# 先天性角化不全症

## 診療の参照ガイド（平成 22 年度版）

先天性角化不全症の診断基準と診療の参照ガイド
作成のためのワーキンググループ

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先天性角化不全症の効果的診断方法の確立と
治療ガイドラインの作成に関する研究
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## 1．緒言

先天性角化不全症（Dyskeratosis congenita; DC）は、爪の萎縮、口腔内白斑、皮膚色素沈着を３徴とする先天性造血不全症候群である。DC患者ではこれらの古典的症状を併せ持つ典型例以外にも、多彩な全身症状を呈する例から血球減少のみの例まで多彩な臨床像を示すため、しばしば臨床診断は困難である[1]。近年、低身長、小脳低形成、小頭症、網膜症などを伴い、独立した疾患と考えられてきたHoyeraal-Hreidarsson症候群、Revesz症候群において、DCと同じ遺伝子変異がみられる事が明らかとなった。さらに、近年の遺伝子変異のスクリーニングにより、特発性再生不良性貧血患者や特発性肺線維症と考えられていた患者のなかに、本症の不全型が含まれている事が明らかにされた[2-4]。

本症における死亡原因としては造血不全が最も高く、60～70％を占める[6,7]。骨髄不全に対する治療として唯一治癒が期待できるのは造血幹細胞移植である。DC患者では治療関連毒性が強く、従来の骨髄破壊的前処置を用いた治療成績は非常に不良であったが、近年の骨髄非破壊的前処置を用いた移植では、治療関連毒性を軽減しつつ良好な生着が得られたとする報告が相次いでいる。しかし、DCは極めてまれな疾患であり、治療研究として得られている情報はきわめて乏しい。

このような事から、海外のデータをもとに我が国のDC患者に対し現時点で最も推奨されると思われる診療ガイドラインを作成した。

## 2．診断

１）疾患概念（図１）

テロメア長の維持機能の障害を背景とし、主に皮膚、爪、口腔粘膜に特徴的な所見を有する遺伝性骨髄不全症候群である。DCは古典的なDCの他に図に示すような最重症型であるHoyeraal-Hreidarsson症候群、Revesz症候群の他、不全型である再生不良性貧血や家族性肺線維症などが存在する。これらの疾患は病像が異なるものの、共通してテロメア長の短縮や、テロメア関連遺伝子の変異がみられることから、一連の疾患と考えられている。

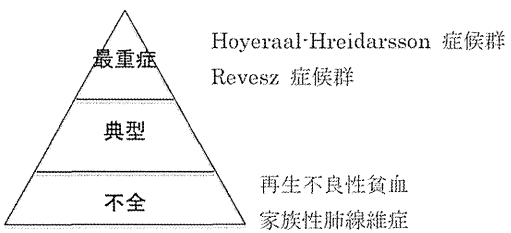


図１　先天性角化不全症の病型

２）診断基準

爪の萎縮、口腔内白斑、皮膚色素沈着などの身体的特徴、汎血球減少がそろっている場合には臨床症状は比較的容易であろうと思われる。しかし、実際にはこれらの身体的特徴がそろわない場合も多く、また症状は多彩かつ重度のものから軽微なものまであるため、そのような患者での診断は臨床症状のみからでは困難である。血球減少、悪性疾患、肺線維症、肝疾患、免疫不全、若年の白髪などの家族歴にも注意すべきである。現在提唱されている診断基準を**表１**に示す[8,9]。診断のための検査として、末梢血を用いたFlow-FISHまたはサザンブロッティングによるテロメア長測定は、簡便で有用である。他の骨髄不全症候群でも時にテロメア長短縮をきたすことがあるため注意が必要であるが、DC患者のテロメア長は他の骨髄不全症候群より特に短縮していることが特徴である[10,11]。

**表１　先天性角化不全症の診断基準**

A．骨髄不全症
　一系統以上の血球減少と骨髄低形成を認める
B．大症状（皮膚、粘膜所見）
　１．網状色素沈着
　２．爪の萎縮
　３．口腔粘膜白斑症
C．小症状（その他の身体所見）
　１．頭髪の喪失、白髪
　２．歯芽の異常
　３．肺病変
　４．低身長、発育遅延
　５．肝障害
　６．食道狭窄
　７．悪性腫瘍
　８．小頭症
　９．小脳失調
　10．骨粗鬆症

狭義な意味での先天性角化不全症は以下の場合に診断する。
骨髄不全および１つ以上の大症状と２つ以上の小症状を満たす

先天性角化不全症の亜型であるHoyeraal-Hreidarsson syndromeやRevesz syndrome、上記の大症状や小症状を伴わない再生不良性貧血、肺線維症は“テロメア病”として広義の意味では先天性角化不全症の類縁疾患であるが、上記の診断基準は適用されない。

３）重症度分類

疾患の重症度としては、概念図を参照されたい。骨髄不全の重症度としては、再生不良性貧血の重症度分類（**表２**）に準じる。

**表２　重症度分類（平成16年度修正）**

| | | |
|---|---|---|
| stage 1 | 軽　症 | 下記以外 |
| stage 2 | 中等症 | 以下の２項目以上を満たす |
| | | 網赤血球　60,000/μl 未満 |
| | | 好中球　1,000/μl 未満 |
| | | 血小板　50,000/μl 未満 |
| stage 3 | やや重症 | 以下の２項目以上を満たし、定期的な赤血球輸血を必要とする |
| | | 網赤血球　60,000/μl 未満 |
| | | 好中球　1,000/μl 未満 |
| | | 血小板　50,000/μl 未満 |
| stage 4 | 重　症 | 以下の２項目以上を満たす |
| | | 網赤血球　20,000/μl 未満 |
| | | 好中球　500/μl 未満 |
| | | 血小板　20,000/μl 未満 |
| stage 5 | 最重症 | 好中球　200/μl 未満に加えて、以下の１項目以上を満たす |
| | | 網赤血球　20,000/μl 未満 |
| | | 血小板　20,000/μl 未満 |

注1　定期的な赤血球輸血とは毎月２単位以上の輸血が必要なときを指す。
注2　この基準は平成10(1998)年度に設定された５段階基準を修正したものである。

4） 診断のフローチャート（図2）

特徴的な身体的異常、骨髄不全、家族歴などからDCが疑われる場合には、末梢血を用いてFlow-FISH またはサザンブロッティングを用いた血球テロメア長測定を行う。また、身体的特徴を有さない再生不良性貧血患者のなかにも、テロメア長の短縮とテロメア関連遺伝子の異常を有する患者がいることがあきらかになっているため、再生不良性貧血患者に対しては、診断時にテロメア長測定を行う事が望ましい。我が国では検査会社でこのような検査は行っていないため、検査が行える施設に問い合わせて検査を依頼する。特徴的な身体所見があり、テロメア長の著明な短縮が証明できれば診断が確定する。遺伝子診断は男性であればDKC 1の変異解析を行う。*DKC1*に変異がない男性患者、または女性であればそれ以外の遺伝子変異について解析を進めるが、既知の遺伝子異常は約半数にしか見られない。

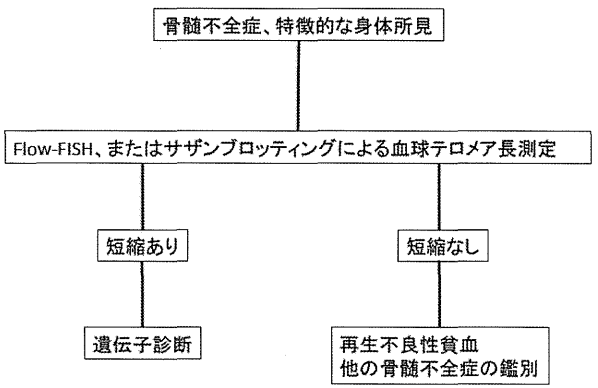


図2　診断のフローチャート

5） 鑑別診断

身体的異常を伴う骨髄不全症として、Fanconi貧血、Schwachman-Diamond症候群、先天性無巨核球性血小板減少症、Pearson症候群などの疾患を鑑別する必要がある。それぞれ特徴的な臨床像があるのでまず臨床像から鑑別していくが、疾患特異的な検査所見や、遺伝子診断もできるようになってきている。

## 3．疫学

1） 発生頻度

我が国においての患者数についてpublishされたものはないが、海外の登録事業からすると、発症頻度は100万人に1人とされる[12]。

2） 自然歴・予後

典型例では身体的異常は幼少期から出現する。爪の萎縮と皮膚色素沈着が10才までに出現し、20才までに骨髄不全が出現し、30才までには90%の症例が骨髄不全を発症する[13]。しかし、症状の種類や、発症時期については患者間で異なり、骨髄不全が初発症状であったり、爪の変化や皮膚色素沈着が重度であっても骨髄不全をきたさないような症例もある。死因としては骨髄不全／免疫不全が60～70%、肺線維症が10～15%、



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悪性疾患が10%とされている[14]。最近の報告では、生存年齢の中央値は49才とされている[1]。

## 4．病因・病態

DC患者細胞のテロメア長は著明に短縮しており、テロメア長の維持機能の障害が疾患の病因であると考えられている。テロメアは染色体末端のTTAGGG 繰り返し配列で、細胞分裂時に起こる染色体の融合や再構成を防いでいる。テロメアの摩耗した細胞では染色体の不安定性が惹起され、アポトーシスに陥る。そのために細胞増殖が盛んな皮膚、骨髄などの組織が高率に犯されるものと考えられている[15-18]。図3に示すように、テロメラーゼ複合体、shelterinという2つの重要なコンポーネントが、正常なテロメア長の維持の役割を担っている。テロメラーゼ複合体はRNA コンポーネントであるTERC を鋳型とし、TERT の逆転写酵素活性によりテロメアを伸長する。shelterinは物理的にテロメアの安定性に関与していると考えられている。現在までにテロメラーゼ複合体をコードする遺伝子のうち、*DKC1*[19]、TERC[17]、*TERT*[8,20,21]、*NOP10*[22]、*NHP2*[23] が、またshelterinの重要なコンポーネントであるTIN 2をコードする*TINF2*[24,25] の遺伝子異常が明らかとなっている（表3）。

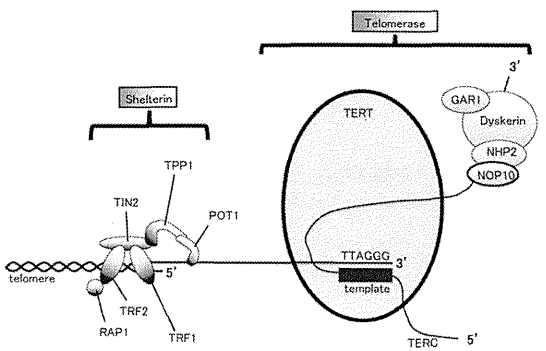


図3　テロメラーゼ複合体の構造

表3　先天性角化不全症の原因遺伝子

| 遺伝子名 | 染色体上の位置 | RNA | アミノ酸 | 機能 | 遺伝形式* | 頻度 |
|---|---|---|---|---|---|---|
| *DKC1* | Xq28 | 1545nt | 514aa | rRNA の pseudouridination<br>テロメラーゼ複合体の安定化<br>TERT の発現抑制 | XR | 30% |
| *TERC* | 3q26.2 | 451nt | 翻訳されない | テロメア複製の鋳型 | AD | ～5% |
| *TERT* | 5p15.33 | 3399nt | 1132aa | テロメア DNA の合成酵素 | AD>AR | ～5% |
| *NHP2* | 5q35.3 | 462nt | 153aa | テロメラーゼ複合体の安定化 | AR | 稀 |
| *NOP10* | 15q14-q15 | 195nt | 64aa | テロメラーゼ複合体の安定化 | AR | 稀 |
| *TINF2* | 14q12 | 1065nt | 354aa | テロメア末端の保護 | AD | ～11% |

＊孤発例の場合も多く、必ずしも遺伝形式を特定できない。
XR：X連鎖劣性、AD：常染色体優性、AR：常染色体劣性

## 5．臨床症状

爪の萎縮、口腔内白斑、皮膚色素沈着が３徴であるが、その他にも診断基準に示すように全身性に異常をきたす。これらの症状の出現時期は年齢に依存し、出現後は通常年齢をおって重症度が増していく。悪性疾患は通常20～40才台に出現する。DC患者では健常人に比較して11倍の罹患率とされる[26]。扁平上皮癌、骨髄異形成症候群、骨髄性白血病の頻度が高い。

## 6．治療法・治療指針

DCに対する根本的な治療法はないため、合併症に対するサポートが中心となる。骨髄不全に対する治療としては、再生不良性貧血の重症度分類による中等症の症例に対してはダナゾールなどの蛋白同化ホルモンを投与する。蛋白同化ホルモンの投与により、約半数の患者で一時的な血液学的反応がみられることがある。血液学的反応がみられるまでに２-３ヶ月を要する事もある。副作用としては、肝障害、男性化、気分の変容などがあり、これらの症状が出ないように投与量を調節する。

重症と判断される場合には、現時点では造血幹細胞移植が唯一の治療である。しかしながら、DCは極めて稀な疾患であるため、過去の報告は極めて少ない。過去の報告から、骨髄破壊的前処置の治療成績は極めて不良で、21例中14例が死亡しており、特に非血縁ドナーからの移植での生存者はない[14,27]。Alterらの過去の文献を含めたすべての前処置を含む65症例のreviewによると、血縁者間移植では５年生存率71％に対し、非血縁者間移植では２年生存率は31％であった[28]。近年、骨髄非破壊的前処置が行われるようになってきており、少ない合併症で血液学的回復を得る事が可能となってきている[28-30]。表４に推奨する前処置を示す。移植ドナーはHLA一致同胞が第一選択であるが、潜在的な患者である事を除外するため、家族内のテロメア長スクリーニングを行うべきである。

表４　先天性角化不全症に対する治療方針

1. 軽症
   経過観察

2. 中等症
   酢酸メテノロンまたはダナゾールの投与

3. やや重症型、重症、最重症
   ・40歳未満で臓器障害（肝臓、肺等）がなければ、HLA一致血縁あるいは非血縁ドナーからの同種骨髄移植*
   ・40歳以上あるいは臓器障害があれば酢酸メテノロンまたはダナゾールの投与

   移植前治療はリン酸フルダラビンを含む骨髄非破壊的前治療が望ましい。
   例）・HLA一致血縁ドナー　　Flu：25mg/m²×4日、CY：750mg/m²×4日
   ・HLA一座不一致血縁ドナー　Flu：25mg/m²×4日、CY：750mg/m²×4日、ATG：2.5mg/kg×4日
   ・HLA一致非血縁ドナー　　TBI：3 Gy

Flu：fludarabine、CY：cyclophosphamide、ATG：antithymocyteglobluine、TBI：Total body irradiation

## 7．問題点・将来展望

我が国のDC患者は、小児血液学会の再生不良性貧血委員会において患者数の把握や追跡調査がされている。しかし、DCは小児に特有の疾患ではなく、成人で診断される場合も多い。特に、悪性腫瘍、肺線維症の合併や、自然歴の把握のためには、皮膚科、呼吸器内科、耳鼻咽喉科などを含めた疾患登録システムが望まれる。また、骨髄非破壊的前処置を用いた移植により短期的な予後に関しては改善が見られているが、移植がDCの自然歴に及ぼす長期的な影響、予後に関しては不明であり、小児から成人への受け渡しなど、長期的なフォローアップシステムが必要である。



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## 参考文献


1）Shimamura A, Alter BP. Pathophysiologyand managementof inheritedbone marrow failure syndromes Blood reviews. 2010 May；24(3)：101-22.

2）Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerasereverse transcriptase, in aplastic anemia The New England journal of medicine 2005 Apr 7；352(14)：1413-24.

3）Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome Blood. 2003 Aug 1；102(3)：916-8.

4）Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. The New England journal of medicine 2007 Mar 29;356(13)：1317-26.

5）Cossu F, Vulliamy TJ, Marrone A, Badiali M, Cao A, Dokal I. A novel DKC 1 mutation severe combined immunodeficiency (T+B-NK- SCID) and bone marrow transplantationin an infant with Hoyeraal-Hreidarsson syndrome British journal of haematology 2002 Dec；119(3)：765-8.

6）Knight S, Vulliamy T, Copplestone A, Gluckman E, Mason P, Dokal I. Dyskeratosis Congenita (DC) Registry：identification of new features of DC. British journal of haematology 1998 Dec；103(4)：990-6.

7）Dokal I. Dyskeratosis congenita: recent advances and future directions J Pediatr Hematol Oncol. 1999 Sep-Oct；21(5)：344-50.

8）Vulliamy TJ, Marrone A, Knight SW, Walne A, Mason PJ, Dokal I. Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation Blood. 2006 Apr 1；107(7)：2680-5.

9）Dokal I. Dyskeratosis congenitain all its forms. British journal of haematology 2000 Sep；110(4)：768-79.

10）Baerlocher GM, Vulto I, de Jong G, Lansdorp PM. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). Nature protocols 2006；1(5)：2365-76.

11）Savage SA, Dokal I, Armanios M, Aubert G, Cowen EW, Domingo DL, et al. Dyskeratosis congenita: The first NIH clinical research workshop Pediatric blood & cancer. 2009 May 4.

12）Walne AJ, Marrone A, Dokal I. Dyskeratosis congenita: a disorder of defective telomere maintenance? Int J Hematol 2005 Oct；82(3)：184-9.

13）Kirwan M, Dokal I. Dyskeratosis congenita: a genetic disorder of many faces. Clinical genetics 2008 Feb；73(2)：103-12.

14）Walne AJ, Dokal I. Advances in the understandingof dyskeratosis congenita British journal of haematology 2009 Feb 4.

15）Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita

Nature. 1999 Dec 2 ; 402(6761) : 551-5.

16) Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL. Telomerase is required to slow telomere shorteningand extendreplicative lifespan of HSCs duringserial transplantation. Blood. 2003 Jul 15 ; 102(2) : 517-20.

17) Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nature genetics 2004 May ; 36(5) : 447-9.

18) Goldman FD, Aubert G, Klingelhutz AJ, Hills M, Cooper SR, Hamilton WS, et al. Characterization of primitive hematopoietic cells from patients with dyskeratosis congenita. Blood. 2008 Feb 29.

19) Heiss NS, Knight SW, Vulliamy TJ, Klauck SM, Wiemann S, Mason PJ, et al. X-linked dyskeratosis congenitais caused by mutationsin a highly conservedgene with putativenucleolar functions. Nature genetics 1998 May ; 19(1) : 32-8.

20) Marrone A, Walne A, Tamary H, Masunari Y, Kirwan M, Beswick R, et al. Telomerase reverse-transcriptase homozygousmutationsin autosomalrecessive dyskeratosis congenitaand Hoyeraal-Hreidarsson syndrome. Blood. 2007 Dec 15 ; 110(13) : 4198-205.

21) Armanios M, Chen JL, Chang YP, Brodsky RA, Hawkins A, Griffin CA, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proceedings of the National Academy of Sciences of the United States of America. 2005 Nov 1 ; 102(44) : 15960-4.

22) Walne AJ, Vulliamy T, Marrone A, Beswick R, Kirwan M, Masunari Y, et al. Genetic heterogeneityin autosomal recessive dyskeratosis congenita with one subtype due to mutations in the telomerase-associated protein NOP10. Hum Mol Genet. 2007 Jul 1 ; 16(13) : 1619-29.

23) Vulliamy T, Beswick R, Kirwan M, Marrone A, Digweed M, Walne A, et al. Mutations in the telomerase componentNHP2 cause the premature ageing syndrome dyskeratosis congenita. Proceedings of the National Academy of Sciences of the United States of America. 2008 Jun 10 ; 105(23) : 8073-8.

24) Walne AJ, Vulliamy TJ, Beswick R, Kirwan M, Dokal I. TINF2 mutationsresult in very short telomeres: Analysis of a large cohort of patients with dyskeratosis congenitaand related bone marrow failure syndromes. Blood. 2008 Jul 30.

25) Savage SA, Giri N, Baerlocher GM, Orr N, Lansdorp PM, Alter BP. TINF2, a componentof the shelterin telomereprotectioncomplex, is mutatedin dyskeratosis congenita. American journal of human genetics 2008 Feb ; 82(2) : 501-9.

26) Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. Blood. 2009 Jun 25 ; 113 (26) : 6549-57.

27) de la Fuente J, Dokal I. Dyskeratosis congenita: advancesin the understandingof the telomerasedefect and the role of stem cell transplantation. Pediatr Transplant. 2007 Sep ; 11(6) : 584-94.

28) Dietz AC, Orchard PJ, Baker KS, Giller RH, Savage SA, Alter BP, et al. Disease-specific hematopoietic cell transplantation: nonmyeloablative conditioning regimen for dyskeratosis congenita. Bone marrow transplantation. 2010 Apr 12.

29) Ostronoff F, Ostronoff M, Calixto R, Florencio R, DominguesMC, Souto Maior AP, et al. Fludarabine, cyclophosphamide and antithymocyteglobulin for a patientwith dyskeratosis congenitaand severe bone marrow failure. Biol Blood Marrow Transplant. 2007 Mar ; 13(3) : 366-8.

30) Nobili B, Rossi G, De Stefano P, Zecca M, Giorgiani G, Perrotta S, et al. Successful umbilical cord blood transplantationin a child with dyskeratosis congenita after a fludarabine-basedreduced-intensityconditioning regimen. British journal of haematology. 2002 Nov ; 119(2) : 573-4.

# Acceptable HLA-mismatching in unrelated donor bone marrow transplantation for patients with acquired severe aplastic anemia

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**We retrospectively analyzed the effect of HLA mismatching (HLA-A, -B, -C, -DRB1, -DQB1) with molecular typing on transplantation outcome for 301 patients with acquired severe aplastic anemia (SAA) who received an unrelated BM transplant through the Japan Marrow Donor Program. Additional effect of HLA-DPB1 mismatching was analyzed for 10 of 10 or 9 of 10 HLA allele-matched pairs (n = 169). Of the 301 recipient/donor pairs, 101 (33.6%) were completely matched at 10 of 10 alleles, 69 (23%) were mismatched at 1 allele, and 131 (43.5%) were mismatched at ≥ 2 alleles. Subjects were classified into 5 subgroups: complete match group (group I); single-allele mismatch group (groups II and III); multiple alleles restricted to HLA-C, -DRB1, and -DQB1 mismatch group (group IV); and others (group V). Multivariate analysis indicated that only HLA disparity of group V was a significant risk factor for poor survival and grade II-IV acute GVHD. HLA-DPB1 mismatching was not associated with any clinical outcome. We recommend the use of an HLA 10 of 10 allele-matched unrelated donor. However, if such a donor is not available, any single-allele or multiple-allele (HLA-C, -DRB1, -DQB1) mismatched donor is acceptable as an unrelated donor for patients with severe aplastic anemia. (*Blood*. 2011;118(11):3186-3190)**

## Introduction

BM transplantation from an unrelated donor (UBMT) is indicated as salvage therapy for patients with severe aplastic anemia (SAA) who fail to respond to immunosuppressive therapy. Early results of UBMT have not been encouraging because of a high incidence of graft failure and GVHD.[1-3] The Center for International Blood and Marrow Transplant Research (CIBMTR) reported the outcome of 232 patients with SAA who received an UBM transplant between 1988 and 1998.[3] The 5-year probabilities of overall survival (OS) were 39% and 36% after matched unrelated and mismatched unrelated donor transplantations, respectively. We previously reported the outcome of 154 patients with SAA who received an UBM transplant between 1993 and 2000 through the Japan Marrow Donor Program (JMDP).[4] The 5-year OS rate was 56% in that study.

In several recent studies, the effect of HLA high-resolution matching on outcome of patients who received an UBM transplant has been elucidated.[5-8] However, results have been derived primarily from an analysis of patients with hematologic malignancies. Major obstacles for UBMT are different between patients with hematologic malignancies and patients with SAA. Relapse is a main cause of death for patients with hematologic malignancies, and GVL effect may result in decrease of relapse rate. In contrast, graft failure is the main problem, and GVHD is the only negative effect for patients with SAA. Therefore, optimal HLA matching may be different between these 2 populations. Algorithms for donor selection derived from an analysis of patients with hematologic malignancies might not be useful for patients with SAA. However, a few studies have focused on the clinical significance of HLA-allele compatibility in patients with SAA.[2,4,9,10]

In a previous study, we analyzed the clinical significance of HLA allele mismatching in 142 patients with SAA, in whom data of high-resolution typing of HLA-A, -B, and -DRB1 were available.[4] Mismatching of HLA-A or -B alleles between donor and recipient was a strong risk factor for acute and chronic GVHD and OS, whereas mismatching of the HLA-DRB1 allele did not have a significant effect on patient outcomes. In the study from the National Marrow Donor Program, mismatching of HLA-DRB1 was the most crucial risk factor for OS.[2] These results indicate that better donor selection through high-resolution typing might result in improved outcome in patients with SAA who receive an UBM transplant. In fact, several recent studies showed a significantly improved outcome in patients with SAA who received and UBM transplant over time.[11,12] In particular, better HLA matching by high-resolution typing has been thought to contribute to these improvements.[4,9-11]

On the contrary, restricting BMT to donor-recipient pairs perfectly matched at high-resolution typing reduces the chance of undergoing UBMT for many patients. Therefore, strategies for selecting a partially HLA allele mismatched donor are required when a full matched donor cannot be identified. Here, we report a detailed analysis of outcome in 301 patients with SAA who were typed for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 by a molecular technique and underwent UBMT through the JMDP.

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## Methods

### Patients

From February 1993 to April 2005, 380 consecutive patients with acquired SAA received an UBM transplant through the JMDP. Patients with inherited AA, such as Fanconi anemia, and patients who received a BM transplant > 2 times were excluded. This study includes 301 patients in whom molecular analysis of HLA-A, -B, -C, DRB1, and -DQB1 were performed by DNA-based methods. HLA-DPB1 was analyzed in 299 of these patients. The previous study included 142 patients in whom molecular typing was performed only for HLA-A, -B, and -DRB1.

Characteristics of the 301 patients and donors are shown in Table 1. Briefly, patients (173 males and 128 females) were between birth and 64 years of age (median, 17 years of age). The median disease duration before BMT was 43 months (range, 4-436 months). All patients failed conventional immunosuppressive therapies and were considered candidates for UBMT. All patients or their guardians gave informed consent for transplantation and submission of the data to the JMDP.

### Transplantation procedure

Characteristics of the transplantation procedures are also shown in Table 1. Patients underwent transplantations at individual centers following the local protocols for preconditioning regimens and GVHD prophylaxis. The various preconditioning regimens used by individual centers were classified into 5 categories: TBI or LFI + CY + ATG (n = 128), TBI or LFI + CY (n = 103), TBI or LFI + CY + Flu with or without ATG (n = 39), CY + Flu + ATG (n = 8), and others (n = 23). In 130 patients, CsA and MTX were used for prophylaxis against GVHD; 134 patients received FK instead of CsA. The remaining 35 patients received other GVHD prophylaxis. Ex vivo T-cell depletion was not used for any patient. The median number of infused nucleated marrow cells was $3.1 \times 10^8$/kg. One-half (n = 150) of the transplantations were performed before 2000, and 151 were done after 2001.

### HLA typing and definition of mismatching

HLA matching between patients and donors was based on HLA serotyping according to the standard technique. Partial HLA-A and -B alleles and complete HLA-DRB1 alleles were identified as confirmatory HLA typing during the coordination process, and HLA-A, -B, -C, -DQB1, and -DPB1 alleles were retrospectively reconfirmed or identified after transplantation. Molecular typing of HLA-A, -B, -C, -DQB1, -DRB1, and -DPB1 alleles was performed by the Luminex microbead method (Luminex 100 system) adjusted for the JMDP and in part by the sequencing-based typing method. Mismatching was defined as the presence of donor antigens or alleles not shared by the recipient (rejection vector) or the presence of recipient antigens or alleles not shared by the donor (GVHD vector).

### Definition of transplantation-related events

The day of engraftment was defined as the first day of 3 consecutive days on which neutrophil count exceeded $0.5 \times 10^9$/L. Patients who did not reach neutrophil counts > $0.5 \times 10^9$/L for 3 consecutive days after transplantation were considered to have primary graft failure. Patients with initial engraftment in whom absolute neutrophil counts declined to < $0.5 \times 10^9$/L subsequently were considered to have secondary graft failure. Acute GVHD was evaluated according to standard criteria in patients who achieved engraftment, and chronic GVHD was evaluated according to standard criteria in patients who achieved engraftment and survived > 100 days after transplantation.

### Data collection and statistical analysis

Transplantation data were collected with the use of standardized forms provided by the JMDP. Patient baseline information and follow-up reports were submitted at 100 days and annually after transplantation. Analysis of patient outcome was performed with the date of last reported follow-up or date of death. Data were analyzed as of July 1, 2007.

Probability of OS and 95% confidence interval (95% CI) were estimated from the time of transplantation according to the Kaplan-Meier method. Cumulative incidence of neutrophil engraftment at day 42 was analyzed in the whole of patients by treating deaths until day 42 as a competing risk. Cumulative incidence of acute GVHD at day 100 was analyzed in patients who sustained engraftment by treating deaths until day 100 as a competing risk. Cumulative incidence of chronic GVHD at day 365 was analyzed in patients who sustained engraftment and survived longer than day 100 by treating deaths until day 365 as a competing risk. In univariate analysis, the log-rank test or Gray test was used to assess the significance of HLA allele mismatching on clinical outcomes. The Mann-Whitney *U* test was used to compare the median days of neutrophil engraftment. The chi-square test or Mann-Whitney *U* test was used to compare patient characteristics and transplantation procedures between the patient groups. All *P* values < .05 were considered statistically significant, whereas *P* values between .05 and .1 were considered as marginally significant.

Multivariate analyses were performed to assess the effect of HLA allele mismatching on the clinical outcome by Cox proportional hazard model (each mismatched group vs fully matched group; hazard risk = 1.0 as a reference group). Factors other than HLA mismatching included in the models were patient age, patient sex, donor age, donor sex, disease duration before BMT, infused cell dose, matching of ABO blood type, GVHD prophylaxis, and preconditioning regimens.

## Results

### HLA matching by DNA typing

Of the 301 recipient/donor pairs, 101 pairs (33%) were completely matched at HLA-A, -B, -C, -DRB1, and -DQB1 allele; 69 pairs (23%) were mismatched at 1 HLA allele; 59 pairs (20%) were mismatched at 2 HLA alleles; and 72 pairs (24%) were mismatched at ≥ 3 alleles (Table 2). The number and frequency of 1-allele and 2-allele mismatches in either GVHD or rejection vector or both vectors in each HLA allele were 55 (18.3%) and 7 (2.3%) in HLA-A allele, 32 (10.6%) and 2 (0.7%) in HLA-B allele, 130 (43.2%) and 10 (3.3%) in HLA-C allele, 68 (22.6%) and 5 (1.7%) in HLA-DRB1 allele, 80 (26.6%) and 13 (4.3%) in HLA-DQB1 allele, and 179 (59.5%) and 44 (14.6%) in HLA-DPB1 allele, respectively. Because the frequency of mismatching was too high at the DPB1 allele, analysis of DPB1 mismatching was separated from that of other alleles. In addition, because the number of single-allele mismatched pairs of HLA-A, -B, -C, -DRB1, and -DQB1 were too small for separate analyses, HLA-A and -B were grouped into the mismatch of the HLA-A or HLA-B allele (A/B) and HLA-DRB1 and -DQB1 into the mismatch of the HLA-DRB1 or HLA-DQB1 allele (DRB1/DQB1), respectively.

### Survival

Of the 301 patients, 202 are alive at the time of analysis with an observation time from 3 to 128 months (median, 44 months) after transplantation. Five-year OS was 66.3% (95% CI, 60.7%-72.5%) in the whole population (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Subgroup analyses were performed in 8 main subgroups (> 15 recipients) as follows: (1) complete match group (n = 101), (2) single locus (A/B) mismatch group (n = 20), (3) single (C) mismatch group (n = 42), (4) 2 loci (A/B + C) mismatch group (n = 20), (5) 2 loci (DRB1/DQB1) mismatch group (n = 19), (6) 3 loci (A/B + C) mismatch group (n = 15), (7) 3 loci (C + DRB1/DQB1) mismatch group (n = 29), and

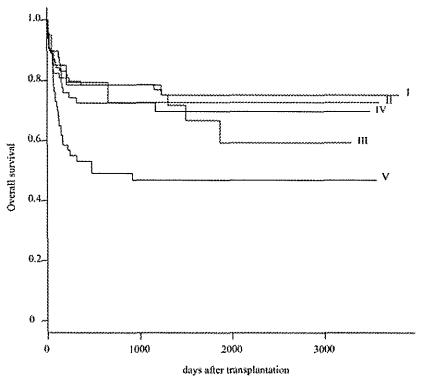


**Figure 1. Kaplan-Meier estimates of OS in 5 HLA groups.**

(8) 3 loci (A/B + C + DRB1/DQB1) mismatch group (n = 21). OS was significantly worse in the following groups than in the complete match group (75.2%): 2 loci (A/B + C) mismatch group (49.0%; $P = .022$), ≥ 3 loci (A/B + C) mismatch group (40.0%; $P = .002$), and A/B + C + DRB1/DQB1 mismatch group (56.1%; $P = .031$; supplemental Table 1).

On the basis of these primary results, 301 patients were reclassified into 5 subgroups: HLA complete match group (group I; n = 101), single-allele (A/B) mismatch group (group II; n = 20), single-allele (C or DRB1/DQB1) mismatch group (group III; n = 49), multiple-allele (restricted to C or DRB1/DQB1) mismatch group (group IV; n = 68), and others (group V; n = 63). The probability of OS at 5 years was 75.2% (95% CI, 84.8%-66.7%) in group I, 72.7% (95% CI, 96.7%-54.7%) in group II, 66.7% (95% CI, 85.1%-52.3%) in group III, 69.7% (95% CI, 82.6%-58.8%) in group IV, and 46.8% (95% CI, 61.7%-35.5%) in group V, respectively (Table 3; Figure 1). Survival rate was significantly inferior in group V than in group I ($P = .003$).

To avoid or minimize the effect of other HLA alleles mismatching, the effect of HLA-DPB1 mismatching was evaluated in group I (n = 101) and groups II + III (n = 69), independently. HLA-DPB1 was matched in 51 recipient/donor pairs (30%) and mismatched in 118 pairs (70%). Patient characteristics and transplantation procedures were not different between HLA-DPB1 matched and mismatched groups (supplemental Table 2). The probability of OS at 5 years in group I was equivalent between the HLA-DPB1 matched group (74.4%; 95% CI, 93.2%-59.4%) and the HLA-DPB1 mismatched group (75.7%; 95% CI, 87.2%-65.8%; $P = .894$; Table 4; Figure 2A). It was also equivalent in groups II + III (71.4%; 95% CI, 93.6%-54.5% in the HLA-DPB1 matched group and in the HLA-DPB1 mismatched group (67.1%; 95% CI, 85.6%-52.5%; $P = .826$; Table 4; Figure 2B). Multivariate analysis identified significant unfavorable variables as follows: recipient age (0-10 years: relative risk [RR] = 1.0; 11-20 years: RR = 4.092, $P = .002$; 21-40 years: RR = 3.970, $P = .004$; > 41 years: RR = 5.241, $P = .003$), conditioning regimen (Flu + CY + TBI/LFI ± ATG: RR = 1.0; CY + TBI/LFI: RR = 4.074, $P = .058$; others: RR = 6.895, $P = .013$), HLA mismatching (group I: RR = 1.0; group V: RR = 1.967, $P = .023$), donor sex (female: RR = 1.0; male: RR = 1.850, $P = .016$), and GVHD prophylaxis (FK + MTX: RR = 1.0; other: RR = 1.754, $P = .024$), blood type (ABO match or minor mismatch: RR = 1.0; major mismatch or bidirection: RR = 1.948, $P = .005$), and disease duration (< 7 years: RR = 1.0; > 7 years: RR = 1.540, $P = .084$; Table 5).

### Engraftment

The cumulative incidence of neutrophil engraftment at day 42 was evaluated in 300 patients. It was 90.3% (95% CI, 93.7%-86.9%) in the whole population. Subgroup analyses showed that it was 93.0% (95% CI, 98.2%-87.8%) in group I, 90.0% (95% CI, 100%-74.6%) in group II, 89.8% (95% CI, 98.9%-80.7%) in group III, 92.6% (95% CI, 99.2%-86.0%) in group IV, and 84.1% (95% CI, 93.4%-74.8%) in group V ($P = .185$; Table 3). The median time to engraftment was 17 days in group I; 18 days in groups II, III, and IV; and 19 days in group V. Engraftment was marginally delayed in group V compared with group I ($P = .053$). Additional HLA-DPB1 mismatching did not affect the cumulative incidence of engraftment in the 10 of 10 and 9 of 10 matched groups, respectively (Table 4). In multivariate analysis, blood type (ABO match or

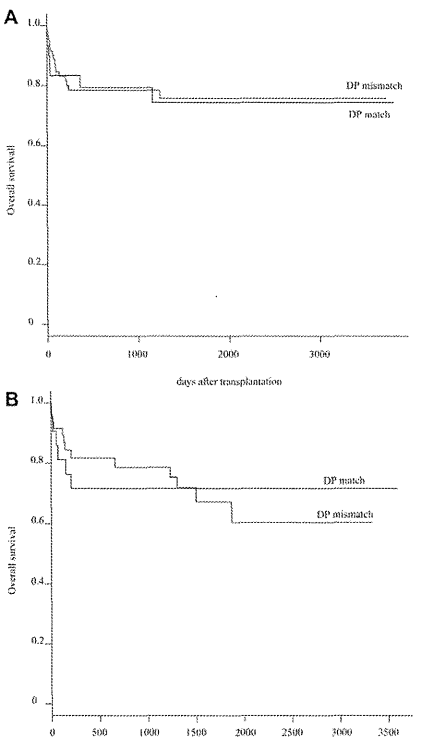


**Figure 2. OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group.** (A) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 10 of 10 HLA allele matched pairs. (B) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 9 of 10 HLA allele matched pairs.







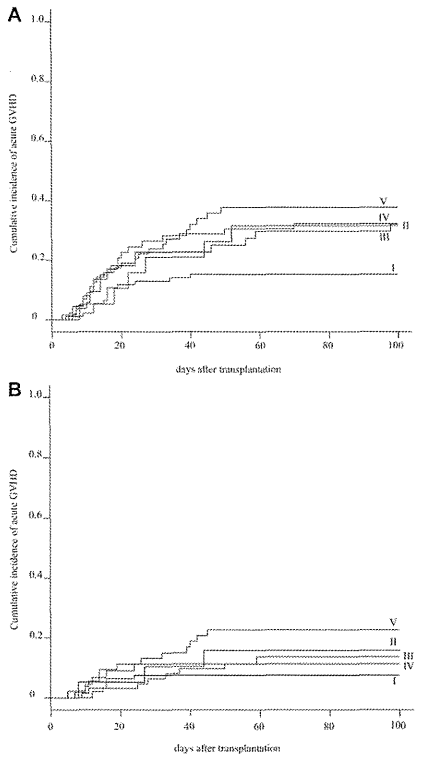


**Figure 3. Cumulative incidence of acute GVHD.** (A) Cumulative incidence of grade II-IV acute GVHD in 5 HLA groups. (B) Cumulative incidence of grade III-IV acute GVHD in 5 HLA groups.

minor mismatch: RR = 1.0; major mismatch or bidirection pair: RR = 5.102, $P = .039$) and HLA mismatching (group I: RR = 1.0; group V: RR = 4.906, $P = .035$) were significant risk factors for engraftment.

### Acute GVHD

The cumulative incidence of acute GVHD at day 100 was evaluated in 272 patients. The cumulative incidence of grade II-IV and grade III-IV acute GVHD was 27.2% (95% CI, 32.5%-21.9%) and 12.9% (95% CI, 16.9%-8.9%) in the whole population, respectively (supplemental Figure 2). Subgroup analyses showed that the cumulative incidence of grades II-IV acute GVHD was statistically lower in group I (15.1%; 95% CI, 22.4%-7.8%) than in group V (37.7%; 95% CI, 50.9%-24.5%; $P = .037$), and marginally lower than in group III (31.8%; 95% CI, 45.8%-17.8%) and group IV (31.7%; 95% CI, 43.3%-20.1%; Table 3; Figure 3A). Whereas the cumulative incidence of grade III-IV acute GVHD was not significantly different among 5 groups: 7.5% (95% CI, 24.6%-0%) in group I, 15.8% (95% CI, 32.7%-0%) in group II, 13.6% (95% CI, 23.9%-3.3%) in group III, 11.1% (95% CI, 18.9%-3.3%) in group IV, and 22.6% (95% CI, 34.0%-11.2%) in group V ($P = .139$; Table 3; Figure 3B). Additional HLA-DPB1 mismatching evaluated in 155 patients did not affect the cumulative incidence of grade II-IV acute GVHD in the 10 of 10 and 9 of 10 matched groups, respectively (Table 4). Multivariate analysis showed that a significantly higher incidence of grade II-IV acute GVHD was associated with HLA mismatching (group I: RR = 1.0; group III: RR = 3.975, $P = .002$; group IV: RR = 3.334, $P = .004$; group V: RR = 3.665, $P = .002$). Other significant risk factors were the preconditioning regimen (Flu +CY + TBI/LFI ± ATG: RR = 1.0; TBI/LFI + CY: RR = 5.224, $P = .003$), and donor sex (female: RR = 1.0; male: RR = 1.844, $P = .034$; supplemental Table 3).

### Chronic GVHD

The cumulative incidence of chronic GVHD at day 365 was evaluated in 232 patients. It was 24.5% (95% CI, 30.3%-18.7%) in the whole population. Subgroup analyses showed that it was comparable among the 5 HLA groups: 19.8% (95% CI, 28.8%-10.8%) in group I, 26.3% (95% CI, 49.3%-3.3%) in group II, 28.2% (95% CI, 43.3%-13.1%) in group III, 26.9% (95% CI, 39.2%-14.6%) in group IV, and 27.3% (95% CI, 42.1%-12.5%) in group V ($P = .922$; Table 3; supplemental Figure 3). HLA-DPB1 mismatching did not affect the cumulative incidence of chronic GVHD (Table 4).

## Discussion

The survival rate in UBMT has increased substantially over the past 10 years in patients with SAA.[8-15] A 5-year survival rate of 90% has been reported in a small series of children.[16,17] A recent meta-analysis showed that detailed HLA-matching facilitated by DNA-based typing has contributed to the improved survival rate in patients with SAA who received an UBM transplant.[18] However, many patients with SAA who need hematopoietic stem cell transplantation do not have an HLA-complete matched donor. Our multivariate analysis indicated that among 4 HLA-mismatched groups, only HLA disparity of group V was a statistically significant unfavorable variable. We conclude that any type of HLA single-allele mismatch or multiple-allele mismatch within HLA-C and HLA class II (DRB1 or DQB1) is acceptable as an unrelated donor when an HLA complete match donor is unavailable.

We previously reported that HLA class I allele mismatching (HLA-A or -B) but not class II allele (HLA-DRB1) mismatching was a significant risk factor for survival when 6 alleles were analyzed.[4] HLA-A or -B mismatching pairs in the previous study were separated into 2 groups in the current study in which 10 alleles were analyzed. One group was a true single-allele mismatching pair of HLA-A or -B alleles (group II), and another was a multiple-allele mismatching pair of HLA-A or -B plus HLA-C and/or class II HLA alleles (group V). Because HLA-C and -DQB1 alleles were not typed, this type of multiple-allele mismatching might be mistaken as a single-allele mismatching pair, which was the reason for the inferior outcome of HLA-class I mismatching pairs in our previous study.

As the same in our previous study, mismatching of HLA-DRB1 did not provide a significant impact on clinical outcome. An HLA-DRB1 mismatching pair was also classified into a true single-allele mismatching of HLA-DRB1 (group III) and HLA-DRB1 plus HLA-C and/or HLA-DQB1 mismatching pairs (group IV). Interestingly, multiple mismatching of group IV was not

associated with increased mortality, which may explain why mismatching of HLA-DRB1 did not have a deleterious effect in the previous study.

The effect of HLA-DPB1 mismatching was also evaluated in HLA complete matched pairs (n = 101) and single-allele mismatched pairs (n = 69). The importance of DPB1 matching in the UBMT setting has been mainly discussed in patients with hematologic malignancies. Although results were controversial in early reports, recent studies support a significant effect of DPB1 mismatching on the incidence of acute GVHD, disease relapse, and OS.[19-22] In a large dataset of the International Histocompatibility Working Group, there was a statistically significant higher risk of both grade II-IV and grade III-IV acute GVHD.[19] The increased risk of acute GVHD was accompanied by a statistically significant decrease in disease relapse, probably because of the GVL effect, which offset the deleterious effect of acute GVHD. Survival rate was significantly better in DPB1-matched transplantations in patients with standard-risk leukemia but not in advanced leukemia. Conversely, in the HLA-mismatched group, there was a significant survival advantage in DPB1 mismatched pairs.

We expected that DPB1 matching might be beneficial for patients with AA who do not need the GVL effect. However, clinical outcomes, including incidence of acute GVHD, were not affected by DPB1 mismatching. HLA-DPB1 typing may not be essential to the donor selection algorithm for patients with SAA. Indeed, HLA-DPB1 mismatching was observed in 74% of recipient/donor pairs, and it may be practically difficult to find HLA 12 of 12 matched donors.

In conclusion, this retrospective study confirms the importance of HLA matching between recipients and donors to improve the outcome of UBMT for patients with SAA patients. However, this study showed that only 33% of patients received transplants from an HLA 10 of 10 matched donor. The availability of unrelated hematopoietic stem cell transplants can be increased through the judicious selection of donors with HLA mismatches that do not substantially lower survival.

## Authorship

Contribution: H. Yagasaki analyzed the data and wrote the paper; S. Kojima designed the research and analyzed the data; and H. Yabe, K.K., H.K., H.S., M.T., S. Kato, T.K., Y.M., and Y.K performed and supervised the research.

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

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## References


1. Hows J, Szydlo R, Anasetti C, Camitta B, Gajewski J, Gluckman E. Unrelated donor marrow transplants for severe acquired aplastic anemia. *Bone Marrow Transplant.* 1992;10(suppl 1): 102-106.
2. Deeg HJ, Seidel K, Casper J, et al. Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy. *Biol Blood Marrow Transplant.* 1999;5(4):243-252.
3. Passweg JR, Pérez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant.* 2006;37(7):641-649.
4. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood.* 2002; 100(3):799-803.
5. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood.* 2004;104(7):1923-1930.
6. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood.* 2002;99(11):4200-4206.
7. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant.* 2007;13(3):315-328.
8. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood.* 2007;110(13): 4576-4583.
9. Kim SY, Lee JW, Lim J, et al. Unrelated donor bone marrow transplants for severe aplastic anemia with conditioning using total body irradiation and cyclophosphamide. *Biol Blood Marrow Transplant.* 2007;13(7):863-867.
10. Perez-Albuerne ED, Eapen M, Klein J, et al. Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. *Br J Haematol.* 2008;141(2):216-223.
11. Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica.* 2007;92(5): 589-96.
12. Viollier R, Socie G, Tichelli A, et al. Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. *Bone Marrow Transplant.* 2008;41(1):45-50.
13. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant.* 2005;36(11):947-950.
14. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood.* 2006;108(5):1485-1491.
15. Lee JW, Cho BS, Lee SE, et al. The outcome of unrelated hematopoietic stem cell transplants with total body irradiation (800 cGy) and cyclophosphamide (120 mg/kg) in adult patients with acquired severe aplastic anemia. *Biol Blood Marrow Transplant.* 2011;17(1):101-108.
16. Yagasaki H, Takahashi Y, Hama A, et al. Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia. *Bone Marrow Transplant.* 2010;45(10):1508-1513.
17. Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. *Biol Blood Marrow Transplant.* 2006;12(12):1277-1284.
18. Peinemann F, Grouven U, Kröger N, Pittler M, Zschorlich B, Lange S. Unrelated donor stem cell transplantation in acquired severe aplastic anemia: a systematic review. *Haematologica.* 2009; 94(12):1732-1742.
19. Shaw BE, Gooley TA, Malkki M, et al. The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation. *Blood.* 2007;110(13): 4560-4566.
20. Kawase T, Matsuo K, Kashiwase K, et al. HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism. *Blood.* 2009;113(12):2851-2285.
21. Crocchiolo R, Zino E, Vago L, et al. Nonpermissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation. *Blood.* 2009; 114(7):1437-1444.
22. Shaw BE, Mayor NP, Russell NH, et al. Diverging effects of HLA-DPB1 matching status on outcome following unrelated donor transplantation depending on disease stage and the degree of matching for other HLA alleles. *Leukemia.* 2010;24(1):58-65.

# Relapse of aplastic anemia in children after immunosuppressive therapy: a report from the Japan Childhood Aplastic Anemia Study Group

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## ABSTRACT

### Background
Although the therapeutic outcome of acquired aplastic anemia has improved markedly with the introduction of immunosuppressive therapy using antithymocyte globulin and cyclosporine, a significant proportion of patients subsequently relapse and require second-line therapy. However, detailed analyses of relapses in aplastic anemia children are limited.

### Design and Methods
We previously conducted two prospective multicenter trials of immunosuppressive therapy for children with aplastic anemia: AA-92 and AA-97, which began in 1992 and 1997, respectively. In this study, we assessed the relapse rate, risk factors for relapse, and the response to second-line treatment in children with aplastic anemia treated with antithymocyte globulin and cyclosporine.

### Results
From 1992 to 2007, we treated 441 children with aplastic anemia with standard immunosuppressive therapy. Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) relapsed. The cumulative incidence of relapse was 11.9% at 10 years. Multivariate analysis revealed that relapse risk was significantly associated with an immunosuppressive therapy regimen using danazol (relative risk, 3.15; $P$=0.001) and non-severe aplastic anemia (relative risk, 2.51; $P$=0.02). Seventeen relapsed patients received additional immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight patients responded within 6 months. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation and five are alive. Eleven patients underwent hematopoietic stem cell transplantation directly and seven are alive.

### Conclusions
In the present study, the cumulative incidence of relapse at 10 years was relatively low compared to that in other studies mainly involving adult patients. A multicenter prospective study is warranted to establish optimal therapy for children with aplastic anemia.

Key words: children, aplastic anemia, relapse, risk factors, immunosuppressive therapy.

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## Introduction

Aplastic anemia (AA) is thought to be an immune-mediated bone marrow disease, characterized by bone marrow aplasia and peripheral blood pancytopenia. Currently, two effective treatments are available for this disorder: allogeneic bone marrow transplantation and immunosuppressive therapy. Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling donor can cure the majority of transplanted patients with severe AA.[1] The outcome after bone marrow transplantation has been markedly better in children than in adults, with less frequent and severe graft-*versus*-host disease and better overall survival.[2,3] However, most children with severe AA have no matched sibling donor and rely on immunosuppressive therapy as first-line treatment.

The combination of antithymocyte globulin and cyclosporine is now considered the standard immunosuppressive regimen for children with severe AA who lack a matched sibling donor.[4] Recent large trials of combined immunosuppressive therapy for severe AA in children demonstrated that the response rate is greater than 60% and the 3- to 5-year survival rate is approximately 90%.[5-7] However, relapse and clonal evolution with transformation to myelodysplasia or acute myeloid leukemia remain significant problems after immunosuppressive therapy, and long-term, event-free survival is less impressive than after bone marrow transplantation.[4,8] We previously reported the results of a multicenter trial of immunosuppressive therapy for children with AA (AA-92 study).[5] In the AA-92 study, the response rate at 6 months was 71%, with the probability of survival at 4 years being greater than 90%. However, a significant proportion of patients subsequently relapsed and required second-line therapy. To select the optimal therapy for such patients, a detailed analysis concerning relapse after response to immunosuppressive therapy is very important; however, analyses of relapse of AA in children after the standard combined immunosuppressive regimen are very limited.[9-11] Although the European Group for Blood and Marrow Transplantation (EMBT) reported an analysis of relapse of AA after immunosuppressive therapy in a large number of patients, the study populations were primarily adults treated in the 1970s and 1980s with antithymocyte globulin monotherapy.[9] A report from the Italian Association of Pediatric Hematology and Oncology focused mainly on the response to cyclosporine and dependence after immunosuppressive therapy.[10] A single-center retrospective analysis from the National Institutes of Health showed excellent long-term survival with a 33% cumulative incidence of relapse at 10 years in children with severe AA who responded to the standard immunosuppressive therapy; however, a detailed analysis of relapse that included risk factors was not provided.[11]

We previously conducted two prospective multicenter studies: the AA-92 and AA-97, which began in November 1992 and October 1997, respectively.[5,12] From 1992 to 2007, 473 children with AA were treated with immunosuppressive therapy in these studies, and 441 of the children were treated with antithymocyte globulin plus cyclosporine. In the present study, we assessed the relapse rate, risk factors for relapse, response to second-line treatment, and prognosis after relapse in AA children treated with an antithymocyte globulin/ cyclosporine-based regimen.

## Design and Methods

### Patients
Two consecutive prospective studies were designed by the Japan Childhood Aplastic Anemia Study Group and involved 79 hospitals in Japan. The eligibility criteria have been described previously.[5] The severity of disease was determined according to currently used criteria.[13,14] Disease was considered severe if at least two of the following were present: (i) neutrophil count less than $0.5\times10^9$/L; (ii) platelet count less than $20\times10^9$/L; and (iii) reticulocyte count less than $20\times10^9$/L with a hypocellular bone marrow. AA was considered very severe if the above criteria for severe disease were fulfilled and the neutrophil count was less than $20\times10^9$/L. Non-severe disease was defined by at least two of the following: (i) neutrophil count less than $1.0\times10^9$/L, (ii) platelet count less than $50\times10^9$/L; and (iii) reticulocyte count less than $60\times10^9$/L with a hypocellular bone marrow. Allogeneic bone marrow transplantation was recommended for those patients with severe or very severe disease who had a matched sibling donor. This study was approved by the Ethic Committee of Hyogo Children Hospital.

### Treatment
The details of the immunosuppressive therapy administered were described in previous reports.[5,12] Immunosuppressive therapy consisted of horse antithymocyte globulin (Lymphoglobulin; Genzyme Corp., Cambridge, MA, USA) (15 mg/kg per day, days 1 to 5), cyclosporine (6 mg/kg per day, days 1 to 180, with subsequent adjustments to maintain the whole blood cyclosporine concentration between 100 to 200 ng/mL), and methylprednisolone for prophylaxis against allergic reactions (2 mg/kg per day for 5 days, with subsequent halving of the dose every week until discontinuation on day 28). Patients with very severe AA were treated with immunosuppressive therapy plus granulocyte-colony stimulating factor (G-CSF) (Filgrastim; Kirin, Tokyo, Japan) [400 μg/m² on day 1, with responding patients (neutrophil count > $1.0\times10^9$/mL) receiving the same dose three times a week for 3 months in the AA-92 study and for 60 days in the AA-97 study]. In the AA-92 study, the addition of G-CSF to immunosuppressive therapy for patients with severe AA and non-severe AA was randomized, while in the AA-97 study, G-CSF was not given to patients with severe AA or non-severe AA except to those with documented severe infection. All patients in the AA-92 study received danazol at a dose of 5 mg/kg/day for 6 months, and danazol was discontinued without tapering.

### Assessments
A complete response was defined for all patients as a neutrophil count greater than $1.5\times10^9$/L, a platelet count greater than $100\times10^9$/L, and a hemoglobin level greater than 11.0 g/dL. For patients with severe AA and very severe AA, a partial response was defined as a neutrophil count greater than $0.5\times10^9$/L, a platelet count greater than $20\times10^9$/L, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions. For patients with non-severe AA, a partial response was defined as a neutrophil count greater than $1.0\times10^9$/L, a platelet count greater than $30\times10^9$/L, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions.[5] In patients with a complete response on day 180, the cyclosporine dose was tapered down slowly (10% of adjusted dose per month). In those with a partial response, cyclosporine was continued for another 6 months to allow further improvement of blood counts. Tapering of cyclosporine was started on day 360 (10% every 2 weeks) regardless of response.

The hematologic response was evaluated 6 months after the

initiation of therapy. Relapse was defined by conversion to no response from a partial or complete response and/or the requirement for blood transfusions.[5]

### Statistical analysis

Failure-free survival curves were calculated by the Kaplan-Meier method, and evaluated by the log-rank test. The Cox proportional hazards model was used to assess the risk factors for relapse after immunosuppressive therapy using both univariate and multivariate analyses. The estimated magnitude of the relative risk (RR) is shown along with the 97.5% confidence interval (CI). Cumulative incidence using the competing risk method, as described by Fine and Gray,[15] was used for the assessment of factors predicting relapse. The competing events of relapse were death and transplantation.

## Results

### Patients' characteristics

In the AA-92 and AA-97 studies, 441 AA children were treated with antithymocyte globulin plus cyclosporine between 1992 and 2007. The characteristics of all the patients studied are summarized in Table 1. There were 112 and 329 patients in the AA-92 and AA-97 studies, respectively. The median age of all these patients was 8.3 years (range, 0 to 17 years). Patients with very severe (n=210), severe (n=149) and non-severe disease (n=82) received initial immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 264 patients (59.9%) had achieved a complete response (n = 91) or partial response (n=173). Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) subsequently relapsed. The cumulative incidence of relapse was 11.9% at 10 years and the median time from diagnosis to relapse was 21 months (range, 6 to 138 months). The median time from response to antithymocyte globulin therapy to relapse was 22 months (range, 2 to 135 months).

### Risk factors for relapse

Two hundred and sixty-four patients with a total of 42 events were eligible for analyses of risk factors for relapse. In univariate analysis, two parameters, non-severe disease (RR=2.98, 97.5% CI 1.40 - 6.34, $P$=0.0047) and use of danazol (RR=3.44, 97.5% CI 1.78 - 6.65, $P$=0.00023), were statistically significant risk factors (Table 2). In contrast, the relative risk of relapse for patients with post-hepatitis AA was significantly lower than the relative risk for patients with idiopathic AA (RR=0.234, $P$=0.043). Gender, age, duration of AA prior to initial treatment, early response (within 90 days after immunosuppressive therapy), use of G-CSF, and HLA-DR2 could not be identified as risk factors. In multivariate analysis, two factors, non-severe AA (RR=2.51, 97.5% CI 1.15 - 5.46, $P$=0.02) and use of danazol (RR=3.15, 97.5% CI 1.62 - 6.12, $P$=0.001) remained statistically significant. Figure 1A shows the cumulative incidence of relapse of patients with non-severe AA (35.3%), severe AA (12.9%), and very severe AA (12.0%) 10 years after the first immunosuppressive therapy. The cumulative relapse rate of patients with non-severe AA was significantly higher than that of patients with severe AA ($P$=0.025) or very severe AA ($P$=0.005). Figure 1B shows the actuarial risk of relapse at 10 years among patients treated with danazol (29.0%) and in the group not treated with danazol (9.8%) ($P$<0.001).

### Repeated immunosuppressive therapy versus hematopoietic stem cell transplantation as second-line therapy

Among 42 relapsed patients, 17 received a second course of immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight of these 17 patients responded within 6 months and are alive. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation (HSCT) as salvage therapy. The hematopoietic stem cell donors were HLA-matched unrelated bone marrow donors (n=4), unrelated cord blood donors (n=2) and one matched sibling donor. Five of seven patients are alive following HSCT. Eleven patients underwent HSCT directly from an alternative donor (unrelated bone marrow donor, n=7; unrelated cord blood donor, n=1, HLA-mismatched family donor, n=3) and seven are alive. The estimated failure-free survival from the beginning of second-line therapy was 63.6% in the HSCT group compared with 47.1% in the groups treatment with repeated immunosuppressive therapy ($P$=0.96).

Table 1. Patients' pretreatment characteristics.

| | Very severe AA | Severe AA | Non-severe AA |
|---|---|---|---|
| Registered | 210 | 149 | 82 |
| Sex (male/female) | 115/95 | 83/66 | 47/35 |
| Median age, years (range) | 8.1 (0-17) | 8.3 (1-17) | 8.5 (2-16) |
| Etiology of AA | | | |
| Ideopathic | 168 | 125 | 74 |
| Hepatitis | 37 | 21 | 7 |
| Viral infection | 2 | 1 | 0 |
| Drug | 3 | 2 | 1 |
| Median days from diagnosis to treatment (range) | 20.4 (1-146) | 30.6 (1-180) | 44.8 (3-180) |
| Study (AA-92/AA-97) | 46/164 | 38/111 | 28/54 |
| Response (complete/partial) (%) | 128 (40/88) (61.0%) | 91 (38/53) (61.1%) | 45 (13/32) (54.9%) |
| Relapse (AA-92/AA-97) | 6/8 | 9/5 | 11/3 |

Table 2. Risk factors for relapse in patients with aplastic anemia by univariate analysis.

| Variable | Relative risk (97.5% CI) | P |
|---|---|---|
| Sex, male | 0.977 (0.514-1.86) | 0.94 |
| Age | 1.01 (0.947-1.08) | 0.78 |
| Etiology of AA | | |
| Ideopathic | 4.97 (1.22-20.2) | 0.025 |
| Hepatitis | 0.234 (0.0577-0.952) | 0.043 |
| Duration of AA prior to initial treatment | 1.01 (0.998-1.02) | 0.11 |
| Response at 90 days | 1.07 (0.517-2.21) | 0.86 |
| Severity of disease | | |
| Non-severe | 2.98(1.40-6.34) | 0.0047 |
| Severe | 1.21(0.561-2.63) | 0.62 |
| Very severe | 1 | |
| Study, AA-92 (Danazol+) | 3.44 (1.78-6.65) | 0.00023 |
| G-CSF (+) | 0.915 (0.363-2.31) | 0.85 |
| HLA-DR2 | 0.905 (0.307-2.67) | 0.86 |





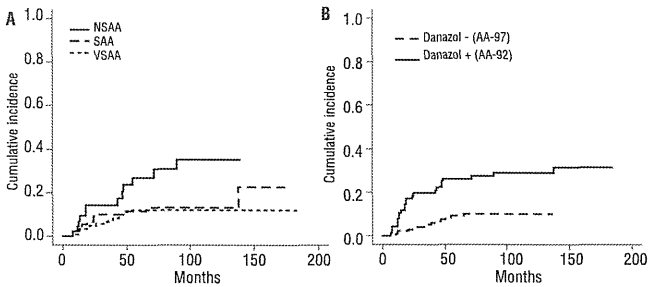


Figure 1. Cumulative incidence of relapse after immunosuppressive therapy in children with aplastic anemia. (A) The cumulative relapse rate of patients with non-severe aplastic anemia (NSAA) was significantly higher than that of patients with severe aplastic anemia (SAA) ($P$=0.025) and very severe aplastic anemia (VSAA) ($P$=0.005) 10 years after the first immunosuppressive therapy. (B) The actuarial risk of relapse at 10 years was significantly higher in the group treated with danazol (29.0%) than in the group not treated with danazol (9.8%) ($P$<0.001).

The overall survival rate did not differ between the immunosuppressive therapy group (84.7%) and the HSCT group (63.6%) after second-line treatment ($P$=0.07). Other patients were treated with cyclosporine alone (n=6) or bone marrow transplantation from a matched sibling donor (n=6). Two patients did not receive second-line treatments. One patient developed clonal evolution to myelodysplastic syndrome after 65 months, and the second developed acute myeloid leukemia after 37 months. Two patients showed clonal evolution to paroxysmal nocturnal hemoglobinuria after 138 months and 55 months. There were seven deaths among the 42 patients who initially relapsed. The causes of death were HSCT-related complications (n=5), acute myeloid leukemia (n=1) and bacteremia (n=1). The overall 10-year survival rates for patients with very severe AA, severe AA, and non-severe AA were 82.2±3.3%, 82.1±4.7% and 98.2±1.8%, respectively.

## Discussion

Analysis of relapse in children with AA responding to immunosuppressive therapy will provide valuable information for the management of childhood AA. Here, we present the results of a comprehensive analysis of the largest consecutive series of AA children treated with standard immunosuppressive therapy. Relapse of AA after immunosuppressive therapy is relatively common, with actuarial risks of 30 - 40% having been reported.[16-18] In the present study, the cumulative incidence of relapse at 10 years was 11.9%, which is relatively low compared with that found in other studies that primarily involved adult patients.[16-18] Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies. A recent Italian study of childhood AA showed a 16% cumulative incidence of relapse, which is comparable with that found in our study.[10]

Multivariate analysis of the data from this retrospective multicenter study shows that the use of danazol was the most statistically significant risk factor for relapse. From 1992 to 2007, 441 children with newly diagnosed AA were treated with immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine with (the AA-92 study) or without danazol (the AA-97 study). There are several reports of the efficacy of anabolic steroids in the treatment of AA. A randomized trial from the EBMT SAA working party demonstrated that the addition of an anabolic steroid (oxymetholone) to antithymocyte globulin treatment improved the response rate of patients with treated AA.[14] In our study, consistent with that report, the response rate at 6 months was higher in the patients who received immunosuppressive therapy with danazol (67.9%) than in the group of patients who received immunosuppressive therapy without danazol (57.1%). Furthermore, our results also showed that the cumulative relapse rate was significantly higher in the patients treated with immunosuppressive therapy plus danazol (Figure 1B). The reason danazol has an impact on relapse is unknown. However, it is possible that a number of cases with an androgen-responsive congenital bone marrow failure syndrome such as dyskeratosis congenita were hidden in our series of AA patients, and discontinuation of danazol was responsible for relapse. Recent reports have shown that a bone marrow failure syndrome of variable severity due to dyskeratosis congenita may be present in otherwise phenotypically normal individuals, and can masquerade as acquired AA.[19-22] We found mutations in the telomerase reverse transcriptase (*TERT*) gene, which is one of the genes causing dyskeratosis congenita, in two of 96 Japanese children with acquired AA.[23] Recently, more dyskeratosis congenita genes have been discovered. It is possible that more cases with an androgen-responsive dyskeratosis congenita were hidden in our series of AA patients. Alternatively, danazol may inhibit complete eradication of pathological T-cell clones by antithymocyte globulin through an unknown mechanism. Understanding the effects of androgens and developing androgen-mimetic drugs could be of significant benefit.

In our cohort of patients with non-severe AA, most patients were transfusion-dependent. In the AA-92 and AA-97 studies, 82 patients with non-severe AA were treated with the standard immunosuppressive regimen consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 13 patients had achieved a complete response and 32 patients achieved a partial response. Among the 32 patients who achieved a partial response, 14 patients later relapsed. However, 18 patients with non-severe AA patients who achieved a partial response maintained their hematologic response, and 12 of them subsequently achieved a complete response. When childhood non-severe AA is treated with supportive care, 67% of patients progress to develop severe AA, suggesting that it is important to consider early immunosuppressive therapy.[24] Our data indicate that

immunosuppressive therapy is beneficial for some patients with non-severe AA.

A previous Japanese study showed that the addition of G-CSF to immunosuppressive therapy increased the hematologic response rate after 6 months and reduced the relapse rate in adult patients with severe AA.[25] Recently, Gurion *et al.* conducted a systematic review and meta-analysis of randomized controlled trials comparing treatments with immunosuppressive therapy with or without hematopoietic growth factors in patients with AA. The addition of hematopoietic growth factors did not affect mortality, response rate, or occurrence of infections, but did significantly decrease the risk of relapse.[26] The data from our AA-92 trial were included in this meta-analysis. In contrast to the other five studies in the meta-analysis, only our study included patients with non-severe AA, who had a significantly higher relapse rate than that of patients with either severe AA or very severe AA. Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies in the meta-analysis. To compare our results with the other studies, we excluded patients with non-severe AA from the statistical analysis, and compared the risk of relapse between patients who did or did not receive G-CSF. The results again showed no significant differences in the relative risk between them (RR=2.71, 97.5% CI 0.614 - 12.0, *P*=0.19).

The majority of patients who experienced relapse responded to reintroduction of immunosuppressive agents.[27] Our present study also demonstrates that a second course of immunosuppressive therapy was a safe and effective treatment for the patients who relapsed after the first immunosuppressive therapy. However, an optimal second immunosuppressive therapy regimen has not yet been established. Furthermore, about half of the relapsing patients eventually received HSCT in our study. The treatment choice was based on center-related preferences or on anecdotal evidence. A multicenter prospective study is warranted to establish optimal therapy for these patients.



### Appendix

*The following centers and persons participated in the Japan Childhood Aplastic Anemia Study Group: Japanese Red Cross Nagoya First Hospital (K. Kato); Kyoto Prefectural University of Medicine (S. Morimoto); Kobe University School of Medicine (Y. Takeshima); Hyogo College of Medicine (Y. Ohtsuka); Tokai University (H. Yabe); Shizuoka Children's Hospital (J. Mimaya); Fukushima Medical University (A. Kikuta); Tokyo Metropolitan Children's Medical Center, Tokyo (T. Kaneko); Osaka City General Hospital (J. Hara); Nagoya University (S. Kojima) ; Jichi Medical School (T. Yamauchi); Kagoshima University (Y. Kawano); Okayama University (M. Oda); Hokkaido University (R. Kobayashi); Hiroshima University (S. Nishimura); Kanazawa University (S. Koizumi); Keio University (T. Mori); Hiroshima Red Cross Atomic Bomb Hospital (K. Hamamoto); Chiba University (T. Sato); Hirosaki University (E. Ito); Teikyo University School of Medicine (F. Ohta); Tottori University (T. Kawakami); Dokkyo University School of Medicine (K. Sugita); Kumamoto National Hospital (K. Takagi); Seirei Hamamatsu Hospital (T. Matsubayashi); Hyogo Children's Hospital (Y. Kosaka); Yokohama City University (K. Ikuta); Yamaguchi University (H. Ayukawa); Kanagawa Children's Medical Center (T. Kigasawa); Hirakata City Hospital (C. Kawakami); Nakadohri General Hospital (A. Watanabe); Gumma Children's Hospital (T. Shitara); National Defence Medical College (I. Sekine); Gifu University School of Medicine (K. Isogai); Kumamoto University School of Medicine (S. Morinaga); University of Ryukyu (N. Hyakuna); Narita Red Cross Hospital (K. Sunami); Asahikawa Medical College (M. Yoshida); Nagoya City University (Y. Ito).*

## Authorship and Disclosures

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

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## References


1. Storb R. Bone marrow transplantation for aplastic anemia. Cell Transplant. 1993;2(5): 365-79.
2. Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. Semin Hematol. 2000;37(1): 30-42.
3. Ades L, Mary JY, Robin M, Ferry C, Porcher R, Esperou H, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. Blood. 2004;103(7):2490-7.
4. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anaemia in children. Br J Haematol. 2007; 136(4):549-64.
5. Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96(6):2049-54.
6. Bacigalupo A, Bruno B, Saracco P, Di Bona E, Locasciulli A, Locatelli F, et al. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midollo Osseo (GITMO). Blood. 2000;95(6):1931-4.
7. Führer M, Rampf U, Baumann I, Faldum A, Niemeyer C, Janka-Schaub G, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106(6): 2102-4.
8. Kojima S, Ohara A, Tsuchida M, Kudoh T, Hanada R, Okimoto Y, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. Blood. 2002;100(3):786-90.
9. Schrezenmeier H, Marin P, Raghavachar A, McCann S, Hows J, Gluckman E, et al. Relapse of aplastic anaemia after immunosuppressive treatment: a report from the European Bone Marrow Transplantation Group SAA Working Party. Br J Haematol. 1993;85(2):371-7.
10. Saracco P, Quarello P, Iori AP, Zecca M, Longoni D, Svahn J, et al. Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow-up. Br J Haematol. 2008;140(2):197-205.
11. Scheinberg P, Wu CO, Nunez O, Young NS. Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr. 2008;153(6):814-9.
12. Kosaka Y, Yagasaki H, Sano K, Kobayashi R, Ayukawa H, Kaneko T, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood. 2008;111(3):1054-9.
13. Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, et al. A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. Blood. 1979;53(3): 504-14.
14. Bacigalupo A, Chaple M, Hows J, Van Lint MT, McCann S, Milligan D, et al. Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. Br J Haematol. 1993; 83(1):145-51.
15. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94(446): 496-509.
16. Schrezenmeier H, Marin P, Raghavachar A, McCann S, Hows J, Gluckman E, et al. Relapse of aplastic anaemia after immunosuppressive treatment: a report from the European Bone Marrow Transplantation Group SAA Working Party. Br J Haematol. 1993;85(2):371-7.
17. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood. 2003;101(4):1236-42.
18. Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia; association between hematologic response and long-term outcome. JAMA. 2003;289 (3): 1130-5.
19. Vulliamy T, Marrone A, Dokal I, Mason PJ. Association between aplastic anaemia and mutations in telomerase RNA. Lancet. 2002;359(9324):2168-70.
20. Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood. 2003;102 (3):916-8.
21. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413-24.
22. Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet. 2003;362(9396):1628-30.
23. Liang J, Yagasaki H, Kamachi Y, Hama A, Matsumoto K, Kato K, et al. Mutations in telomerase catalytic protein in Japanese children with aplastic anemia. Haematologica. 2006;91(5):656-8.
24. Howard SC, Naidu PE, Hu XJ, Jeng MR, Rodriguez-Galindo C, Rieman MD, et al. Natural history of moderate aplastic anemia in children. Pediatr Blood Cancer. 2004; 43(5):545-51.
25. Teramura M, Kimura A, Iwase S, Yonemura Y, Nakao S, Urabe A, et al. Treatment of severe aplastic anemia with antithymocyte globulin and cyclosporin A with or without G-CSF in adults: a multicenter randomized study in Japan. Blood. 2007;110(6):1756-61.
26. Gurion R, Gafter-Gvili A, Paul M, Vidal L, Ben-Bassat I, Yeshurun M, et al. Hematopoietic growth factors in aplastic anemia patients treated with immunosuppressive therapy-systematic review and meta-analysis. Haematologica. 2009;94(5): 712-9.
27. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit anti-thymocyte globulin and cyclosporin for patients with relapsed or refractory severe aplastic anaemia. Br J Haematol. 2006;133(6):622-7.

# Predicting response to immunosuppressive therapy in childhood aplastic anemia

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## ABSTRACT

In aplastic anemia, predictive markers of response to immunosuppressive therapy have not been well defined. We retrospectively evaluated whether clinical and laboratory findings before treatment could predict response in a pediatric cohort from the multicenter AA-97 study in Japan. Between 1997 and 2006, 312 newly diagnosed children were enrolled and treated with a combination of antithymocyte globulin and cyclosporine. In multivariate analyses, lower white blood cell count was the most significant predictive marker of better response; patients with white blood cell count less than $2.0\times10^9$/L showed a higher response rate than those with white blood cell count of $2.0\times10^9$/L or more ($P$=0.0003), followed by shorter interval between diagnosis and therapy ($P$=0.01), and male sex ($P$=0.03). In conclusion, pre-treatment clinical and laboratory findings influence response to therapy. The finding that response rate worsens with increasing interval between diagnosis and treatment highlights the importance of prompt immunosuppressive therapy for patients with aplastic anemia.

Key words: aplastic anemia, children, immunosuppressive therapy, predictive marker.

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## Introduction

Aplastic anemia (AA) is defined as peripheral blood pancytopenia caused by bone marrow failure, and the pathogenesis is thought to involve autoimmune processes.[1-3] Several studies have confirmed immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine (CyA) as a promising therapeutic option for patients lacking HLA-identical related donors.[4-8] Although several potential markers of IST response that appear to reflect the immune pathophysiology of aplastic anemia have been suggested, mainly from adult studies,[9-11] none have been widely accepted. We have already investigated the clinical relevance of HLA, a minor population of paroxysmal nocturnal hemoglobinuria-type cells, and a specific autoantibody associated with aplastic anemia in pediatric patients, finding no correlation between these markers and response to therapy.[12]

Some groups have recently shown that pre-treatment laboratory variables are associated with good response to immunosuppressive therapy, but those results remain controversial, as the numbers of children included in the study was relatively small and the drugs used for immunosuppressive therapy have not been consistent.[13-15] The present study, therefore, evaluated whether clinical and laboratory findings before treatment could predict immunosuppressive therapy response in a large population of children with aplastic anemia enrolled in a multi-center study.

## Design and Methods

### Patients

Between October 1997 and September 2006, a total of 312 Japanese children with aplastic anemia (AA) from 118 hospitals were enrolled in the AA-97 multicenter study conducted by the Japan Childhood Aplastic Anemia Study Group. Patients with acquired AA were eligible if the following criteria were met: age under 18 years; newly diagnosed disease (≤180 days) without specific prior treatment; and moderate to very severe AA. The disease was considered severe if at least 2 of the following were noted: neutrophil count less than $0.5\times10^9$/L; platelet count less than $20\times10^9$/L; or reticulocyte count less than $20\times10^9$/L with hypocellular bone marrow.[16] AA was considered very severe if the criteria for severe disease were fulfilled and neutrophil count was less than $0.2\times10^9$/L. Moderate disease was defined by at least 2 of the following: neutrophil count less than $1.0\times10^9$/L; platelet count less than $50\times10^9$/L; or reticulocyte count less than $60\times10^9$/L.[6] Patients with congenital AA or paroxysmal nocturnal hemoglobinuria were excluded. Allogeneic stem cell transplantation was recommended for patients with severe or very severe disease who had an HLA-matched sibling, so these patients were not included in the AA-97 study. Written informed consent was obtained from all parents and all patients over the age of ten years. All study protocols were approved by the ethics committee of each participating hospital. The study also conforms to the recently revised Declaration of Helsinki.

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### IST

All patients were treated with a combination of intravenous ATG (Lymphoglobulin; Genzyme, Cambridge, USA) at 15 mg/kg/day for five days and oral CyA at 6 mg/kg/day. The dose of CyA was adjusted to maintain trough levels between 100 and 200 ng/mL, and the appropriate dose was administered for at least six months. Granulocyte colony-stimulating factor (Filgrastim; Kirin, Tokyo, Japan) was administered intravenously or subcutaneously at 400 μg/m$^2$ for three months only to patients with very severe disease.[17] Response to IST was evaluated at six months after initiation of therapy. Complete response (CR) was defined as a neutrophil count more than $1.5\times10^9$/L, a platelet count more than $100\times10^9$/L, and a hemoglobin level more than 11.0 g/dL.[17] Partial response (PR) was defined as a neutrophil count more than $0.5\times10^9$/L, a platelet count more than $20\times10^9$/L, and a hemoglobin level more than 8.0 g/dL in patients with severe or very severe AA, and as a neutrophil count more than $1.0\times10^9$/L, a platelet count more than 30 $\times10^9$/L, and a hemoglobin level more than 8.0 g/dL in patients with moderate AA.[17] Overall response was defined as CR or PR at six months after IST.

### Statistical analyses

Parameters for univariate analyses to determine predictors of response to IST included age at diagnosis, sex, interval between diagnosis and treatment, etiology, severity of disease, white blood cell (WBC) count, neutrophil count, lymphocyte count, hemoglobin level, reticulocyte count, and platelet count. Pre-treatment laboratory values were defined as the lowest value without transfusions during the four weeks preceding IST. Continuous variables were divided into quartile categories, and these cut offs were used for categorical analysis. To evaluate correlations between these parameters and response, differences in continuous variables were analyzed using the Mann-Whitney U-test and differences in frequencies were tested using the $\chi^2$ or Fisher's exact test. For multivariate analyses, logistic regression modeling was performed. Important covariates in the multivariate models were chosen using stepwise variable selection procedures. Values of $P$<0.05 were considered statistically significant.

## Results and Discussion

Patients' characteristics are shown in Table 1. A total of 312 patients fulfilled the eligibility criteria. Median age at diagnosis was eight years. Severity of AA was considered very severe in 156 patients, severe in 107 patients, and moderate in 49 patients. The median interval between diagnosis and treatment was 15 days. A total of 176 of the 312 (56.4%) patients improved with IST and achieved PR (n=131) or CR (n=45) at six months. All of them achieved transfusion independence.

To determine predictors of IST response, we compared differences in potential pre-treatment variables between IST responders and non-responders. The following were analyzed both for prevalence in categorical variables and differences in continuous variables: age at diagnosis, interval between diagnosis and treatment, WBC count, neutrophil count, lymphocyte count, hemoglobin level, reticulocyte count, and platelet count. In univariate analyses, WBC count, lymphocyte count, interval between diagnosis and therapy, and gender showed associations with IST response (Table 2). We also performed multivariate logistic regression analysis to assess the simultaneous contributions of each of the variables in predicting response. In these analyses, lower WBC count ($P$=0.0003), shorter interval between diagnosis and therapy ($P$=0.012), and male sex ($P$=0.036) represented significant predictors of better response (Table 2).

Boys displayed better response than girls (Figure 1A). This relationship was also observed in a retrospective European study in which a young female cohort experienced delayed recovery of bone marrow function following IST.[18] Median WBC count before treatment was significantly lower in patients who achieved response ($1.9\times10^9$/L) than in those who did not ($2.3\times10^9$/L; $P$= 0.007). In addition to the analysis with continuous variable, lower WBC count according to categorical analysis also associated with favorable response, with 93 of 144 patients (65%) with WBC less than $2.0\times10^9$/L and 83 of 168 patients (49%) with WBC of $2.0\times10^9$/L or more showing improvement with IST ($P$=0.009; Figure 1B). When lymphocyte count was applied to the analysis instead of WBC count, a correlation between lower lymphocyte count and response to IST was also observed (Table 2); 82 of 123 patients (67%) with lymphocyte count less than $1.5\times10^9$/L improved with IST, a significantly higher frequency than the 94 of 189 patients (50%) with lymphocyte count of $1.5\times10^9$/L or more who improved with IST ($P$=0.004). Neither neutrophil count nor severity of disease was predictive of response.

Regarding the association between pre-treatment neutrophil count and response, conflicting results have been reported. A European study reported superior response rates in children with very severe AA compared to severe AA[5] but, in contrast, some studies including a recent report of a Korean cohort of adult patients have produced the opposite results.[15,19] The present findings differ from those published studies, with favorable responses correlating well with lower WBC count rather than neutrophil count or disease severity. Indeed, WBC count was the strongest predictor of response to IST in multivariate analysis. In patients with AA, pre-treatment WBC count may mainly reflect the size of lymphocyte populations, due to the severe neutropenia in this condition. These results suggest that poor response to IST might possibly be ascribed to higher WBC

Table 1. Patients' characteristics.

| | | |
|---|---|---|
| N. of patients | | 312 |
| Age at diagnosis, years, median (range) | | 8 (1-17) |
| Gender | male / female | 186 /126 |
| Etiology | n. of patients (%) | |
| Idiopathic | | 261 (83.7) |
| Hepatitis | | 44 (14.1) |
| Others | | 7 (2.2) |
| Severity of AA | n. of patients (%) | |
| VSAA | | 156 (50.0) |
| SAA | | 107 (34.3) |
| MAA | | 49 (15.7) |
| Peripheral blood data at diagnosis | | |
| Median WBC count, $\times10^9$/L (range) | | 2.02 (0.20-8.70) |
| Median neutrophil count, $\times10^9$/L (range) | | 0.22 (0.00-3.13) |
| Median lymphocyte count, $\times10^9$/L (range) | | 1.82 (0.10-8.50) |
| Median Hb level, g/dl (range) | | 6.9 (2.1-13.2) |
| Median reticulocyte count, $\times10^9$/L (range) | | 16.0 (0.0-98.0) |
| Median platelet count, $\times10^9$/L (range) | | 11.0 (1.0-109.0) |
| Interval from diagnosis to treatment, days, median (range) | | 15 (1-180) |

*VSAA: very severe aplastic anemia; SAA: severe aplastic anemia; MAA: moderate aplastic anemia; WBC: white blood cell; Hb: hemoglobin.*

Table 2. **Univariate and multivariate analysis for IST response in 312 patients with AA.**

| Univariate variables | Responder | Non-responder | P |
|---|---|---|---|
| N. of patients (%) | 176 (56.4) | 136 (43.6) | |
| Median age at diagnosis, years | 8 | 8 | NS |
| Gender, male / female | 115/61 | 71/65 | 0.025 |
| Etiology, n. of patients (%) | | | |
| Idiopathic | 141(80) | 120(88) | NS |
| Hepatitis | 29(17) | 15(11) | |
| Others | 6(3) | 1(1) | |
| Severity of AA, n. of patients (%) | | | |
| VSAA | 90(51) | 66(49) | NS |
| SAA | 62(35) | 45(33) | |
| MAA | 24(14) | 25(18) | |
| Median WBC count, $\times 10^9$/L | 1.900 | 2.255 | 0.007 |
| $\geq 2.0\times10^9$/L, n. of patients (%) | 87(47) | 85(63) | 0.009 |
| $< 2.0\times10^9$/L, n. of patients (%) | 93(53) | 51(37) | |
| Median lymphocyte count, $\times 10^9$/L | 1.600 | 2.016 | 0.006 |
| Median neutrophil count, $\times 10^9$/L | 0.218 | 0.200 | NS |
| Median Hb level, g/dl | 6.8 | 6.8 | NS |
| Median reticulocyte count, $\times 10^9$/L | 15.730 | 17.600 | NS |
| Median platelet count, $\times 10^9$/L | 10.000 | 11.000 | NS |
| Interval from diagnosis to treatment, days | 13 | 19 | 0.002 |

| Multivariate variables | Odds ratio | 95% CI | P |
|---|---|---|---|
| WBC count, $< 2.0\times10^9$/L | 3.219 | 1.707-6.070 | 0.0003 |
| Interval from diagnosis to treatment, < 30 days | 2.571 | 1.225-5.396 | 0.012 |
| Gender, male | 1.873 | 1.042-3.366 | 0.036 |
| Reticulocyte count, $>25\times10^9$/L | 1.589 | 0.843-2.997 | NS |
| Platelet count, $>20\times10^9$/L | 1.362 | 0.657-2.826 | NS |
| Etiology, hepatitis/others | 1.223 | 0.504-2.966 | NS |

*VSAA: very severe aplastic anemia; SAA, severe aplastic anemia; MAA, moderate aplastic anemia; WBC, white blood cell; Hb, hemoglobin*

count, that is, a relative increase in lymphocytes. Given the dramatic effects of T-cell suppressants including ATG and CyA on *in vivo* hematopoiesis, autoreactive T-cell responses against hematopoietic stem cells have been suggested to play a major role in the pathogenesis of AA, and *in vitro* studies have also supplied supportive evidence for this idea. Early experiments demonstrated inhibitory effects of autologous lymphocytes on hematopoietic progenitor cell growth through overproduction of cytokines such as interferon-γ and tumor necrosis factor-α by activated cytotoxic T cells in AA patients.[10-12] More recently, oligoclonal T-cell expansions have been described in AA patients, disappearing with clinical improvement following IST.[13] Taking our results and previous findings together, a higher WBC count before treatment may indicate the presence of numerous autoreactive T cells that need to be eliminated and thus a high potential to destroy marrow function through lymphocytes, rather than better residual marrow function. In this scenario, patients with a lower WBC count could be seen to have a better probability of hematopoietic recovery following IST.

We identified a significantly inverse correlation between response and interval between diagnosis and treatment; median intervals among responders and non-responders were 13 and 19 days, respectively ($P$=0.002). In categorical analysis, response rates of patients with intervals less than 30 and of 30 days or more were 60% and 43%, respectively ($P$=0.013). Figure 1C clearly indicates the inverse relationship. Notably, response rates to IST were considerably low among AA patients with long-standing disease; only 35% of patients treated 90 days or more after diagnosis responded, suggesting that patients with this condition may receive irreversible damage to hematopoietic progenitor cells or stromal elements that progresses over time, possibly due to

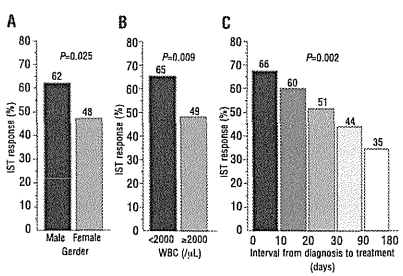


**Figure 1. Response to IST in 312 patients according to WBC count, gender, and interval from diagnosis to treatment. (A) Response rate according to gender. Boys showed better response than girls (62% *vs.* 48%, respectively; $P$=0.025). (B) Response rate according to WBC counts. Patients with WBC count $<2.0\times10^9$/L displayed a significantly higher response rate than patients with WBC $\geq2.0\times10^9$/L (65% *vs.* 49%, respectively; $P$=0.009). (C) Response rate according to the interval between diagnosis and treatment. Response rate was inversely associated with the interval between diagnosis and treatment ($P$=0.002).**





immune attack through autoreactivated lymphocytes. The present study indicates the importance of prompt IST therapy for patients with AA. We, therefore, recommend offering IST as soon as possible in all children with AA who lack a matched sibling donor.

Other variables did not differ significantly between responders and non-responders (Table 2). Particularly with regard to reticulocyte count, 122 patients showed reticulocyte count more than $25\times10^9$/L, of whom 67 (55%) responded to IST, and 186 patients had reticulocyte count of $25\times10^9$/L or less, of whom 107 (58%) responded to IST. Correlations of higher reticulocyte count and higher lymphocyte count at initial diagnosis with better response to IST in patients of all ages have recently been described by the National Institutes of Health (NIH) group.[18] However, when the same analysis was applied to their 77 pediatric patients, lymphocyte count was not predictive.[14] More recently, another relatively small study in adults with AA found no such association.[15] These studies were limited by inconsistency of regimens used for IST. The current study investigated a large cohort of children with AA treated using a unified regimen, but failed to confirm any correlation between reticulocyte count and response to IST, suggesting a limited contribution of this clinical parameter to the prediction of hematopoietic recovery, at least in children.

In conclusion, pre-treatment clinical and laboratory findings influence response to IST. Favorable response correlates better with lower WBC count than with neutrophil count or disease severity, and this blood count parameter might help in clinically assessing bone marrow function. Unlike the situation in adult AA, reticulocyte count is not predictive of response to IST in pediatric patients. IST should be started as soon as possible after diagnosis of AA, given that the response rate worsens as the interval between diagnosis and treatment increases.

## Authorship and Disclosures

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

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## References


1. Kojima S, Horibe K, Inaba J, Yoshimi A, Takahashi Y, Kudo K, et al. Long-term outcome of acquired aplastic anaemia in children: comparison between immunosuppressive therapy and bone marrow transplantation. Br J Haematol. 2000;111(1):321-8.
2. Mathe G, Amiel JL, Schwarzenberg L, Choay J, Trolard P, Schneider M, et al. Bone marrow graft in man after conditioning by antilymphocytic serum. Transplant Proc. 1971;3(1):325-32.
3. Young NS. Acquired aplastic anemia. JAMA. 1999;282(5):271-8.
4. Bacigalupo A, Broccia G, Corda G, Arcese W, Carotenuto M, Gallamini A, et al. Antilymphocyte globulin, cyclosporin, and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party. Blood. 1995;85(5):1348-53.
5. Fuhrer M, Rampf U, Baumann I, Faldum A, Niemeyer C, Janka-Schaub G, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106(6):2102-4.
6. Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96(6):2049-54.
7. Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica. 2007;92(1):11-8.
8. Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. JAMA. 2003;289(9):1130-5.
9. Nakao S, Takamatsu H, Chuhjo T, Ueda M, Shiobara S, Matsuda T, et al. Identification of a specific HLA class II haplotype strongly associated with susceptibility to cyclosporine-dependent aplastic anemia. Blood. 1994;84(12):4257-61.
10. Maciejewski JP, Follmann D, Nakamura R, Saunthararajah Y, Rivera CE, Simonis T, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. Blood. 2001;98(13):3513-9.
11. Oguz FS, Yalman N, Diler AS, Oguz R, Anak S, Dorak MT. HLA-DRB1*15 and pediatric aplastic anemia. Haematologica. 2002;87(7):772-4.
12. Yoshida N, Yagasaki H, Takahashi Y, Yamamoto T, Liang J, Wang Y, et al. Clinical impact of HLA-DR15, a minor population of paroxysmal nocturnal haemoglobinuria-type cells, and an aplastic anaemia-associated autoantibody in children with acquired aplastic anaemia. Br J Haematol. 2008;142(3):427-35.
13. Chang MH, Kim KH, Kim HS, Jun HJ, Kim DH, Jang JH, et al. Predictors of response to immunosuppressive therapy with antithymocyte globulin and cyclosporine and prognostic factors for survival in patients with severe aplastic anemia. Eur J Haematol. 2009;84(2):154-9.
14. Scheinberg P, Wu CO, Nunez O, Young NS. Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr. 2008;153(6):814-9.
15. Scheinberg P, Wu CO, Nunez O, Young NS. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. Br J Haematol. 2009;144(2):206-16.
16. Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, et al. A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. Blood. 1979;53(3):504-14.
17. Kosaka Y, Yagasaki H, Sano K, Kobayashi R, Ayukawa H, Kaneko T, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood. 2008;111(3):1054-9.
18. Nissen C, Gratwohl A, Tichelli A, Stebler C, Wursch A, Moser Y, et al. Gender and response to antilymphocyte globulin (ALG) for severe aplastic anaemia. Br J Haematol. 1993;83(2):319-25.
19. Bacigalupo A, Hows J, Gluckman E, Nissen C, Marsh J, Van Lint MT, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. Br J Haematol. 1988;70(2):177-82.
20. Hinterberger W, Adolf G, Aichinger G, Dudczak R, Geissler K, Hocker P, et al. Further evidence for lymphokine overproduction in severe aplastic anemia. Blood. 1988;72(1):266-72.
21. Hoffman R, Zanjani ED, Lutton JD, Zalusky R, Wasserman LR. Suppression of erythroid-colony formation by lymphocytes from patients with aplastic anemia. N Engl J Med. 1977;296(1):10-3.
22. Zoumbos NC, Gascon P, Djeu JY, Young NS. Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. Proc Natl Acad Sci USA. 1985;82(1):188-92.
23. Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, Young NS. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. Lancet. 2004;364(9431):355-64.

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MEETING REPORT

# The Third Consensus Conference on the treatment of aplastic anemia

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## 1 Introduction

Acquired aplastic anemia (AA) is characterized by bone marrow hypoplasia and peripheral blood pancytopenia. Although the pathogenesis of AA is not well understood, it is thought to be an immune-mediated disease in most patients [1, 2]. The main treatment options for patients with AA include allogeneic bone marrow transplantation (BMT) and immunosuppressive therapy (IST). Recent studies of BMT from an HLA-matched family donor (MFD) showed excellent survival for AA patients. The long-term survival rate of children and young adults with severe aplastic anemia (SAA) after BMT from an MFD ranges from 70 to 90% [3, 4], and BMT currently represents first-line therapy if an MFD is available. The combination of antithymocyte globulin (ATG) and cyclosporine (CsA) results in a response rate of 60–70% in AA patients [5–7] and is indicated as first-line therapy in children and young adults if MFD is unavailable, as well as in all patients older than 40–50 years.

BMT from an HLA-matched unrelated donor (MUD BMT) is indicated for patients who have failed at least one course of ATG and CSA. Better HLA-typing and less-toxic preparative regimens have resulted in substantial increases in survival among patients undergoing MUD BMT [8–10].

Bearing these issues in mind, experts from Europe, America, and Asia presented recent advances in understanding of the pathophysiology and current clinical trials for the treatment of AA (Table 1) at the Third Consensus Conference on the treatment of aplastic anemia on February 21, 2010, in Hamamatsu, Japan. After all speakers had presented, a general consensus was held to establish guidelines for the diagnosis and treatment of AA. Participants included clinicians and scientists from 13 countries, including seven countries in Asia.

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## 2 Pathogenesis of AA

In Session 1, four scientists presented the latest data regarding the pathogenesis of AA. Dr. Hirano identified two AA-associated antigens, kinectin and anti-postmeiotic segregation increased 1 (PMS1), by screening antibodies in a patient's sera against a peptide library of fetal liver cells [11]. The putative T cell epitope derived from kinectin triggered a cytotoxic T cell response in vitro, and inhibited granulocyte–macrophage colony forming unit formation. However, kinectin-specific T cells were not seen in AA patients. These auto-antibodies are present only in sera of AA patients, and become undetectable in the patients who achieve clinical remission, suggesting that these auto-antibodies may serve as a biomarker for AA, and may correlate with or predict disease activity in AA patients. However, a prospective study conducted by the Japan Childhood Aplastic Anemia Study Group failed to demonstrate a correlation between the presence of anti-PMS1 and response to IST [12].

Dr. Nakao discussed the clinical implication of detecting small paroxysmal nocturnal hemoglobinuria (PNH) clones by sensitive flow cytometric analysis. The presence of an increased number of PNH-type cells was predictive of a response to IST and a favorable prognosis among patients with AA. Ninety percent of patients with increased PNH-type cells responded to ATG + CSA, whereas only 50% of patients without PNH-type cells responded. Failure-free survival rates were significantly higher among patients with minor PNH clones than among those without these cells [13].

A single nucleotide polymorphism array (SNP-array) has recently been applied widely as a powerful karyotyping tool that detects deletions, amplifications, and loss of heterozygosity (LOH) at high resolution [14]. Dr. Maciejewski used this new tool in a series of 102 AA patients. Using conventional metaphase cytogenetics, 13% of patients showed cytogenetic abnormalities, which increased to 26% when a SNP-array was used. Early detection of clonal lesions was also possible when using a SNP-array. Interestingly, loss of the short arm of chromosome 6, which encompasses the HLA locus, was detected in three patients before IST. This finding suggests that escape from immune attack may work through the loss of the HLA haplotype in AA patients.

Telomeres are repeated nucleotide sequences that cap the ends of chromosomes and protect them from damage. Telomeres are short in one-third of AA patients [15]. Children with congenital bone marrow failure syndrome, and in particular, dyskeratosis congenita (DC), have extremely short telomeres [16]. Dr. Young demonstrated the presence of mutations in telomerase-complex genes such as TERT and TERC in a small percentage of AA patients without phenotypic characteristics of DC [17, 18]. A family study showed that healthy relatives of patients carrying these mutations also had short telomeres and mild hematologic abnormalities. Although telomere length does not predict response to IST, patients with short telomeres are at high risk of relapse and clonal evolution to myelodysplasia and acute myeloid leukemia after IST [19]. Dr. Young's group recently reported the significant correlation between absolute reticulocyte count (ARC) and absolute lymphocyte count at initial diagnosis and response to IST [20]. A further addition of telomere length increased the predictive capacity. Patients with both high ARC and long telomeres showed excellent survival, whereas those with low ARC and short telomeres had poor outcomes; patients with one of the two variables had intermediate outcomes.

**Table 1** Program

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|---|
| *Session 1: S. Nakao, N. Young* |
| Pathogenesis of aplastic anemia (AA) |
| (1) Autoimmunity in AA |
| N. Hirano, Dana-Farber Cancer Institute, MA, USA |
| (2) Application of SNP-array in bone marrow failure syndromes |
| J.P. Maciejewski, Cleveland Clinic Foundation, OH, USA |
| (3) PNH clones as a marker of autoimmunity |
| S. Nakao, Kanazawa University Graduate School of Medicine, Japan |
| (4) Genetic risk factors for AA |
| N. Young, National Heart, Lung, and Blood Institute, MD, USA |
| *Session 2: S. Kojima, A. Bacigalupo* |
| Stem cell transplantation |
| (1) Optimal conditioning regimen |
| S. Kojima, Nagoya University Graduate School of Medicine, Japan |
| (2) Role of antithymocyte globulin |
| A. Bacigalupo, Ospedale San Martino, Italy |
| (3) Long- term outcome after stem cell transplantation |
| H.J. Deeg, Fred Hutchinson Cancer Research Center, WA, USA |
| *Session 3: K. Ozawa, G. Socie* |
| Immunosuppressive Therapy |
| (1) Optimal dose of rabbit-antithymocyte globulin |
| J.C.W. Marsh, Kings College London, UK |
| (2) ATG + Cyclosporine vs High-dose Cyclophosphamide for treatment of aplastic anemia |
| F.K. Zhang, Institute of Hematology & Blood Disease Hospital, China |
| (3) Role of G-CSF |
| G. Socie, Hospital Saint Louis, France |
| (4) Role of iron chelator |
| J.W. Lee, The Catholic University of Korea, Korea |
| *Session 4: S. Nakao, S. Kojima, A. Bacigalupo* |
| Discussion for General Consensus |

## 3 Stem cell transplantation

In Session 2, three experts from Asia, Europe, and America discussed the optimization of stem cell transplantation for AA. Until the late 1990s, fewer than 40% of AA patients who underwent MUD BMT survived long-term, and there was a high incidence of graft failure and graft versus host disease (GVHD) [21]. Recent data have shown improved results through better selection of HLA-matched donors and changes in conditioning regimens [22, 23].

Dr. Kojima analyzed a Japanese cohort of 301 AA patients who received MUD BMT through the Japan Marrow Donor Program. Using matched-pair analysis, he showed the superiority of a fludarabine (Flu) + cyclophosphamide (CY) + ATG and radiation regimen compared with a CY + ATG + total body irradiation (TBI) regimen. The current recommended regimen in Japan includes Flu (100 mg/m$^2$) + CY (3,000 mg/m$^2$) + rabbit ATG (5 or 10 mg/kg) + 3 Gy TBI. He also used matched-pair analysis to compare tacrolimus (FK)/methotrexate (HTX) with CsA/MTX for the prophylaxis of GVHD in AA patients who received a MUD BMT. Results showed the superiority in overall survival of FK/MTX over CsA/MTX [24].

Dr. Bacigalupo proposed optimized protocols for BMT from an MFD for AA patients. For children and young adults, the recommended regimen is CY (200 mg/kg) + rabbit ATG (7.5 mg/kg). The stem cell source should be bone marrow rather than peripheral blood [25]. GVHD prophylaxis consists of CsA + MTX [26]. There is controversy concerning the upper age limit for BMT in AA patients. A large amount of data from the Europe Group for Blood and Marrow Transplantation (EBMT) showed an inferior outcome in AA patients older than 50 years, although outcomes for patients aged 30–40 years were similar to this aged 40–50 years. To improve outcome, Dr. Bacigalupo proposed a conditioning regimen with Flu (120 mg/kg), CY (1,200 mg/m$^2$), and rabbit ATG (7.5 mg/kg) for older patients. Dr. Bacigalupo also referred to previously published conditioning regimens for MUD BMT [22]. The current EBMT regimen recommended for children is Flu (120 mg/kg), CY (1,200 mg/m$^2$), and rabbit ATG (15 mg/kg). For adult patients, the addition of TBI (2 Gy) with a reduced dose of ATG (7.5 mg/kg) is recommended. However, a recent analysis of 100 patients treated according to these protocols revealed that graft failure and Epstein Barr virus (EBV)-lymphoproliferative disease (LPD) still remain significant causes of death [27]. Consequently, Dr. Bacigalupo modified the current EBMT protocol with an increased dose of CY (from 1,200 mg/m$^2$ to 120 mg/kg), a reduction of rabbit ATG (from 15 to 7.5 mg/kg), and prophylactic administration of rituximab for EBV-LPD.

In the United States, Dr. Deeg previously demonstrated an improved outcome in patients receiving CY + ATG + 2 Gy TBI for MUD BMT, compared with a higher dose of TBI [23]. The ongoing CTNN study in the United States is designed to find the best dose of CY (0, 50, 100, or 150 mg/kg) combined with a regimen of Flu, ATG, and 2 Gy TBI. The 0- and 150-mg trials stopped due to rejection and toxicities, respectively. Both regimens currently undergoing testing in Europe and the United States are similar to the regimen recommended by the Japanese group. Dr. Deeg discussed the late effects of stem cell transplantation and its major adverse effect, i.e., chronic GVHD. There are no benefits associated with chronic GVHD in patients with non-malignant diseases and it increases the risk of secondary malignancy [28]. The most significant risk factor for developing chronic GVHD is the use of peripheral blood stem cells [25]. Dr. Deeg recommended bone marrow, not peripheral blood, as the source of stem cells for AA patients. He analyzed risk factors for chronic GVHD in AA patients who received a matched-related BMT. Patients who received a nucleated marrow cell dose greater than 3.4 × 108/kg developed chronic GVHD 7.7 times more often than those who received a marrow cell dose less than 2.3 × 108/kg ($P = 0.004$). This finding was further reflected in overall survival, which was significantly worse in patients who a received higher dose of bone marrow cells than in those who received a lower dose, although CD34 cell dose was not analyzed in this study.

## 4 Immunosuppressive therapy

Dr. Marsh summarized the clinical trials of IST with rabbit ATG and CsA for AA as the initial course of treatment [29, 30]. The dose of rabbit ATG varied between 10 and 18.75 mg/kg among studies. Response rates ranged from 50 to 70%, which was equivalent with rates seen with of horse ATG, although patient numbers reported from some of these studies were small. However, immunosuppression of rabbit ATG is more potent than horse ATG, resulting in an increased incidence of infectious complications [31]. Dr. Marsh concluded that it is warranted to conduct a prospective study to find the optimal dose of rabbit ATG, and that larger prospective studies comparing rabbit ATG with horse ATG are needed.

High-dose CY (HD-CY) without stem cell rescue has been developed as a promising therapy for AA by the John's Hopkin's group [32]. However, a randomized trial conducted by the National Institutes of Health showed unacceptable toxicities, leading to early closure [33]. Dr. Zhang compared HD-CY + CsA with ATG + CsA for treatment of AA. The dose of CY was decreased to 120 mg/kg from the original report of 200 mg/kg. The costs of drugs were much cheaper in the CY group than the ATG group in China. The response rate at 6 months was comparable between both groups at 70%. The overall survival at 3 years was also comparable between the two groups, at 85%. It is noteworthy that the rate of early death was less than 5% in the CY group. Dr. Zhang's data justify conducting a randomized study to compare a modified dose of CY therapy with standard ATG therapy for newly diagnosed.

## 5 AA patients

To date, three prospective randomized studies have addressed the role of granulocyte-colony stimulating factor (G-CSF) in combination with IST [6, 34, 35]. Dr. Socie summarized the results of these studies, which showed faster recovery of neutrophils in the G-CSF group but failed to show significant differences in study endpoints including response rate, incidence of infections, and overall survival between the G-CSF group and the non-G-CSF group. He also presented the latest EBMT study, which enrolled more than 200 newly diagnosed patients with AA [36]. The study also confirmed the results of previous studies; there was no difference in overall survival or event-free survival between the two arms.

Dr. Lee discussed the role of iron chelation therapy in patients with AA. Regular transfusions lead to the development of iron overload, which is increasingly recognized as a risk factor following HSCT [37]. He presented the results of the EPIC trial, which evaluated the efficacy and safety of deferasirox, an oral iron chelator, in a large cohort of AA patients [38]. After 1 year of treatment, median serum ferritin levels decreased significantly with concomitant improvement of liver dysfunction. The therapy was generally well tolerated, but one quarter of patients suffered from an increase in serum creatinine levels. The concomitant use of CsA had a significant impact on serum creatinine levels.

## 6 Consensus panel

After all speakers had presented, a general consensus session was held. This session was chaired by S. Nakao, S. Kojima, and A. Bacigalupo. A number of questions were raised by the chairperson, and the following consensus was reached.

### 6.1 New diagnostic tests

The panelists discussed the relevance of incorporating new diagnostic tests into the management of AA patients. The new diagnostic tests include AA-associated autoantibodies, SNP-array, sensitive flow cytometric assay for PNH clones, and measurement of telomere length. All panelists felt that these new tests may be useful in the investigation of the pathophysiology of AA, but that it is too early to incorporate them into general practice for AA. The findings presented by each speaker must be confirmed by other investigators.

### 6.2 Stem cell transplantation

All panelists agreed that bone marrow should be used as the source of stem cells. The use of peripheral blood is indicated when a voluntary donor donates peripheral blood. A consensus was reached regarding the upper age limit both for BMT from an HLA-identical sibling and from an unrelated donor. The limit should be 50 years.

The chairperson proposed (1) CY + ATG for young patients and (2) CY + Flu + ATG for older patients as conditioning regimens in the case of HLA-matched sibling transplants. For adult patients transplanted from an unrelated donor, CY + Flu + ATG + low-dose TBI regimen was proposed. Although the panelists did not recommend other conditioning regimens, no general consensus was reached on conditioning regimens. Results of ongoing CTNN study in the United States are expected to reveal the optimal conditioning regimen for unrelated BMT. A higher dose of stem cell infusion has been recommended to facilitate engraftment. According to the presentation by Dr. Deeg, however, a higher dose of stem cell infusion was harmful because of the associated increase with chronic GVHD. The panelists discussed the optimal dose of stem cells, but no agreement was reached. Dr. Deeg emphasized that all of the patients who receive HSCT for a rare disease such as AA should be enrolled into prospective studies to address unsolved questions. All panelists agreed that the donor should be matched at 10/10 or 9/10 levels by HLA high-resolution typing. In the case of patients in whom an appropriate donor is unavailable, unrelated cord blood transplantation or haploidentical transplantation may be indicated.

### 6.3 Immunosuppressive therapy

The combination of ATG and CsA remains the gold standard for immunosuppressive therapy. Because the supply of horse ATG was stopped in Europe and Asia, rabbit ATG replaced horse ATG in these areas. Because the optimal dose of rabbit ATG has not been clarified, a prospective study to compare two doses of rabbit ATG is proposed. In addition, the panelists discussed the rationale of performing a randomized study to compare a modified dose of high-dose CY + CsA with ATG + CsA as first-line therapy.

Although several panelists agreed with the need for such a study, the majority of the panelists did not place a high priority on this type of study. Most panelists thought that G-CSF is indicated only in limited cases, for example, patients with severe bacterial or fungal infections. Although all published randomized studies revealed that G-CSF has no proven effect on clonal evolution in AA, several panelists felt that a longer follow-up period is necessary to reach a definitive conclusion on this issue.

## Appendix

The following persons participated in the conference

Hoon Kook (Gwangiu, Korea), Dae Chul Jeong (Seoul, Korea), Jong Wook Lee (Seoul, Korea), Surapol Issaragagrisil (Bankok,Thailand), Xiao-Fan Zhu (Tianjin, China), Feng-Kui Zhang (Tianjin, China), Jing-Yan Tang (Shanghai, China), Jianping Shen (Hangzhou, China), Minghui Duan (Beijing, China), Jun Ma (Harbin, China), Honorata Baylon (Manila, Philippine), See Voon Seow (Singapore), Michelle Poon (Singapore), Lily Wong Lee (Sabah, Malaysia), Naoto Hirano (Boston, USA), Jaroslaw Maciejewski (Cleveland, USA), Neal Young (Bethesda, USA),

Joachim Deeg (Seattle, USA), Andrea Bacigalupo (Genova, Italy), Judith Marsh (London, UK), Gerard Socie (Paris, France), Keiya Ozawa (Tochigi, Japan), Masao Tomonaga (Nagasaki, Japan), Shinji Nakao (Kanazawa, Japan), Hiroto Yamazaki (Kanazawa, Japan), Akio Urabe (Tokyo, Japan), Seishi Ogawa (Tokyo, Japan), Hiroki Yamaguchi (Tokyo, Japan), Masanao Teramura (Tokyo, Japan), Kensuke Usuki (Tokyo, Japan), Chitose Ogawa (Tokyo, Japan), Ohara Akira (Tokyo, Japan), Tatsutoshi Nakahata (Kyoto, Japan), Hiromasa Yabe (Isehara, Japan), Etsuro Ito (Hirosaki, Japan), Kazuko Kudo (Shizuoka, Japan), Seiji Kojima (Nagoya, Japan), Yoshiyuki Takahashi (Nagoya, Japan), Haruhiko Ohashi (Nagoya, Japan), Koichi Miyamura (Nagoya, Japan).

## References


1. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006;105:2509–19.
2. Young NS, Bacigalupo A, Marsh JC. Aplastic anemia: pathophysiology and treatment. Biol Blood Marrow Transplant. 2010; 16:S119–25.
3. Kahl C, Leisenring W, Deeg HJ, Chauncey TR, Flowers ME, Martin PJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anemia: a long-term follow-up. Br J Haematol. 2005;130:747–51.
4. Yagasaki H, Takahashi Y, Hama A, Kudo K, Nishio N, Muramatsu H, et al. Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia. Bone Marrow Transplant. 2010 Feb 1 [Epub ahead of print].
5. Bacigalupo A, Broccia G, Corda G, Arcese W, Carotenuto M, Gallamini A, et al. Antilymphocyte globulin, cyclosporin, and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party. Blood. 1995;85:1348–53.
6. Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96:2049–54.
7. Führer M, Rampf U, Baumann I, Faldum A, Niemeyer C, Janka-Schaub G, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106:2102–4.
8. Deeg HJ, Amylon ID, Harris RE, Collins R, Beatty PG, Feig S, et al. Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. Biol Blood Marrow Transplant. 2001;7:208–15.
9. Kojima S, Matsuyama T, Kato S, Kigasawa H, Kobayashi R, Kikuta A, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. Blood. 2002;100:799–803.
10. Maury S, Balère-Appert ML, Chir Z, Boiron JM, Galambrun C, Yakouben K, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica. 2007;92:589–96.
11. Hirano N, Butler MO, Von Bergwelt-Baildon MS, Maecker B, Schultze JL, O'Connor KC, et al. Autoantibodies frequently detected in patients with aplastic anemia. Blood. 2003;102: 4567–75.
12. Yoshida N, Yagasaki H, Takahashi Y, Yamamoto T, Liang J, Wang Y, et al. Clinical impact of HLA DR15, a minor population of paroxysmal nocturnal haemoglobinuria-type cells, and an aplastic anaemia-associated autoantibody in children with acquired aplastic anemia. Br J Haematol. 2008;142:427–35.
13. Sugimori C, Chuhjo T, Feng X, Yamazaki H, Takami A, Teramura M, et al. Minor population of CD55-CD59-blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. Blood. 2006;107:1308–14.
14. Maciejewski JP, Mufti GJ. Whole genome scanning as a cytogenetic tool in hematologic malignancies. Blood. 2008;112:965–74.
15. Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC. Progressive telomere shortening in aplastic anemia. Blood. 1998;91:3582–92.
16. Alter BP, Baerlocher GM, Savage SA, Chanock SJ, Weksler BB, Willner JP, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. Blood. 2007;110:1439–47.
17. Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet. 2003;362:1628–30.
18. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352:1413–24.
19. Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT, Young NS. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. JAMA. 2010;304: 1358–64.
20. Scheinberg P, Wu CO, Nunez O, Young NS. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. Br J Haematol. 2009;144:206–16.
21. Passweg JR, Pérez WS, Eapen M, Camitta BM, Gluckman E, Hinterberger W, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. Bone Marrow Transplant. 2006;37:641–9.
22. Bacigalupo A, Locatelli F, Lanino E, Marsh J, Socié G, Maury S, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. Bone Marrow Transplant. 2005;36:947–50.
23. Deeg HJ, O'Donnell M, Tolar J, Agarwal R, Harris RE, Feig SA, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. Blood. 2006;108: 1485–91.
24. Yagasaki H, Kojima S, Yabe H, Kato K, Kigasawa H, Sakamaki H, et al. 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. 2009;15:1603–8.
25. Schrezenmeier H, Passweg JR, Marsh JC, Bacigalupo A, Bredeson CN, Bullorsky E, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood. 2007; 110:1397–400.
26. Locatelli F, Bruno B, Zecca M, Van-Lint MT, McCann S, Arcese W, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. Blood. 2000;96:1690–7.
27. Bacigalupo A, Socie' G, Lanino E, Prete A, Locatelli F, Locasciulli A, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation for alternative donor transplants in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. Haematologica. 2010;95:976–82.
28. Deeg HJ, Socié G, Schoch G, Henry-Amar M, Witherspoon RP, Devergie A, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood. 1996;87:386–92.
29. Di Bona E, Rodeghiero F, Bruno B, Gabbas A, Foa P, Locasciulli A, et al. Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy. Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Br J Haematol. 1999; 107:330–4.
30. Garg R, Faderl S, Garcia-Manero G, Cortes J, Koller C, Huang X, et al. Phase II study of rabbit anti-thymocyte globulin, cyclosporine and granulocyte colony-stimulating factor in patients with aplastic anemia and myelodysplastic syndrome. Leukemia. 2009; 23:1297–302.
31. Scheinberg P, Fischer SH, Li L, Nunez O, Wu CO, Sloand EM, et al. Distinct EBV and CMV reactivation patterns following antibody-based immunosuppressive regimens in patients with severe aplastic anemia. Blood. 2007;109:3219–24.







32. Brodsky RA, Chen AR, Dorr D, Fuchs EJ, Huff CA, Luznik L, et al. High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. Blood. 2010;115:2136–41.
33. Tisdale JF, Dunn DE, Geller N, Plante M, Nunez O, Dunbar CE, et al. High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial. Lancet. 2000;356:1554–9.
34. Gluckman E, Rokicka-Milewska R, Hann I, Nikiforakis E, Tavakoli F, Cohen-Scali S, et al. Results and follow-up of a phase III randomized study of recombinant human-granulocyte stimulating factor as support for immunosuppressive therapy in patients with severe aplastic anaemia. Br J Haematol. 2002;119:1075–82.
35. Teramura M, Kimura A, Iwase S, Yonemura Y, Nakao S, Urabe A, et al. Treatment of severe aplastic anemia with antithymocyte globulin and cyclosporin A with or without G-CSF in adults: a multicenter randomized study in Japan. Blood. 2007;110:1756–61.
36. Tichelli A, Schrezenmeier H, Socie G, Marsh J, Bacigalupo A, Daehrsen U, et al. Use of G-CSF in patients with severe aplastic anemia treatment with ATG and cyclosporine increases neutrophils and decreases infection rates and hospital days but dose not improve long-term outcome: Results of a prospective randomizes clinical trial of the EBMT. Blood. 2009;114:205a.
37. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood. 2007;109:4586–8.
38. Lee JW, Yoon SS, Shen ZX, Ganser A, Hsu HC, Habr D, et al. Iron chelation therapy with deferasirox in patients with aplastic anemia: a subgroup analysis of 116 patients from the EPIC trial. Blood. 2010 Jun 21 [Epub ahead of print].

5 Quintanilla-Martinez L, Kimura H, Jaffe E. EBV-positive T-cell lymphoproliferative disorders of childhood. In: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (Swerdlow S, Campo E, Harris N, Jaffe E, Pileri S, Stein H et al., eds), 4th edn. Lyon: IARC Press, 2008; 278–80.
6 Koyama M, Takeshita Y, Sakata A *et al.* Cytotoxic chemotherapy successfully induces durable complete remission in 2 patients with mosquito allergy resulting from Epstein–Barr virus-associated T-/natural killer cell lymphoproliferative disease. *Int J Hematol* 2005; **82**:437–40.
7 Kimura H, Miyake K, Yamauchi Y *et al.* Identification of Epstein–Barr virus (EBV)-infected lymphocyte subtypes by flow cytometric in situ hybridization in EBV-associated lymphoproliferative diseases. *J Infect Dis* 2009; **200**:1078–87.
8 Iwatsuki K, Satoh M, Yamamoto T *et al.* Pathogenic link between hydroa vacciniforme and Epstein–Barr virus-associated hematologic disorders. *Arch Dermatol* 2006; **142**:587–95.

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## Novel adenosine triphosphate (ATP)-binding cassette, subfamily A, member 12 (*ABCA12*) mutations associated with congenital ichthyosiform erythroderma

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MADAM, Autosomal recessive congenital ichthyosis (ARCI) is a keratinization disorder, characterized by general desquamation. ARCI is a heterogeneous entity, including harlequin ichthyosis (HI, MIM 242500), lamellar ichthyosis type 2 (LI2, MIM 601277) and congenital ichthyosiform erythroderma (CIE, MIM 242100). The reported mutations in CIE include adenosine triphosphate (ATP)-binding cassette, subfamily A, member 12 (*ABCA12*),[1] transglutaminase 1 (TGM1),[2] lipoxygenase-3, 12(R)-lipoxygenase,[3] *NIPAL4*[4] and *CYP4F22*.[5] Mutations in *ABCA12* also result in LI2 and HI.[6,7] We report *ABCA12* mutations in four unrelated Japanese patients with CIE and identified five unreported and two recurrent mutations.

Patient 1 is a 3-year-old girl with generalized scales on erythroderma, ectropion, eclabium, severely deformed ears and alopecia (Fig. 1a–c). Her elder sister displayed similar symptoms and died after dehydration and infection. Patient 2 is a 9-year-old girl with generalized scales on an erythrodermic skin, mild ectropion, alopecia of the forehead and mild auricular malformation. Her younger sister died after severe skin symptoms and subsequent complications. Patient 3 is a 4-month-old boy, born as a collodion baby, with systemic whitish scales and generalized erythrodermic skin. There is no family history. Patient 4 is a 3-month-old boy, born as a collodion baby, with generalized whitish scales on a mild erythrodermic skin (Fig. 1d,e). Ectropion, eclabium and auricular malformation were not seen. There is no family history. Pathological findings of all patients revealed hyperkeratosis, mild acanthosis and perivascular lymphocytic infiltration.

We initially examined for *ABCA12* mutation, because *ABCA12* mutations have been found frequently in Japanese patients with CIE. For analysis of the *ABCA12* gene, polymerase chain reaction (PCR) fragments were amplified with 53 primer pairs, as previously reported.[6] We identified five unreported and two recurrent mutations (Table 1). Patient 1 had compound heterozygosity of missense/small deletion mutations [(p.Thr1575Pro)+(c.6031delG)]. Patients 2 and 3 had compound heterozygosity of missense/splice-site mutations [(p.Arg986Trp)+(c.5940–1G>C), (p.Asn1380Ser)+(c.5128+3A>G), respectively]. Patient 4 had compound heterozygosity of missense mutations [(p.Thr1575Pro)+(p.Gly1651Ser)]. Each of the parents was a heterozygous carrier. Five mutations (p.Thr1575Pro, c.6031delG, p.Arg986Trp, c.5940–1G>C and c.5128+3A>G) have not been reported previously. Two recurrent mutations (p.Asn1380Ser and p.Gly1651Ser) have been reported previously in LI2.[6] These mutations were not found in 200 normal, unrelated Japanese alleles.

In cDNA from the skin of patient 2, reverse transcriptase-PCR (RT-PCR) across the c.5940–1G>C mutation site showed a single band of 526 bp. Subcloning and direct sequencing

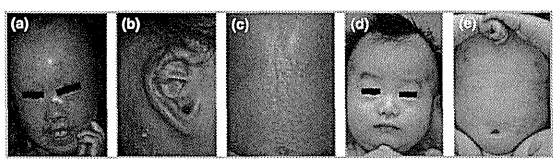


Fig 1. (a–c) Clinical features of patient 1. The whole body was covered with whitish scales on the erythrodermic skin. Ectropion, eclabium and alopecia of the forehead were seen. (d,e) Clinical features of patient 4. Whitish scales and generalized erythrodermic skin were seen.

Table 1 Summary of mutation analysis of *ABCA12* in the present study

| Patient | Age, sex | Mutation | Maternal | Paternal |
|---|---|---|---|---|
| 1 | 3 years, girl | Compound heterozygous | p.Thr1575Pro (c.4723A>C) | c.6031delG |
| 2 | 9 years, girl | Compound heterozygous | p.Arg986Trp (c.2956C>T) | c.5940–1G>C |
| 3 | 4 months, boy | Compound heterozygous | p.Asn1380Ser (c.4139A>G) | c.5128+3A>G |
| 4 | 3 months, boy | Compound heterozygous | p.Thr1575Pro (c.4723A>C) | p.Gly1651Ser (c.4951G>A) |






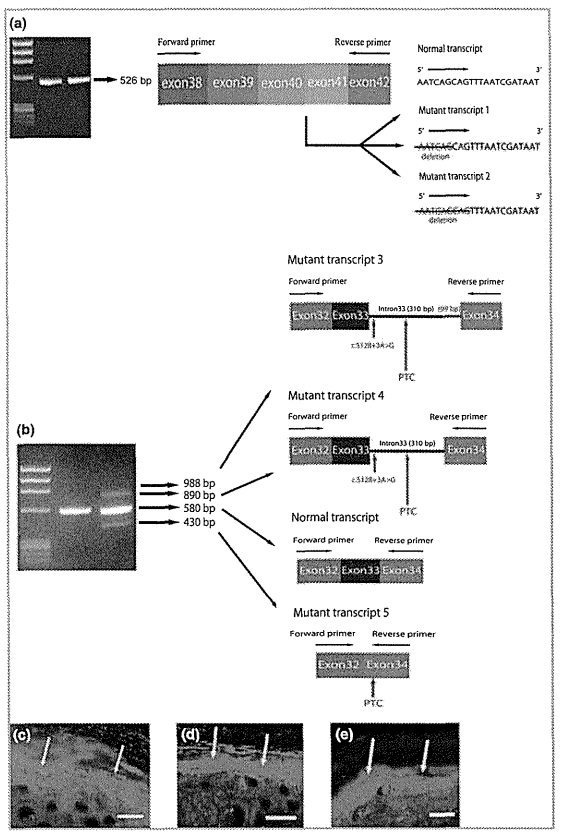


Fig 2. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of mRNA fragments around the splice-site mutations and immunofluorescent analysis. (a) In patient 2, RT-PCR, subcloning and direct sequencing through the exon 40–41 boundary revealed two mutant transcripts as well as a normal transcript. Mutant transcript 1 had lost 6-bp nucleotides from exon 41, which resulted in a 2-amino acid deletion (Ile1981_Ser1982del). Mutant transcript 2 had lost 9-bp nucleotides from exon 41, which resulted in a 3-amino acid deletion (Ile1981_Ser1983del). Both mutant transcripts were within-frame deletions. (b) In patient 3, three aberrant mutant transcripts, all of which led to a premature termination codon, were identified by RT-PCR, subcloning and direct sequencing through the exon 33–34 boundary. Mutant transcript 3 was 988 bp in length with the inclusion of 310 bp and another 99 bp of intron 33. Mutant transcript 4 was 890 bp in length with the inclusion of 310 bp of intron 33. Mutant transcript 5 had exon 33 skipping. (c–e) Immunofluorescent labelling of ABCA12 in the skin. (c,d) A dot-like pattern of ABCA12 staining was seen in the cytoplasm of keratinocytes in the upper epidermis in patient 1 (c) and patient 2 (d). (e) In the normal control epidermis, ABCA12 staining was relatively strong in the granular layers and seemed to be dominant at the cell periphery. Bar = 5 μm.

revealed two mutant transcripts with in-frame deletions (Fig. 2a). In cDNA from the skin of patient 3, RT-PCR across the c.5128+3A>G mutation site identified four bands of 988, 890, 580 and 430 bp, with a single 580-bp band in the control sample (Fig. 2b). Subcloning and direct sequencing revealed three aberrant mutant transcripts, all of which led to premature termination codons. Immunofluorescence using anti-ABCA12 antibody revealed a diffuse staining of ABCA12 in the granular layers of control skin (Fig. 2e) and of the non-ABCA12 form (TGM1) from patient CIE (data not shown), while a dot-like staining in the cytoplasm was observed in patients 1 and 2 (Fig. 2c,d).

ABCA12 is a membrane lipid transporter that functions in the lipid transport from the *trans*-Golgi network to lamellar granules.[8] *ABCA12* mutations result in heterogeneity, including LI2, HI and CIE.[1,6,7] LI2 is characterized by generalized scales without serious erythroderma, and caused by either homozygote or compound heterozygote for missense mutations within the first nucleotide-binding folds of *ABCA12*.[6] HI is the severest form of ARCI, characterized by generalized large, plate-like scales with ectropion, eclabium and flattened ears.[7] HI is usually caused by homozygous or compound heterozygous truncation mutations in *ABCA12*.[7] In contrast, CIE with *ABCA12* mutation clinically shows milder manifestations.[1] Thus far, 17 different mutations in *ABCA12* have been reported in 12 cases of CIE. Eleven of 12 cases have at least one missense mutation. Only three of 17 mutations (p.Asn1380Ser, p.Ile1494Thr and p.Arg1514His) were located in the first nucleotide-binding folds. Other mutations were located outside ABCA12 active transporter sites: two nucleotide-binding folds and 12 transmembrane domains. The mutation p.Thr1575Pro was identified in two unrelated patients with different clinical severity. Patient 1 with severer features had a heterozygous truncation mutation (c.6031delG) on another allele, while patient 4, with a milder phenotype, had another heterozygous missense mutation (p.Gly1651Ser). We suggest that the phenotypic variability in these two patients was caused by different mutations.

We identified two *ABCA12* splice-site mutations, which were not reported in CIE: c.5128+3A>G and c.5940−1G>C. RT-PCR analysis across the site of the c.5940−1G>C mutation in patient 2 revealed two mutant transcripts. These findings demonstrate expression of the in-frame shorter transcript lacking two or three amino acids due to this splice-site mutation, which may account for the mild phenotype. In contrast, RT-PCR analysis across the site of the c.5128+3A>G mutation in patient 3 revealed three aberrant mutant transcripts, all of which led to premature termination codons. Therefore, patient 3 had a compound heterozygosity for missense/truncated combinations of mutations.

Using high-throughput sequencing analyses, screening of all ARCI-related genes is currently possible, but the cost is still expensive.[9] Once this is overcome, the elucidation of the pathogenesis of ARCI will greatly progress in the near future.

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## References

1 Natsuga K, Akiyama M, Kato N *et al.* Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. *J Invest Dermatol* 2007; **127**:2669–73.
2 Laiho E, Ignatius J, Mikkola H *et al.* Transglutaminase 1 mutations in autosomal recessive congenital ichthyosis: private and recurrent mutations in an isolated population. *Am J Hum Genet* 1997; **61**:529–38.
3 Jobard F, Lefèvre C, Karaduman A *et al.* Lipoxygenase-3 (ALOXE3) and 12(R)-lipoxygenase (ALOX12B) are mutated in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. *Hum Mol Genet* 2002; **11**:107–13.
4 Lefèvre C, Bouadjar B, Karaduman A *et al.* Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. *Hum Mol Genet* 2004; **13**:2473–82.
5 Lefèvre C, Bouadjar B, Ferrand V *et al.* Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006; **15**:767–76.
6 Lefèvre C, Audebert S, Jobard F *et al.* Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. *Hum Mol Genet* 2003; **12**:2369–78.
7 Akiyama M, Sugiyama-Nakagiri Y, Sakai K *et al.* Mutations in ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest* 2005; **115**:1777–84.
8 Sakai K, Akiyama M, Sugiyama-Nakagiri Y *et al.* Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes. *Exp Dermatol* 2007; **16**:920–6.
9 Pastinen T. Genome-wide allele-specific analysis: insights into regulatory variation. *Nat Rev Genet* 2010; **11**:533–8.

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## Iatrogenic androgenetic alopecia in a male phenotype 46XX true hermaphrodite

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MADAM, Androgenetic alopecia (AGA) is a term that describes the androgen-dependent and genetically determined nature of the disease.[1] However, although it is known that androgen replacement therapy can induce AGA, no report has previously been issued regarding the development of iatrogenic AGA in a hermaphrodite undergoing androgen therapy. Herein, we describe a unique case of a castrated male phenotype 46XX true hermaphrodite receiving exogenous androgen supplementation who developed male-type hair loss.

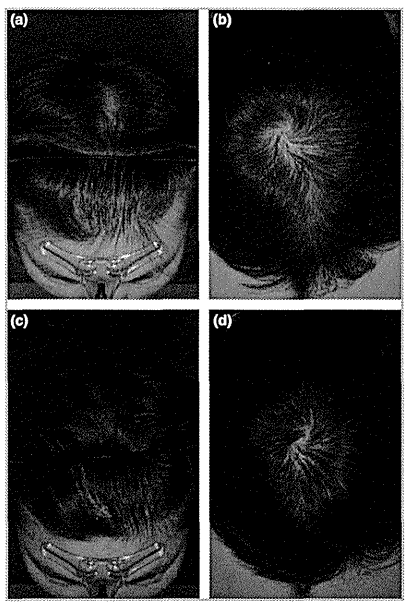


Fig 1. Iatrogenic androgenetic alopecia in a male phenotype 46XX true hermaphrodite showed a great improvement compared with baseline (a, b) after 4 months of finasteride treatment (c, d).

A 21-year-old male phenotype 46XX true hermaphrodite presented with a 3-year history of progressive hair loss. At the age of 16 years he was diagnosed as a 46XX true hermaphrodite with bilateral ovotestis, and subsequently underwent bilateral orchiectomy and testis prosthesis insertion. In addition, he was then given testosterone replacement therapy (testosterone enanthate, Jenasteron®; Jenapharm, Jena, Germany) for surgically induced andropausal status, which halted the development of secondary sexual characteristics. After 3 years of androgen therapy, progressive hair thinning developed on the scalp. Hair examination revealed nonscarring Norwood–Hamilton type III vertex alopecia with frontotemporal recession or BASP classification M1V2 alopecia (Fig. 1a, b).[2] Digital microscopy (Folliscope®; LeadM Corporation, Seoul, Korea) showed miniaturized hair shafts, and hair shaft size variation over the vertex scalp (Fig. 2). Serum testosterone, at the time, was 4·1 ng mL$^{-1}$ (normal 2·7–10·7) and serum dehydroepiandrosterone sulphate was 184·5 ng mL$^{-1}$ (normal 800–5600). Under a diagnosis of iatrogenic androgen-induced alopecia, finasteride (1 mg daily) therapy was started. After 4 months of treatment, the hair loss stabilized and scalp hair regrowth was observed, despite the continuance of testosterone replacement therapy (Fig. 1c, d).

True hermaphroditism is an extremely rare disorder, which is defined as the coexistence of testicular and ovarian tissue in the same subject. The most frequent karyotype of true hermaphrodites is 46XX.[3] Gender assignments for hermaphrodites are made according to genetic, gonadal, social and psychologically determined sex, and the requests of patients and their relatives.[4] To be reared as male or female, surgical correction of ambiguous external genitalia, surgical removal of dysgenetic gonads, and sex hormone replacement for the surgically induced andropausal or menopausal state are required. The unwanted dermatological side-effects of testosterone replacement therapy include acne, excessive hair growth and male pattern baldness. As in our case, to be reared

Fig 2. Photomicrograph showing miniaturized hair shafts, and variations in hair shaft size over the vertex scalp (original magnification ×50).

REVIEW ARTICLE

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# Malignant skin tumours in patients with inherited ichthyosis

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## Summary

Inherited ichthyoses are rare genodermatoses caused by mutations in the genes involved in epidermal development. Although there have been case reports on patients with ichthyosis who developed skin malignancies, it is still unknown whether or not patients with ichthyosis have an increased risk of skin malignancies. Here, we review case series of skin malignancies in patients with ichthyosis and show biological findings which might lead to cancer susceptibility. A survey of the literature revealed 28 cases of inherited ichthyoses with skin malignancy, including 12 cases of keratitis–ichthyosis–deafness (KID) syndrome, seven of autosomal recessive congenital ichthyosis, three of Netherton syndrome and six of miscellaneous ichthyosis. Twenty-four of the 28 cases developed single or multiple squamous cell carcinomas (SCCs). The age at diagnosis of the first skin malignancy ranged from 15 to 54 years. As patients with these particular subtypes of ichthyosis seem to be prone to skin malignancies, including SCC, at an unusually young age, routine cancer surveillance of these patients is strongly recommended.

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Skin cancer poses a serious problem in patients with inherited disorders, such as Gorlin syndrome, Cowden syndrome, xeroderma pigmentosum and epidermolysis bullosa. The prognosis for these patients is greatly influenced by skin malignancies, which develop at an unusually early age.

Ichthyoses are disorders characterized by skin dryness. Congenital ichthyoses are caused by mutations in the genes organizing keratinocyte differentiation and skin barrier function, although some of the causative genes are still undetermined.[1] There have been sporadic case reports of skin malignancies in patients with congenital ichthyosis. However, the epidemiology among these patients remains unknown because of the limited number of cases.

This review article summarizes skin malignancies in congenital ichthyoses described in the English language literature and discusses the biological background underlying skin barrier defects and carcinogenesis.

## Skin malitgnancies in each ichthyosis subtype

Twenty-eight cases of skin malignancy in congenital ichthyoses were found in the literature: 12 cases of keratitis–ichthyosis–deafness (KID) syndrome, seven of autosomal recessive congenital ichthyosis (ARCI), three of Netherton syndrome (NS) and six of miscellaneous ichthyosis. The first malignancies were diagnosed at the ages of 15–54 years. Reported skin malignancies include squamous cell carcinoma (SCC), basal cell carcinoma (BCC), malignant proliferating trichilemmal tumour (MPTT), malignant melanoma (MM), malignant fibrous histiocytoma and cutaneous lymphoma, although single or multiple SCC was the malignancy in most of the cases (24 out of 28). Table 1 summarizes the skin malignancies in patients with ichthyosis described in the literature.

## Keratitis–ichthyosis–deafness syndrome

Keratitis–ichthyosis–deafness syndrome (KID) syndrome is an autosomal dominant disease characterized by congenital erythrokeratoderma as well as sensorineural deafness and eye involvement.[2,3] Heterozygous mutations in *GJB2*, which encodes connexin 26 (Cx26), are responsible for the disease.[4,5] Mutations in *GJB6*, the gene encoding connexin 30 (Cx30), are causal in some cases which overlap with Clouston syndrome.[6,7]

There are 12 reports of patients with sporadic KID syndrome in the literature who developed skin malignancies, including SCC and MPTT (Table 1).[5,8–15] The age of onset for SCC in KID syndrome is 15–43 years, which is earlier than that for SCC in the normal population (around the age of 70 years).[16,17] p.Asp50Asn in Cx26, the most prevalent mutation in KID syndrome, was found in six patients who developed SCC or MPTT.[5,12,14,15] SCC was reported in roughly 10% of patients with KID syndrome and has been proposed as a distinguishing manifestation of the disease.[5] In a recent case series, three out of 14 (21%) patients with KID syndrome developed SCC.[14] Recurrent and chronic infection of the skin in KID syndrome has been suggested to be partly responsible for the increased risk of SCC[8,13] or to be one of the many factors involved in multiple-step carcinogenesis.[15] Also, alteration of E-cadherin expression due to dysfunctional Cx26 is hypothesized to lead to cancer susceptibility.[5] Mutated Cx26 might lead to tumorigenesis through a decrease in gap junction communication, a possibility that is supported by a mouse carcinogenesis model.[18] Overexpression of Cx26 has been shown to suppress tumour growth and induce apoptosis in prostate cancer cells through Bcl-2 downregulation.[19]

In a mouse model for KID syndrome in which Cx26 harbouring the p.Ser17Phe mutation was introduced as a heterozygous mutation under control of the endogenous Cx26 promoter, the basal layer showed increased cell proliferation.[20] However, progressive skin growth and increased susceptibility to SCC were not observed.[20]

Table 1 Skin malignancies in patients with ichthyosis

| Ichthyosis subtype | Age at the diagnosis of first skin malignancy (years) | Skin malignancy | Causative gene | Reference |
|---|---|---|---|---|
| KID | 35 | SCC | NE | 8 |
| KID | 28 | Multiple SCC | NE | 9 |
| KID | 43 | SCC | NE | 10 |
| KID | 38 | SCC | GJB2 | 5 |
| KID | 31 | Multiple SCC | GJB2 | 12 |
| KID | 31 | Multiple MPTT | NE | 11 |
| KID | 15 | SCC | NE | 13 |
| KID | 28 | Multiple SCC/MPTT | GJB2 | 15 |
| KID | 24 | Multiple MPTT | ND | 15 |
| KID | 30 | Multiple SCC | GJB2 | 14 |
| KID | 38 | SCC | GJB2 | 14 |
| KID | 40 | SCC | GJB2 | 14 |
| CIE | 44 | SCC, MM | *ABCA12* | 23, 32 |
| CIE | 37 | MM, cutaneous lymphoma | *ABCA12* | 23 |
| CIE | 43 | Multiple SCC/BCC | NE | 31 |
| CIE | 51 | Multiple SCC/BCC | NE | 31 |
| CIE | 25 | SCC, MFH | NE | 29 |
| LI | 27 | Multiple SCC/BCC | NE | 33 |
| LI | 33 | Multiple BCC | NE | 30 |
| NS | 23 | Multiple SCC/BCC | NE | 52 |
| NS | 29 | Multiple SCC | NE | 53 |
| NS | 29 | Multiple SCC/BCC | NE | 54 |
| ICM | 54 | Multiple SCC | NE | 63 |
| ICM | 40 | Multiple SCC | NE | 62 |
| MAUIE | 21? | SCC | NE | 33 |
| MAUIE | 26 | Multiple SCC | NE | 64 |
| EI | 49? | Multiple SCC/BCC | NE | 68 |
| CHILD | 29 | SCC | NE | 71 |

KID, keratitis–ichthyosis–deafness syndrome; CIE, congenital ichthyosiform erythroderma; LI, lamellar ichthyosis; NS, Netherton syndrome; ICM, ichthyosis Curth–Macklin; MAUIE, micropinnae, alopecia universalis, congenital ichthyosis and ectropion; EI, epidermolytic ichthyosis; CHILD, congenital hemidysplasia with ichthyosiform erythroderma and limb defects; SCC, squamous cell carcinoma; MPTT, malignant proliferating trichilemmal tumour; MM, malignant melanoma; MFH, malignant fibrous histiocytoma; BCC, basal cell carcinoma; NE, not examined; ND, not detected.

## Autosomal recessive congenital ichthyosis

Congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI) are two major types of ARCI.[1] CIE is characterized by fine, white scaling with erythroderma. In contrast, the typical manifestation of LI is coarse brown/dark scaling. Their causative genes are *ALOXE3*,[21] *ALOX12B*,[21] *ABCA12*,[22,23] *CYP4F22*,[24] *NIPAL4*[25] and *TGM1*.[26,27] CIE and LI have been proposed as representing variations of a single group of disorders, although the typical cases of each type have distinct clinical features.[28]

In the literature, five patients have been reported with CIE and two with LI who developed skin malignancies (Table 1).[23,29–33] They began to suffer from SCC between the ages of 25 and 51 years.[29,31–33] There is the possibility that chronic inflammation due to skin barrier defects is associated with skin carcinogenesis in CIE/LI patients,[23] as discussed in the section on KID syndrome. Scarring from chronic inflammation was suggested to underlie SCC in one CIE case,[32] although scar formation was not histologically evident in SCC specimens from two other patients with CIE.[31] The increased proliferation observed in CIE keratinocytes *in vitro*[34] might account for the early onset of SCC. It is notable that the long-term administration of systemic retinoids did not prevent SCC development in some patients with CIE,[31,32] although the retinoids might have reduced the number or severity of the SCCs.

Genetic analysis was performed on only two of the patients with CIE, both of whom had missense mutations in *ABCA12*.[23] The patients developed MM at the ages of 47 and 37, respectively. It is unclear why the ABCA12-deficient patients with CIE developed skin malignancies at those early ages. ABCA12 is an ATP-binding cassette (ABC) transporter that is thought to play a pivotal role in keratinocyte lipid transport.[35,36] ABCA12 is expressed mainly in keratinocytes, and not in melanocytes or lymphocytes.[35,37,38] A recent study also confirmed that ABCA12 is only weakly expressed in normal melanocytes and is largely absent in melanoma cells.[39] ABCA transporters are involved in regulating lipid transport and metabolism, and cholesterol levels may be a limiting factor in membrane maintenance in rapidly dividing cancer cells.[40] From these facts, it is unlikely that ABCA12 deficiency directly promotes skin tumorigenesis including that of MM. Other ABCA members that compensate for ABCA12 dysfunction might be related to tumorigenesis in patients with CIE.

*Abca12*-deficient mouse models have been developed, all of which showed neonatal lethality,[41–44] and these models






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