, Japan; US, United States of America; UK, United Kingdom; Ger, Germany; Sw, Sweden; Sp, Spain; Rus, Russia; Isr, Israel; and NL, The Netherlands.

*There was a total of 173 patients.

†There was a total of 128 families.