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G. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

稀少小児遺伝性血液疾患の迅速な原因究明及び診断・治療法の開発に関する研究

先天性血小板減少症の遺伝子解析

研究分担者　國島　伸治（国立病院機構名古屋医療センター臨床研究センター高度診断研究部　室長）

研究要旨：　我々が現在施行中の先天性血小板減少症の遺伝子解析に、次世代シークエンサーを用いた網羅的遺伝子解析を追加した倫理審査承認を得た後に、既知の先天性血小板減少症の原因遺伝子に異常を認めず、両親検体も同時に保存された38家系、117人分のDNA検体を東京大学小川誠司研究室に送付した。

A. 研究目的

先天性血小板減少症は病因不明な疾患が多く、特発性血小板減少性紫斑病（ITP）と診断され不必要な治療を受ける症例も少なくない。本研究は、既知の原因遺伝子に異常を認めない原因不明の先天性血小板減少症において、次世代シークエンサーを用いた網羅的遺伝子解析を施行することにより新規原因遺伝子を同定、病態を解析し、鑑別診断法を確立することを目的とする。

B. 研究方法

我々が現在施行中の先天性血小板減少症の遺伝子解析に、次世代シークエンサーを用いた網羅的遺伝子解析を追加した倫理審査承認を得た後に、既知の先天性血小板減少症の原因遺伝子に異常を認めず、両親検体も同時に保存された 38 家系、117 人分の DNA 検体を東京大学小川誠司研究室に送付した。

（倫理面への配慮）

本研究を行なうにあたっては、当施設で施行中の先天性血小板減少症の遺伝子解析に関する研究に、次世代シークエンサーを用いた網羅的遺伝子解析方法を追加することを当院ヒトゲノム・遺伝子解析研究審査委員会に申請し、審査承認を得た。東京大学へ送付する DNA は、匿名化し、罹患の有無、親子関係、性別のみの情報を付与した。

C. 研究結果

東京大学小川誠司研究室にて次世代シークエンサーを用いたエクソーム解析を施行中であり、候補遺伝子が同定された場合にはサンガー法によりバリデーションを行なう予定である。

D. 考察

先天性血小板減少症は病因不明な疾患がおおいため、鑑別診断される症例は半数に満たない。現在までに、血小板蛋白の生化学および分子生物学的解析やポジショナルクローニングに代表される遺伝学解析によりいくつかの原因遺伝子が同定されてきた。しかし、血小板減少のため解析に供する血小板分離困難や、連鎖解析が可能となる大家系は稀であることにより、その他の稀少疾患の原因遺伝子の同定は困難であった。次世代シークエンサーを用いた網羅的遺伝子解析はこれら稀少な先天性血小板減少症の原因遺伝子同定に進展をもたらすと考えられる。

E. 結論

候補遺伝子が同定された場合にはサンガー法によりバリデーションを行なう。

F. 研究発表

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G. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

稀少小児遺伝性血液疾患の迅速な原因究明及び診断・治療法の開発に関する研究

稀少小児遺伝性血液疾患の疫学データベース構築
研究分担者　小原　明（東邦大学医療センター大森病院　輸血部・小児科　教授）

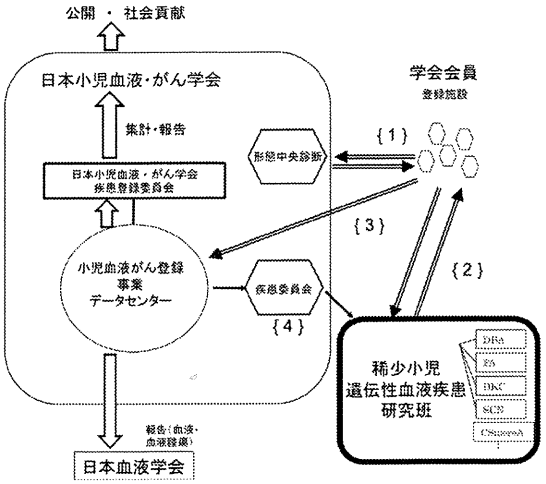
研究要旨：　小児血液がん学会疾患登録事業を一次登録とする、稀少小児遺伝性血液疾患のデータベース構築が開始された。当該一次登録では2006年から2010年の期間にFanconi anemia 18例、DiamondBlackfan anemia 65例、Severe Congenital Neutropenia 8例、Schwachman Diamond anemia 2例、Dyskeratosis congenital 3例が既に登録されている。さらに学会形態診断事業を通じて診断されている該当疾患を調査し、登録を促すことに因り、日本の小児遺伝性血液疾患疫学データベースを完成させ、以後の研究に資する事が可能となった。

A. 研究目的

稀少な小児遺伝性血液疾患の原因究明及び診断治療法の開発の基盤となる、症例登録による疫学データベースを構築する。

B. 研究方法

I. 小児血液がん学会会員を対象にした、血液疾患登録事業ならびに形態中央診断事業を活用し、日本全国から悉皆性をもって稀少な遺伝性血液疾患を渉猟する（図参照）。



図解説

(1) 遺伝性血液疾患（疑）の症例が発生した学会会員施設は、学会中央診断にコンサルトして、意見書を受け取る。

(2) または、直接当研究班にコンサルトする。

(3) 意見書を受け取った後に小児血液がん登録事業データセンター（名古屋 NPO 法人 OSCR 委託）に症例登録を行う（以上が学会疾患登録：一次データ）

(4) 学会疾患委員会（例：再不貧 MDS 委員会）は本研究班が対象とする遺伝性小児血液疾患の疾患登録一次データを受け取り、症例を渉猟して当研究班疫学データベース担当分担研究者に提供する。上記(1)(2)と合わせて悉皆性のあるデータベースを完成する。

II. 当研究班疫学データベース担当分担研究者は疾患別データベースを作成し、疾患別の分担研究者は必要に応じて疾患に適した二次調査を実施する。

III. この一次二次調査を基礎データとして、その後のゲノム研究などへ貢献する。

（倫理面への配慮）

本研究は疫学研究倫理指針に準拠して実施し、本データベース症例登録は診療施設内連結可能匿名化された状態でなされる。データベース構築の元になる小児血液がん学会血液疾患登録事業は既に学会倫

理委員会審査承認が得られている。

C. 研究結果

2006年から2010年診断症例の学会疾患登録（一次調査）により、表に示す症例が渉猟されている。DBA研究者は既にこれを元に二次調査を開始した。

D. 考察

既存の小児血液がん学会疾患登録事業と、形態中央診断事業と連携することにより、全国の稀少小児遺伝性血液疾患を悉皆性をもって渉猟し、データベースを構築することが可能となる。すでに一次調査は実施されており、DBAでは二次調査も開始されていることから、今後この研究班で実施されるゲノム研究に、このデータベースが貢献できる。症例の匿名性、個人情報保護については診断施設内連結可能匿名化されており、ゲノム研究研究者には個人を特定する情報がわたることはない。次年度ではDBA以外の疾患の二次調査を進めるべく、一次調査データの抽出に努める。

E. 結論

小児血液がん学会疾患登録事業を一次登録とする、稀少小児遺伝性血液疾患のデータベース構築が開始された。

F. 研究発表

1. 論文発表

なし

2. 学会発表

なし

G. 知的財産権の出願・登録状況

なし

表

Diagnosis / Year	**2006**	**2007**	**2008**	**2009**	**2010**
Hospitals (registered/member)	184 / 223	204 / 231	212 / 235	213 / 236	216 / 239
(%)	83%	88%	90%	90%	90%
Idiopathic AA	58	57	59	51	48
Hepatitis AA	5	8	11	6	12
AA / PNH	2	1	1	0	1
Fanconi Anemia	**5**	**4**	**4**	**1**	**4**
Diamond-Blackfan	**14**	**10**	**14**	**18**	**9**
Svere Cong. Neutropenia	**2**	**1**	**2**	**0**	**3**
Cyclic Neutropenia	**1**	**3**	**2**	**3**	**2**
Schwachman-Diamond	**0**	**1**	**0**	**1**	**0**
Dyskeratosis congenita	**1**	**0**	**0**	**1**	**1**

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

稀少小児遺伝性血液疾患の迅速な原因究明及び診断・治療法の開発に関する研究

次世代シーケンサーを用いた稀少小児遺伝性血液疾患の原因遺伝子探索

研究分担者　小川　誠司（東京大学医学部付属病院 Cancer Board 特任准教授）

研究要旨：　小児遺伝性血液疾患の多くは、年間発症数が10例以下と極めて稀であるが、致死的経過をたどることが少なくない。ここ数年、原因遺伝子の解明が進みつつあるが、いまだ多くは原因不明であり、的確な診断・有効な治療につながる原因遺伝子の発見が求められている。一方、近年多くの遺伝性疾患で次世代シーケンサーを利用した新たな原因遺伝子の発見が報告されており、単一遺伝子病である遺伝性血液疾患は、次世代シークエンサーを用いた新規遺伝子探索が極めて有用であると考えられている。本研究では、既知の原因遺伝子の異常が同定されなかった小児遺伝性血液疾患症例に対して、家族発症例を中心に次世代シーケンサーを用いた全エクソンシーケンスによる網羅的な原因検索を行う。

A. 研究目的

小児遺伝性血液疾患の多くは極めて稀であるが、致死的経過をたどることが少なくない。ここ数年、原因遺伝子の解明が進みつつあるが、いまだ多くは原因不明である。本研究では、Fanconi 貧血（FA）、先天性赤芽球ろう（DBA）、先天性角化不全症(DKC),遺伝性鉄芽球性貧血(CSA)、先天性好中球減少症(SCN)、先天性顆粒放出異常症、毛細血管拡張性小脳失調症（AT）、一過性骨髄異常増殖症（TAM）、Congenital dyserythropoietic anemia(CDA)　、Shwachman-Diamond syndrome(SBDS)、先天性血小板減少症、先天性溶血性貧血などの疾患を対象に、原因遺伝子が不明な症例について SNP array 解析、次世代シークエンサーを用いた既知の変異遺伝子異常の網羅的な既知の遺伝子異常のスクリーニング解析を行う。さらに、既知の原因遺伝子の異常が同定されなかった症例について、家族発症例を中心に次世代シーケンサーを用いた全エクソンシーケンスをおこない、原因遺伝子を同定することを目的とする。

B. 研究方法

本年度は本研究の環境整備のため、当研究班で保存されている検体の原因遺伝子の判明状況の調査、および研究開始のための研究計画書の策定と倫理委員会の承認を行った。

また、倫理委員会で承認が得られた疾患では解析を開始し、これまでに Diamond-Blackfan 貧血 44 例について SNP array 解析を行った。解析には一塩基多型（SNP）プローブを高密度に配置した Affymetrix 社の SNP アレイである GeneChip 250K アレイを用い、解析アルゴリズムとして CNAG/AsCNAR を用いた。ゲノム全域にわたる網羅的なゲノムコピー数やアレル不均衡の解析を行い、ゲノム異常の詳細について検討を行った。

次世代シーケンサーを用いた全エクソンシーケンスは、ヒト全エクソン領域をターゲットとするビオチン化された cRNA（Agilent 社 SureSelect ®）を用いて濃縮したのち、高速シーケンサー（illumina 社 GA IIx, Hiseq 2000）で解析を行う予定である。

（倫理面への配慮）

本研究で行った臨床検体を用いた実験は、東京大学の倫理審査委員会で審査され、「ヒトゲノム・遺伝子解析研究に関する倫理指針（2001 年、

2008年改訂)」を遵守することを条件に承認された。検体提供者への人権擁護、個人情報保護に細心の注意を払って本研究を実施した。

C. 研究結果

当研究班内には、全国調査で把握された症例の80%の検体が保存されているが、これまでに収集した検体の約半数にあたる579検体では原因遺伝子が未だ検出されていないことが判明した。

本研究の当研究施設での倫理委員会での承認が得られ、また検体集積施設でもCSA（東北大学血液・免疫学分野・張替秀郎教授)、FA（京都大学放射線生物研究センター・高田穣教授、東海大学医学部基盤診療学系細胞移植科・矢部みはる准教授)、DBA(弘前大学小児科学・伊藤悦朗教授)、先天性血小板減少症（名古屋医療センター・國島伸治室長)、AT（東京医科歯科大学発生発達病態学・森尾友宏准教授）については各施設で承認が得られたため、それぞれの施設から221検体が当研究室にこれまでに送付された。このうち、これまでに既知のDBA原因遺伝子の変異が見つかっていない44例のDBA症例のSNP array解析を行い、2例でRPS19（chr19 q13.2）を含む領域の欠失を認めた。SNP arrayで異常が認められなかった症例については今後全エクソンシーケンスを行う予定である。

D. 考察

DBAでこれまでに既知の原因遺伝子の変異が認められなかった症例についてSNP array解析を行ったが、原因遺伝子を含む領域の欠失を認めた症例は少なく、次世代シーケンサーを用いた網羅的な解析が必要であると考えられた。

E. 結論

次世代シーケンサーを用いた網羅的な全エクソンシーケンスは遺伝性疾患での原因遺伝子の検索に極めて有用な方法であり、今後順次解析を行っていく予定である。

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G. 知的財産権の出願・登録状況

なし

Ⅲ．研究成果の刊行に関する一覧

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Yagasaki H, Kojima S, Yabe H, Kato K, Kigasawa H, Sakamaki H, Tsuchida M, Kato S, Kawase T, Morishima Y, Kodera Y.	Acceptable HLA-mismatching in unrelated donor bone marrow transplantation for patients with acquired severe aplastic anemia.	Blood	118	3186-90	2011
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Ⅳ. 研究成果の刊行物・別刷り

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

Submitted April 22, 2011; accepted June 18, 2011. Prepublished online as *Blood* First Edition paper, July 14, 2011; DOI 10.1182/blood-2011-04-349316.

The online version of this article contains a data supplement.

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typed for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 by a molecular technique and underwent UBMT through the JMDP.

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 3.1×10^8/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 0.5×10^9/L. Patients who did not reach neutrophil counts > 0.5×10^9/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 < 0.5×10^9/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

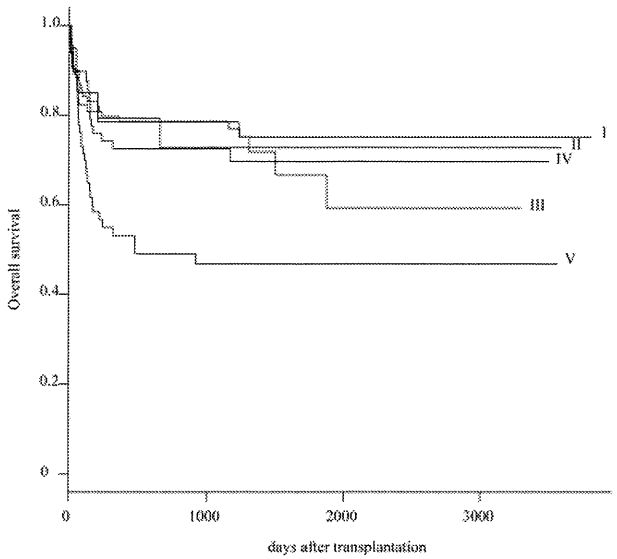

Figure 1. Kaplan-Meier estimates of OS in 5 HLA groups.

(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

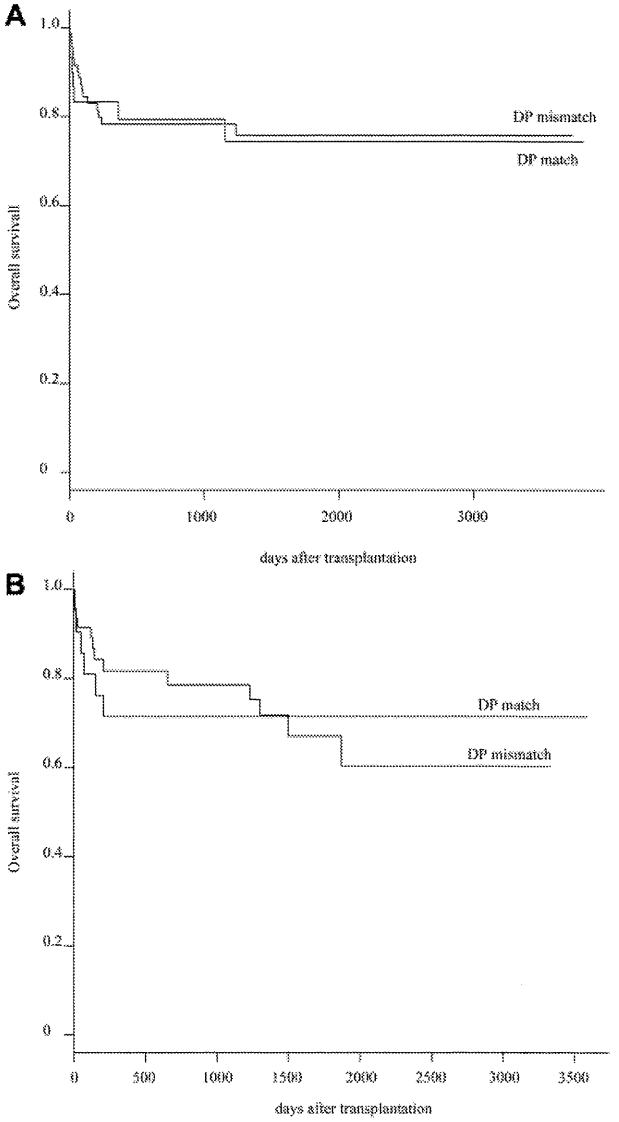

Figure 2. OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group. (A) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 10 of 10 HLA allele matched pairs. (B) Difference of OS between HLA-DPB1 matched group and HLA-DPB1 mismatched group in 9 of 10 HLA allele matched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

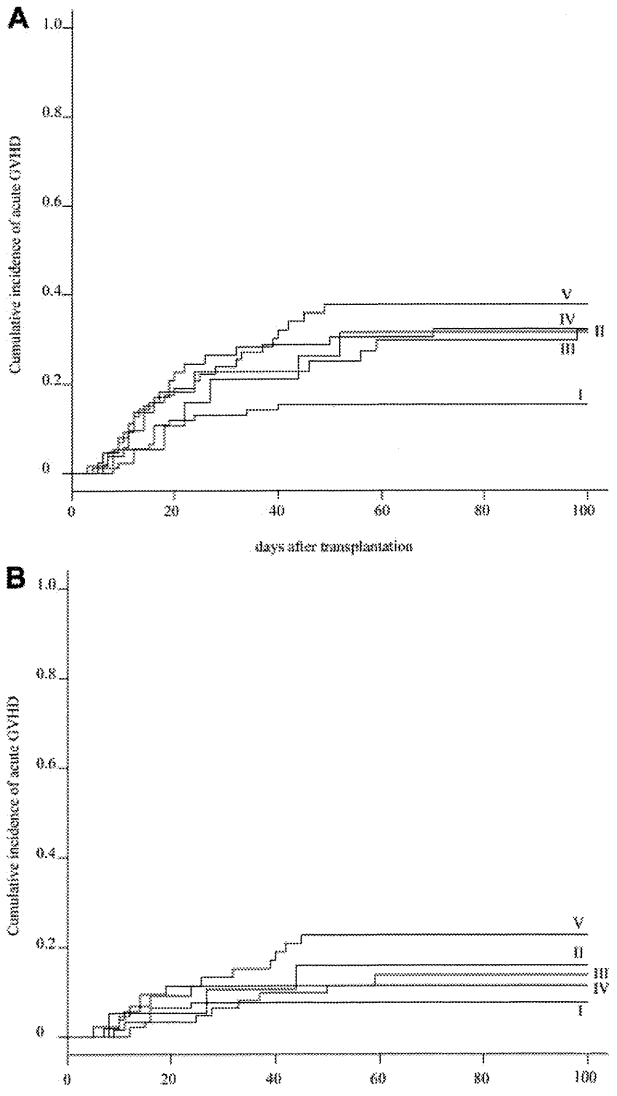

Figure 3. Cumulative incidence of acute GVHD. (A) Cumulative incidence of grade II-IV acute GVHD in 5 HLA groups. (B) Cumulative incidence of grade III-IV acute GVHD in 5 HLA groups.

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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