厚生労働科学研究費補助金

免疫・アレルギー疾患等予防・治療研究事業

組織適合性に基づく非血縁同種造血幹細胞移植の成績向上に関する研究

平成20年度～22年度　総合研究報告書

研究代表者　森 島 泰 雄

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（免疫アレルギー疾患等予防・治療研究事業（H20-免疫-一般-014）

総合研究報告書

組織適合性に基づく非血縁同種造血幹細胞移植の成績向上に関する研究

研究代表者　森島泰雄　愛知県がんセンター中央病院副院長・部長

研究要旨：非血縁者間骨髄移植を受けた患者とドナーのＨＬＡ遺伝子型とその他の組織適合性抗原を精緻な細胞遺伝学的な手法（HLAとその分子解析、HLAハプロタイプ解析、HLA遺伝子以外の多型解析（Whole genome SNPs 、マイクロサテライト、サイトカイン受容体、NK細胞受容体）、In vitro解析）で解析することにより得られたＧＶＨＤ、ＧＶＬに関与する組織適合性抗原の同定や新知見は、JMDPでのドナー選択HLA検査や移植の臨床に応用され、移植成績の向上をはかることができた。

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（H21年度～H22年度）

A．研究目的

日本骨髄バンク（JMDP）からの非血縁者間骨髄移植が18年間に約12000例、日本さい帯血ネットワークからの移植が約6000例実施されているが、長期生存率はそれぞれ約60％と40％に留まり満足すべきものではない。成績を悪化させる主な原因は同種移植に伴う重症移植片対宿主病（GVHD）の発症、移植片の拒絶と移植後の造血器腫瘍の再発であり、ドナーと患者の組織適合性抗原の違いがこれら免疫反応と生存に大きく関与することが本研究班におけるJMDP症例を用いた解析で次第に明らかになってきた。

本研究は、JMDPを介した患者とドナーのペアーの同意が得られた検体を保存し、より精緻な細胞遺伝学的な手法（HLAとその分子解析、HLAハプロタイプ解析、HLA遺伝子以外の多型解析（Whole genome SNPs 、マイクロサテライト、サイトカイン受容体、NK細胞受容体）、In vitro解析）を用い、HLAとHLA以外の組織適合性

抗原とその多型が移植免疫反応に与える影響を明らかにする。これら患者とドナーの組織学的な情報と臨床情報に基づき、適切なドナー選択の個別化アルゴリズム（基準）を構築することを目的とする。

B．研究方法

1．非血縁者間骨髄移植症例とドナーの検体保存

同意が得られた非血縁者間骨髄移植実施患者とドナーの血液検体を細胞、DNAに分離し、凍結保存する（骨髄移植推進財団検体保存事業として実施）。

2．HLA抗原座およびHLA抗原型の違いが移植免疫反応と生存に及ぼす影響の解析

JMDPを介した非血縁者間骨髄移植症例におけるドナーと患者のHLA型と臨床データからなるデータベースを作成する。HLA-A, B, C, DRB1, DQB1, DPB1の遺伝子型を同定し、whole genome増幅法を用い、検体を増量し、解析用サンプルセットを作成する。

Cox hazard model等を用いた多変量解析法により移植免疫反応に関与する不適合HLA抗原の組み合わせを同定する。非血縁者間移植における「許容されないHLA型の不適合の組み合わせ」と「許容されるHLA型の組み合わせ」を検索し、従来HLA抗原座の不適合により選択されなかったドナーからの移植を可能にさせる。

3．HLAハプロタイプの均一性の検討

HLA領域のmulti-SNPsデータとHLA型を用いて解析.

4．国際ワークショップにおける検討

本研究で得られる知見が、日本人間だけではなく他の民族においても認められる普遍的なものであることを検証するため、国際組織適合性ワークショップにおいて国際的な移植データベースによる解析を行った。

5．HLA以外の組織適合性に関与する抗原 と多型の移植成績に及ぼす影響の検討

以下の解析手法を用いて解明する。

1）NK細胞受容体関連抗原の関与：KIR関連遺伝子とその多型の解析

2）HLA領域とその他の領域のマイクロサテライト解析

3）サイトカイン受容体多型等の解析

4）Whole genome SNPs解析

6．統合解析

本研究で有意になった多数の組織適合性抗原多型の中で臨床的に有用な多型の重みづけ解析（統合解析）を行い、影響の大きい多型を見出しJMDPでの組織適合検査に組み込むことを目的として、本研究班で同定された、あるいは論文化されている50種の多型を抽出し、HLA完全適合450ペアーのDNAを用いてTagman法で解析を実施している。。

（倫理面への配慮）

本研究で用いるデータと試料は「ヒトゲノム・遺伝子解析研究に関する倫理指針」「疫学研究に関する倫理指針」により骨髄バンク等により患者、ドナーへの説明と同意が得られたものを用いた。

本研究に用いるデータ・試料は連結可能匿名化されたものを用いた。

本研究は研究実施施設におけるヒトゲノム遺伝子倫理審査委員会ならびに該当バンクの承認を得て実施した。

C．研究結果

1．非血縁者間骨髄移植症例とそのドナーの検体整備

患者とドナーの血液検体（約900ペアー　1800検体/年度）を細胞、DNAに分離し、保存した。

2。 HLA適合度が移植免疫反応に与える影響の解析

1）検体の調整とHLA検査法の確認

約1000ペアのHLA-A, B, C, DRB1, DQB1, DPB1の遺伝子型を同定した。さらに、従来の保存DNAも加えて、whole genome 増幅法を用い、約2000検体を増量し、解析用サンプルセット作成を可能にした。

健常者503人を対象として最も高精度とされるSBT法（自家製）と現在HLA-A,-B,-DR検査に用いられている蛍光ビーズ法「WAKFlow　HLAタイピング試薬 HLA-C」とによる大規模*HLA-C*頻度調査を実施し、両方法を比較検討した。*HLA－C*タイピングでは503件中、１例に新アリルCw*0303Vが検出された。Cw*0303Vは非同義置換を伴う新アリルでWAKFlow　HLAタイピング試薬 HLA-Cでは通常のCw*0303と判定された。この１例を除き蛍光ビーズ法で得られた結果とSBT法との結果はすべて一致した（一致率99.8%）。このことから、SBT法と比べ多数検体処理能に優れた蛍光ビーズ法においても、HLA-C座の検査が可能であることが確認された。（２）市販のWGAキット3種類を用いて全ゲノム増幅効率と増幅前後検体によるHLAタイピング結果を比較し、最適な系（約1万倍の増幅率）を決定し、東海大学と協力して同系を用いて患者ドナー保存検体の増幅作業を開始した。また解析希望検体セットをロボットシステムで作成、配布する系の構築作業中である。

2）HLA型適合度につき症例数を増して多変量解析しHLA-Aｱﾚﾙ、HLA―Bｱﾚﾙ、HLA-Cｱﾘﾙ、HLA-DPB1ｱﾘﾙのドナーと患者間の違いが急性GVHDの発症頻度を高め、HLA-DRB1とHLA-DPB1の違いは高めないことを確認し、従来移植コーディネート時に実施されているHLA適合確認検査にHLA-C検査を新たに追加するためのデータを提供した。

3）許容されるされないHLA型の組み合わせの同定

許容されないHLA型の組み合わせ群が明らかになっていたが、この許容されない組み合わせを対照として、有意に重症GVHDの頻度が低くなる組み合わせを予備的に「許容される組み合わせ」として12組を明らかにした。許容できない組み合わせは、JMDPのドナー選択アルゴリズムに組み入れられた。

3．非血縁者間骨髄移植におけるＨＬＡ型の違いと移植片対白血病効果（ＧＶＬ）の解析）

造血器腫瘍症例につき多変量解析を用いて解析し

1）ＨＬＡ－Ｃ座とＨＬＡ－ＤＰＢ１座の違

いがＧＶＬを生じる。

２）ＨＬＡ－Ｃはリンパ系腫瘍、ＨＬＡ－ＤＰＢ１は骨髄系腫瘍においてＧＶＬ効果が認められる。

３）ＨＬＡ－Ｃ座不適合の中で、ＧＶＬ効果を生じさせるＣ遺伝子型の組みあわせを明らかにすることが出来た。

４．日本人に特有なHLAハプロタイプとその高度な保存性と移植免疫反応への関与

高頻度なHLA-A～-DPB1ハプロタイプHP-P1, HP-P2, HP-P3ではHLA-A座からHLA-DPB1座までの3.3 Mbが高度に保存されているだけでなく、HLA-A座のテロメア側に広範囲に保存されていた。さらに、HP-1ではHLA-Aのテロメア側でsubtype AとBに分かれることが判明した。

非血縁者間骨髄移植におけるＨＬＡハプロタイプと急性ＧＶＨＤとの関連を解析すると、日本人に高頻度に認められるＨＬＡ－ＡからＨＬＡ－ＤＰＢ１と広範囲に及ぶＨＬＡハプロタイプを同定することができた。さらに、特定のＨＬＡハプロタイプ（ＨＰ－Ｐ２）を有する患者において急性ＧＶＨＤの発症頻度が低く、ＨＰ－Ｐ３では高くなる傾向が認められた。

このHLAハプロタイプのSNPのコンセンサスシークエンスに基づき、２５０個の日本人に存在するHLAハプロタイプとその均一性を明らかにすることができた。これら同定された多数のHLAハプロタイプを用いてHLAがHLA-AからHLA-DPB1までが完全一致していればHLAハプロタイプとして適合しているかどうか検索中である。

５．非血縁者間造血幹細胞移植における急性GVHD発症率、白血病再発率、移植後生存率の人種による違いの解析

ＨＬＡ－ＡからＤＱＢ１まで適合したＴ細胞除去法を用いないＧＶＨＤ予防法をにより非血縁者間移植を実施した5543症例を用いて多変量解析することにより、日本人間の移植の急性GVHDの頻度、白血病の再発率、移植後の死亡率はいずれも白人間の移植に比べて有意に低いことが明らかになった。人種特異的に頻度の高いHLAハプロタイプを有する日本人と白人の移植の比較でも、日本人間の移植は白人間に比し有意に急性GVHDの頻度が低かった。

６．HLA以外の組織適合性に関与する抗原と多型の移植成績に及ぼす影響の解析

１）マイクロサテライト多型解析により、IL1RAPL2, MAPK14, DNAH17, TNF, TBL1X, MMP25, ELTD1, MAP3K4, IL7R, AKT3, S100Z, CAV1に急性GVHD発症と統計学的に有意な関連を認めた。IL2-330のG/TのSNPが慢性GVHD発症に強く関連していることが見いだされた（p=0.00007）。またTNFα-1031とTNFRSF1Bは重症急性GVHDの発症と統計学的に有意に関連していた。

２）日本人の同種造血幹細胞移植において、Tリンパ球の活性化を抑制する分子cytotoxic T-lymphocyte antigen-4（*CTLA-4*）の多型が移植成績に影響を与えるか否か、その遺伝子ハプロタイプに着目して検討した。*CTLA-4*遺伝子の3塩基（-318、+49、CT60）がC-A-Aとなるハプロタイプを有するドナーから移植を受けた場合は、

そうでないドナーから移植を受けた場合と比べ、再発率が低く、GVHD発症率は変わらず、生存率が高いことが確認された。

3）*IL-17*遺伝子多型(rs2275913, G197A)解析を行い、197A陽性 vs. 197A陰性で比較検討した。骨髄破壊的移植(360ペア)群では、患者側の197A陽性は、急性 GVHDの有意な危険因子であった(ハザード比1.33・95%信頼区間1.00-1.76・*P*=0.05)。骨髄非破壊的移植(150ペア)群を対象にこれを検証したところ、患者197陽性が急性GVHDの危険因子であることが裏付けられた(ハザード比2.36・95%信頼区間1.23-4.51・*P*=0.01)。

4）抑制性サイトカインである*IL-10*遺伝子のプロモーター領域SNPおよび*IL-10受容体* 遺伝子SNPと移植成績について解析し、患者*IL-10*遺伝子プロモーターSNPハプロタイプが急性GVHD重症化および無病生存率と関連することを明らかにした。

5）KIRリガンド不適合移植ドナーおよびHLA6座12抗原一致症例ペアの約800検体のタイピングを行った。LILR受容体のうち抑制型*LILRB2*の遺伝子多型と移植成績との関連解析を行い、急性GVHD重症化との関連を見出した。抑制性サイトカイン*IL-10*遺伝子多型と移植成績の関連解析は昨年までに判明した患者遺伝子プロモーター3箇所のSNPハプロタイプに加えて新たに上流側のSNPも急性GVHD重症化と関連することを明らかにした。さらに近接する*IL-19*遺伝子SNPが急性GVHD重症化および無病生存率と関連する結果を得た。

6）HLA 5座適合1600移植に関する全ゲノム関連解析を実施し、GVHD関連遺伝子座の探索を行い、新規マイナー組織適合性抗原遺伝子座に関わると考えられる20個のGVHD関連候補遺伝子座を同定した。さらに、これらの候補遺伝子座について独立な移植症例セットを用いた関連の検証研究を行った。検証解析の結果、5番染色体のSNP座の不適合とGVHDとの関連は再度確認された。

7）NKG2Dは、遺伝子多型により、高NK活性型(NKG2D-HNK1)と低NK活性型(NKG2D-LNK1)がある。HLAアリル一致非血縁者間骨髄破壊的前処置骨髄移植145症例を解析したところ、HNK1ハプロタイプ陽性ドナーから移植を受けた患者（標準リスク群）の生存率(OS)・移植関連死亡(TRM)は有意に優れていた。この結果は第2コホート（360例）解析でも確認された。そこで、移植免疫におけるNKG2D遺伝子多型の影響を検討するため、NK細胞の機能解析を行った。健常人のNK細胞をエフェクターとして、CMV感染線維芽細胞・NKG2D-L誘導（VPA処理）骨髄性白血病細胞株に対する細胞傷害活性を検討したところ、HNK1陽性NK細胞はHNK1陰性NK細胞に比べて、高い細胞傷害活性を示した。活性化NK細胞上のNKG2D発現は、HNK1陽性の方が高く、mRNA発現も同様であった。次に、NKG2Dハプロタイプを決定するSNP(rs1049174)がNKG2D 3' UTR領域にあることを考慮し、NK細胞内NKG2D mRNAに対する影響をレポーター遺伝子解析で検討した。NKG2Dアリル種類にかかわらずNKG2D 3' UTR挿入後レポーター遺伝子発現は低下したが、LNK1アリルの方がHNK1

アリルより、発現低下が顕著であった。標識したNKG2Dアリルプローブと核内抽出物との反応をゲルシフト解析でみたところ、LNK1アリルの方が、核内抽出物と高い結合性を示した。以上から、NKG2D 3' UTR SNP (rs1049174)は機能的なSNPで、核内の抑制因子と結合することにより、NKG2D遺伝子発現を調整している可能性が示唆された。これにより、NKG2D遺伝子多型が、移植後転帰に影響していると考えられた。

7．HLA不適合非血縁移植におけるGVHD発症機構の解明

その免疫学的機序は、ドナーおよびレシピエントにおいて、T細胞が共通に認識するHLA・ペプチド複合体の発現量多寡に基づくとの仮説を提唱し、動物モデルによって検証することを目的として、B6マウスを遺伝背景として、ドナーモデルとしてのβ2mノックアウトマウスのヘテロ接合、レシピエントモデルとして発現量の異なる３系統のH2-Kbトランスジェニックマウスを樹立した。さらにホモ接合H2-Kbトランスジェニックマウスを作成して、同一HLA複合体の発現量が異なる個体間での骨髄移植によるGVHD発症モデルとしての検証を試みた。

8．HLA不適合が関わる移植免疫発症機構のin vitroでの解析

HLA-A, B, DRB1一致、Cw 1座不一致の非血縁ドナーから骨髄移植を受け、移植片対宿主病および移植片対腫瘍効果を認めた急性リンパ性白血病患者の末梢血から、7つの細胞傷害性Tリンパ球クローンを分離した。それらは全て、患者のみが有するHLA-Cw*03分子をその分子上に提示されているペプチドとともに認識していた。

D。考察

わが国のHLA解析研究は世界の非血縁移植のHLA研究を常にリードし、その解析結果はJMDPにおけるHLA検査法とドナー選択への適格で迅速な導入がなされてきた。計画的かつ継続的な保存事業による多数検体の保存と組織適合性研究者による最新の組織適合性研究手法の導入によりはじめて可能になるものである。本研究で得られる新しい精緻な組織適合性検索とドナー選定への応用により、今後いっそう移植成績の向上と両バンクの効率的な運用に寄与するものと考えられる。また、人のGVHD発症機構を明らかにする上で、基礎的にも重要な知見を提供している。

E．結論

非血縁者間骨髄移植を受けた患者とドナーのＨＬＡ遺伝子型とその他の組織適合性抗原を精緻な細胞遺伝学的な手法（HLAとその分子解析、HLAハプロタイプ解析、HLA遺伝子以外の多型解析（Whole genome SNPs 、マイクロサテライト、サイトカイン受容体、NK細胞受容体）、In vitro解析）で解析することにより得られたＧＶＨＤ、ＧＶＬに関与する組織適合性抗原の同定や新知見は、JMDPでのドナー選択HLA検査や移植の臨床に応用され、移植成績の向上をはかることができた。今後、さい帯血移植や非血縁者間

造血幹細胞移植への研究の進展が求められている。

F．健康危機情報

なし

G．　研究発表

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H．知的財産権の取得状況

なし

別紙４

研究成果の刊行に関する一覧表

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ORIGINAL ARTICLE

A single nucleotide polymorphism of IL-17 gene in the recipient is associated with acute GVHD after HLA-matched unrelated BMT

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IL-17 has an important role in the host defense against extracellular pathogens and the pathophysiology of autoimmune diseases. This study retrospectively examined the impact of a single-nucleotide polymorphism (rs2275913, G197A) in the IL-17 gene of a total 510 recipients with hematologic malignancies and their unrelated donors on the clinical outcomes in HLA-matched myeloablative (discovery study) and nonmyeloablative (validation study) BMT through the Japan Marrow Donor Program (JMDP). In the discovery study, the presence of a 197A genotype in the recipient resulted in a higher incidence of grades II–IV acute GVHD (hazard ratio (HR), 1.87; 95% confidence interval (CI), 1.23–2.85; $P=0.004$). The donor IL-17A genotype did not significantly influence the transplant outcomes. The validation study showed a trend toward an association of the recipient 197A genotype with an increased risk of grades III–IV acute GVHD (HR, 5.84; 95% CI, 0.75–45.72; $P=0.09$), as well as a significantly increased risk for chronic GVHD (HR, 3.86; 95% CI, 1.29–11.59; $P=0.02$). These results suggest an association of the 197A genotype in the recipient side with the development of acute GVHD.

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Keywords: IL-17; unrelated donor; single-nucleotide polymorphism

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Introduction

Hematopoietic SCT represents a therapeutic approach that can potentially cure many patients with otherwise fatal hematologic malignancies. However, its utility is limited because of transplant-related life-threatening complications including GVHD, infections and disease relapse.[1] Among these, acute GVHD is the main cause of early mortality and morbidity. Although HLA matching represents the major genetic determinant in clinical outcome after allo-SCT, recent evidence suggests that non-HLA immune-associated genes are also implicated.[2] Previous investigations have revealed that several single-nucleotide polymorphisms (SNPs), which impact on individual immune response to infections and inflammatory reactions are associated with SCT outcomes including the risk of acute GVHD.[3–12]

IL-17, also known as IL-17A, is the hallmark cytokine of a new T-helper subset termed Th17.[13–16] γδT cells, macrophages and neutrophils are sources of IL-17 as well.[17,18] IL-17 receptor (IL-17RA), a ubiquitous type-I membrane glycoprotein, is expressed in particularly high levels in hematopoietic tissues.[13,19,20] IL-17 has important roles in bridging innate and adaptive immunity, and is involved in the host defense against extracellular pathogens, the pathophysiology of autoimmune diseases, and allograft rejection of solid organs.[21–29] Moreover, several reports have so far shown that Th17 cells and IL-17 has a significant impact on the development of acute GVHD in mouse models.[30–35]

Recent reports have shown association of SNPs in the IL-17 gene with autoimmune diseases such as rheumatoid arthritis and ulcerative colitis.[36–39] The promoter SNP of the IL-17 gene, rs2275913 (G197A), was found to be associated with the susceptibility of rheumatoid arthritis in the Norwegian population[38] as well as that of ulcerative colitis in the Japanese population.[36] The finding that GVHD mimics some aspects of autoimmune diseases prompted us to investigate the impact of donor and recipient SNPs in the IL-17 gene (rs2275913,

G197A) on the clinical outcomes in patients following allogeneic myeloablative BMT using an HLA allele-matched unrelated donor. The data herein show that the presence of the 197A allele in the recipient is associated with a significantly higher incidence of acute GVHD.

Design and methods

Patients

In a total 510 recipients with hematologic malignancies and their unrelated donors on whom IL-17 genotyping was performed, 360 recipients in the discovery study cohort received myeloablative transplantation between January 1993 and July 2002, and 150 recipients in the validation study cohort received nonmyeloablative transplantation between January 1996 and December 2007. Transplantation was undertaken through the Japan Marrow Donor Program (JMDP) with T-cell-replete marrow from an HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 allele-matched donor. HLA genotypes of patient and donor were determined by the Luminex microbead method described previously (Luminex 100 System; Luminex, Austin, TX, USA).[40,41] Although the Luminex microbead method does not provide unambiguous HLA four-digit typing for all genotypes, JMDP has confirmed that this method can identify all HLA alleles with >0.1% frequency among the Japanese population.[42] No patients had a history of any previous transplantation. The final clinical survey of these patients was completed by November 1, 2008. Diagnoses were acute myeloid leukemia in 156 (31%), acute lymphoblastic leukemia in 100 (20%), chronic myeloid leukemia in 94 (18%), myelodysplastic syndrome in 79 (15%), malignant lymphoma in 71 (14%), and multiple myeloma in 10 (2%; Table 1). The recipients were defined as having standard risk disease if acute myeloid leukemia and acute lymphoblastic leukemia were in first CR, malignant lymphoma was in any CR and chronic myeloid leukemia was in any chronic phase and myelodysplastic syndrome. All others were designated as high-risk disease. The myeloid malignancies include acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, and the lymphoid malignancies included acute lymphoblastic leukemia and malignant lymphoma. CYA- or tacrolimus-based regimens were used in all patients for GVHD prophylaxis and anti-T-cell therapy such as anti-thymocyte globulin and *ex vivo* T-cell depletion was not. All patients and donors gave their written informed consent to participate in molecular studies of this nature according to the declaration of Helsinki at the time of transplantation. The project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and JMDP.

IL-17 G197A genotyping

Genotyping of IL-17 was performed using the TaqMan-Allelic discrimination method[43] with a 7900-HT Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) and results were analyzed using the Allelic Discrimination software program (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and the specific probe rs2275913 designed for SNP of IL-17 G197A (product No. C_15879983_10; Applied Biosystems).

Data management and statistic analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pretransplant CMV serostatus was routinely tested for only patients but not for their donors. Engraftment was confirmed by an ANC of more than 0.5×10^9/L for at least 3 consecutive days. Acute- and chronic GVHD were diagnosed and graded using established criteria.[44,45] The OS was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplant-related mortality was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. The data on causative microbes of infections and postmortem changes in cause of death, as well as the data on supportive care including infections prophylaxis and therapy of GVHD, which were given on institution basis, were not available in this cohort. The analysis was performed using the Excel 2007 (Microsoft Corp, Redmond, WA, USA), OriginPro version 8.0J (Lightstone Inc., Tokyo, Japan) and R (The R Foundation for Statistical Computing, Perugia, Italy) software programs.[46] The probability of OS was calculated using the Kaplan–Meier method and compared using the log-rank test. The probabilities of transplant-related mortality, disease relapse, acute GVHD, chronic GVHD and each cause of death were compared using the Grey test[47] and analyzed using the cumulative incidence analysis,[46] considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD and death without each cause as respective competing risks. The variables were recipient age at time of transplantation, sex, CMV serostatus before transplantation, disease characteristic (disease type, disease lineage and disease risk at transplantation), donor characteristics (age, sex, sex compatibility and ABO compatibility), transplant characteristics (TBI-containing regimen, tacrolimus vs CYA and total nucleated cell count harvested per recipient weight) and the year of transplant. The median was used as the cutoff point for continuous variables. The χ^2-test and Mann–Whitney test were used to compare two groups. The Hardy–Weinberg equilibrium for the IL-17 gene polymorphism was tested using the Haploview program.[5] Multivariate Cox models were used to evaluate the hazard ratio (HR) associated with the IL-17 polymorphism. Covariates found to be significant in univariate analyses ($P \leqslant 0.20$) were included in the models. For both the univariate and multivariate analyses, P-values were two-sided and outcomes were considered to be significant with $P \leqslant 0.05$.

Results

Discovery study

Frequencies of the IL-17 genotyping. The IL-17 gene polymorphism was analyzed in 360 unrelated BM donor-myeloablative transplant recipient pairs (Table 1). The genotype frequencies of 197A/A, 197A/G and 197G/G were 16, 46 and 38% in recipients and 14, 51 and 36% in

Table 1 Donor and recipient characteristics

	Discovery study (myeloablative transplantation)				P	*Validation study (nonmyeloablative transplantation)*				P
	Recipient IL-17 genotype					*Recipient IL-17 genotype*				
	197A positive n = 223, 62%		*197A negative* n = 137, 38%			*197A positive* n = *87, 58%*		*197A negative* n = *63, 42%*		
	No.	*Ratio (%)*	*No.*	*Ratio (%)*		*No.*	*Ratio (%)*	*No.*	*Ratio (%)*	
Age, years										
Recipient										
Median		33		29	0.12		53		51	0.99
Range		2–65		1–65			1–70		3–68	
Donor										
Median		34		33	0.11		35		33	0.47
Range		20–51		22–51			21–50		20–51	
Year of transplant										
Median		1998		1998	0.65		2004		2004	0.22
Range		1993–2002		1993–2002			1996–2007		1996–2007	
Donor IL-17 genotype										
197A positive	145	65	87	64	0.77	53	61	40	63	0.75
197A negative	78	35	50	36		34	39	23	37	
Sex, male										
Recipient	136	61	74	54	0.81	61	70	39	62	
Donor	141	63	77	56	0.19	26	30	24	38	
Recipient/donor sex										
Sex matched	138	62	86	63	0.99	62	71	43	68	0.20
Male/female	45	20	27	20		14	16	6	10	
Female/male	40	18	24	18		11	13	14	22	
Disease										
Acute myeloid leukemia	73	33	37	27	0.25	23	26	23	37	0.19
Acute lymphoblastic leukemia	48	22	38	28	0.18	9	10	5	8	0.62
Chronic myeloid leukemia	53	24	31	23	0.80	4	5	6	10	0.23
Myelodysplastic syndrome	25	11	16	12	0.89	26	30	12	19	0.13
Malignant lymphoma	23	10	14	10	0.98	19	22	15	24	0.78
Multiple myeloma	1	0	1	1	0.73	6	7	2	3	0.32
ABO matching										
Match	148	66	88	64	0.35	52	60	40	63	0.65
Major mismatch	38	17	17	12		18	21	16	25	
Minor mismatch	32	14	28	20		21	24	10	16	
Bidirectional	5	2	4	3		4	5	3	5	
Conditioning regimen										
With total body irradiation	177	79	115	84	0.28	53	61	39	62	0.90
Without total body irradiation	46	21	22	16		34	39	24	38	
Pretransplant CMV serostatus										
CMV positive recipient	149	67	98	72	0.35	68	78	53	84	0.36
Missing	26	12	18	13	0.68	8	9	10	16	0.21
GVHD prophylaxis										
With cyclosporine	145	65	91	66	0.71	39	45	26	41	0.66
With tacrolimus	78	35	46	34		48	55	37	59	
TNC × 10^8 per kg										
Median		5.7		5.7	0.89		4.2		4.5	0.13
Range		0.1–87.0		0.6–87.0			0.8–74.2		1.3–33	
Engraftment	220	99	136	99	0.59	81	93	59	94	0.89

Abbreviation: TNC = total nucleated cell count harvested.

donors. These were similar to previous reports[38,48] in Japanese populations (15, 52 and 33%, respectively) and Caucasian populations (13, 48 and 39%, respectively), and were in accord with the Hardy–Weinberg equilibrium ($P = 0.91$).

Transplant outcome according to the IL-17 genotype. The median follow-up duration in the cohort was 90 months among the survivors (range 4–171 months), 102 recipients (28%) had relapsed or progressed and 187 (52%) had died. Three patients (1%) died before engraftment.

The transplant outcomes according to the IL-17 genotype are summarized in Table 2. The presence of the 197A genotype in the recipient was associated with a significantly higher incidence of grades II–IV acute GVHD (37 vs 23%, $P=0.004$; Figure 1a) as well as a trend toward a higher incidence of grades III–IV acute GVHD (16 vs 10%, $P=0.08$; Figure 1b), whereas no significant differences between the 197A/A and the 197A/G genotype in the recipient were seen in incidences of grades II–IV (38 vs 34%, $P=0.69$) and grades III–IV (17 vs 16%, $P=0.96$) acute GVHD. The 197A genotype on the recipient side showed a tendency to increase a risk of mortality of acute GVHD as a primary cause of death (6 vs 2%, $P=0.095$). There were no significant differences in the impact of a 197A in the recipient genotype on OS, transplant-related mortality, relapse, chronic GVHD or extensive chronic GVHD (data not shown). The donor genotype showed no significant effects on either of these variables in addition to acute GVHD (Table 2).

Multivariate analysis. All of the factors found to be significant in univariate analyses were included in the model. The 197A genotype in recipients remained statistically significant in the multivariate analyses for the development of grades II–IV acute GVHD (Table 3). The presence of a 197A genotype in the recipient side resulted in a higher incidence of grades II–IV acute GVHD (HR, 1.87; 95% confidence interval (CI), 1.23 to 2.85; $P=0.004$) when adjusted for the other factors in the models. In the combined patient group of acute lymphoblastic leukemia and acute myeloid leukemia, this effect was also positive and was close to statistical significance (HR, 1.84; 95% CI, 0.98–3.43; $P=0.056$).

Validation study

The characteristics of the patients in the validation study were similar to those of the patients in the discovery study except for conditioning regimen and recipient age (Table 1). The univariate analysis showed a significant association between the recipient 197A genotype and a higher incidence of grades III–IV acute GVHD (15 vs 4%, $P=0.04$; Figure 1d), whereas no significant difference in the incidence of grades II–IV acute GVHD (33 vs 26%, $P=0.37$; Figure 1c). In the multivariate analysis, the validation study performed on nonmyeloablative SCT did not confirm the association of recipient 197A with grades II–IV acute GVHD found in the discovery study, although there was a trend toward an association with grades II–IV acute GVHD (HR, 5.84; 95% CI, 0.75–45.72; $P=0.09$; Table 4). The recipient 197A genotype was associated with a significantly increased risk for chronic GVHD (HR, 3.86; 95% CI, 1.29–11.59; $P=0.02$), although this association was not found in the discovery study.

Discussion

The discovery study on the basis of myeloablative transplantation showed that the IL-17 197A genotype on the recipient side was associated with a higher risk of grades

Table 2 Univariate analysis of the association of IL-17 genotype with clinical outcomes after transplantation in the discovery study

	No.	*5-year OS (%)*	P	*5-year TRM (%)*	P	*5-year relapse (%)*	P	*II–IV acute GVHD (%)*	P	*III–IV acute GVHD (%)*	P	*Chronic GVHD (%)*	P
Recipient IL-17A genotype													
197A positive	223	53	0.89	27	0.20	24	0.21	37	0.004	16	0.08	48	0.94
197A negative	137	53		21		31		23		10		48	
Donor IL-17A genotype													
197A positive	232	50	0.13	27	0.09	27	0.93	31	0.71	14	0.70	49	0.66
197A negative	128	56		21		27		34		13		47	

Bold values have statistical significance.

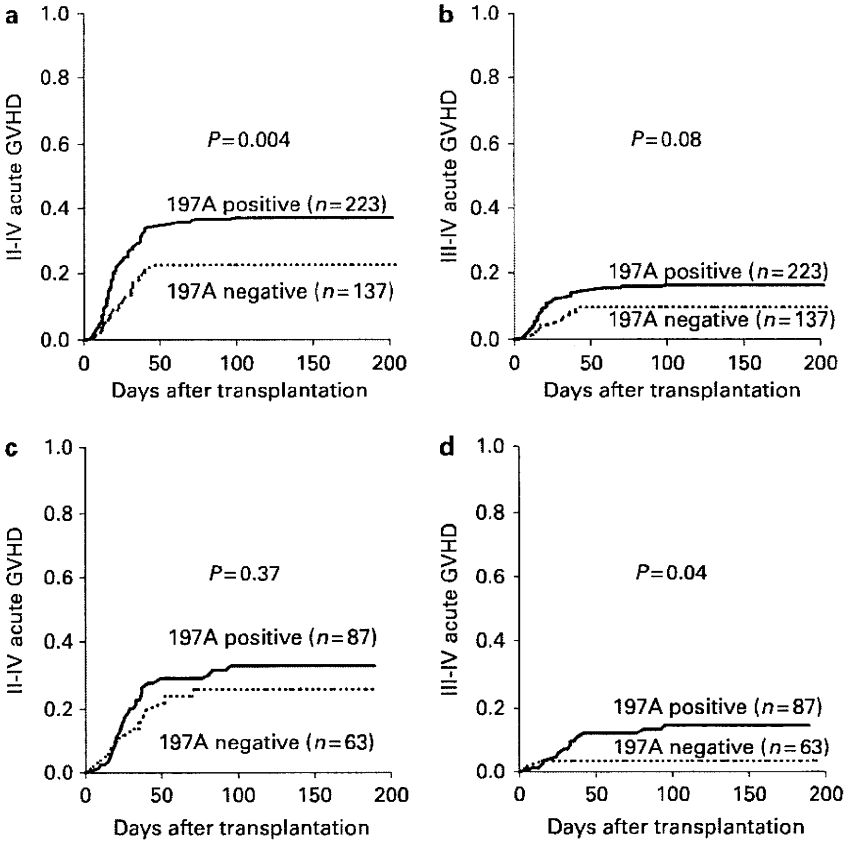

Figure 1 Estimated cumulative incidence curves of grades II–IV (**a**, **c**) and grades III–IV (**b**, **d**) acute GVHD according to the recipient IL-17 genotype in the discovery study (**a**, **b**) and the validation study (**c**, **d**).

Table 3 A multivariate analysis of the association of IL-17 genotype with the clinical outcomes after transplantation in the discovery study

	OS			*TRM*			*Relapse*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95%CI*	P
197A-positive recipient	0.99	0.72–1.37	0.97	1.00	0.64–1.56	0.99	0.92	0.61–1.37	0.67
197A-positive donor	1.22	0.88–1.71	0.24	1.26	0.79–2.00	0.33	1.04	0.69–1.58	0.85
Recipient age, >30 years	**1.63**	**1.17–2.28**	**0.004**	**2.02**	**1.25–3.28**	**0.004**	—	—	—
Donor age, >32 years	—	—	—	1.29	0.81–2.08	0.29	—	—	—
Female-to-male transplant	—	—	—	1.37	0.82–2.28	0.22	0.76	0.42–1.37	0.36
High-risk disease	**2.02**	**1.47–2.79**	**<0.001**	—	—	—	**2.42**	**1.62–3.61**	**<0.001**
Minor ABO incompatibility	1.19	0.81–1.74	0.38	1.28	0.77–2.15	0.34	—	—	—
CMV-positive recipient	**1.84**	**1.18–3.67**	**0.01**	1.35	0.74–2.48	0.33	—	—	—

	II–IV acute GVHD			*III–IV acute GVHD*			*Chronic GVHD*		
197A-positive recipient	**1.87**	**1.23–2.85**	**0.004**	**1.69**	**0.90–3.22**	**0.10**	0.96	0.69–1.35	0.83
197A-positive donor	0.86	0.59–1.27	0.45	1.13	0.60–1.97	0.70	1.10	0.78–1.55	0.59
Recipient age, >30 years	—	—	—	—	—	—	1.38	0.99–1.93	0.06
Donor age, >32 years	1.41	0.94–2.10	0.10	**2.17**	**1.10–4.23**	**0.02**	1.31	0.92–1.86	0.14
Female-to-male transplant	—	—	—	0.63	0.27–1.49	0.29	—	—	—
High-risk disease	1.32	0.91–1.94	0.15	—	—	—	—	—	—
Minor ABO incompatibility	—	—	—	—	—	—	—	—	—
CMV-positive recipient	—	—	—	—	—	—	—	—	—

Abbreviations: CI = confidence intervals; HR = hazard ratio.
Bold values have statistical significance.

II–IV acute GVHD after unrelated HLA-matched myeloablative BMT through JMDP. The validation study for nonmyeloablative transplantation revealed a trend toward the association of the recipient 197A genotype with an increased risk of grades III–IV acute GVHD, although its association on grades II–IV acute GVHD was unclear. Of

Table 4 A multivariate analysis of the association of IL-17 genotype with the clinical outcomes after transplantation in the validation study

	OS		P	*TRM*		P	*Relapse*		P
	HR	*95% CI*		*HR*	*95% CI*		*HR*	*95% CI*	
197A-positive recipient	0.97	0.55–1.69	0.91	0.92	0.45–1.88	0.82	1.09	0.54–2.20	0.81
197A-positive donor	0.99	0.57–1.71	0.98	0.78	0.39–1.55	0.48	1.52	0.73–3.18	0.26
Recipient age, >52 years	1.63	1.17–2.28	0.004	**2.02**	**1.25–3.28**	**0.004**	—	—	—
Donor age, >32 years	—	—	—	—	—	—	—	—	—
Female-to-male transplant	—	—	—	—	—	—	**3.33**	**1.55–7.13**	**0.002**
High-risk disease	1.21	0.70–2.09	0.49	—	—	—	**2.22**	**1.14–4.30**	**0.02**
Major ABO incompatibility	0.60	0.28–1.27	0.18	—	—	—	—	—	—
Minor ABO incompatibility	0.85	0.43–1.67	0.63	—	—	—	—	—	—
CMV-positive recipient	**5.45**	**1.30–22.87**	**0.02**	6.98	0.94 51.93	0.06	—	—	—
TNC, >4.3 x 10^8 per kg									
GVHD prophylaxis with tacrolimus	—	—	—	—	—	—	2.04	1.00–4.13	0.049
	II–IV acute GVHD			*III–IV acute GVHD*			*Chronic GVHD*		
197A-positive recipient	1.42	0.74–2.71	0.29	**5.84**	**0.75–45.72**	**0.09**	**3.86**	**1.29–11.59**	**0.02**
197A-positive donor	1.03	0.55–1.94	0.93	1.12	0.33–3.83	0.86	**0.27**	**0.10–0.74**	**0.01**
Recipient age, >52 years	—	—	—	—	—	—	**0.20**	**0.08–0.53**	**0.001**
Donor age, >32 years	—	—	—	—	—	—	—	—	—
Female-to-male transplant	**2.49**	**1.23–5.04**	**0.01**	—	—	—	—	—	—
High-risk disease	—	—	—	—	—	—	—	—	—
Major ABO incompatibility	0.40	0.15–1.02	0.06	—	—	—	—	—	-
Minor ABO incompatibility	—	—	—	—	—	—	—	—	—
CMV-positive recipient				—	—	—	**0.20**	**0.07–0.60**	**0.004**
TNC, >4.3 x 10^8 per kg				—	—	—	0.48	0.19–1.20	0.12
GVHD prophylaxis with tacrolimus				0.49	0.14–1.68	0.26	0.57	0.22–1.48	0.25

Abbreviations: CI = confidence intervals; HR = hazard ratio.
Bold values have statistical significance.

note, the validation study has demonstrated the association between the recipient 197A genotype and the increased incidence of chronic GVHD. This might reflect the association between the recipient 197A genotype and the risk of late acute GVHD,[49] considering that late acute GVHD occurs frequently after nonmyeloablative conditioning transplantation[50] and that the manifestation of late acute GVHD is usually indistinguishable from chronic GVHD.[51] In this study, the diagnosis of chronic GVHD was based on historical criteria,[45] and data on chronic GVHD classification according to the new NIH criteria[49] were unavailable, thus suggesting that late-onset, prolonged or delayed acute GVHD could have been diagnosed as chronic GVHD. Taken together, it would appear that the validation cohort data is consistent with the discovery cohort data, although additional validation studies are warranted. This is the first report to demonstrate that IL-17 may be involved in the pathophysiology of acute GVHD in humans.

The role of IL-17 in pathogenesis of acute GVHD remains unclear. Several mouse model experiments have revealed that transfer of IL-17-producing cells induced acute GVHD,[33–35] whereas in contrast there is a report[31] showing that donor IL-17-producing cells ameliorated acute GVHD. Host DCs are critical in the initiation of acute GVHD,[52–54] leading to a hypothesis that IL-17-producing cells could modify the function of host DCs through unknown mechanisms. Direct interaction between IL-17 and host DCs may be supported by the fact that DCs expressed IL-17 receptors.[26] As the IL-17 G197A polymorphism is located in the promoter region of IL-17 gene, it is conceivable that it may exert some roles in the transcriptional regulation of IL-17 secretion. Thus, investigating the influence of the IL-17 G197A polymorphism on the expression of IL-17 may offer useful information on this issue.

The current study did not show an association between the risk of acute GVHD and the IL-17 genotype in the donor side, implying an influence of host IL-17-secreting cells such as Th17 cells might be more important than the influence of donor IL-17-secreting cells on the pathophysiology of acute GVHD. However, it is still unclear how IL-17 secreted from the host IL-17-secreting cells is involved in the development of acute GVHD. Patient serum and lymphocytes may offer useful information on this issue, although these samples were not obtained for our study.

This study showed that the increased risk of acute GVHD associated with the host 197A genotype of IL-17 did not significantly benefit those with transplant-related mortality and OS after BMT. This might result from the low incidence of acute GVHD-related mortality regardless of the host IL-17 genotype in this cohort. Further investigations for patients at higher risk for acute GVHD including PBSC or HLA-mismatched transplant recipients should be warranted to clarify this issue.

The discovery study also indentified higher recipient age, high-risk disease and CMV-positive recipient as significant predictive factors for worse transplant outcomes (Table 3), which is consistent with earlier studies.[55–57] In addition, similar to a previous report,[58] higher donor age was associated with the increased risk of grades III–IV acute GVHD, which might result from the replacement of naive T cells by memory T cells with aging.[59]

This study suggests that genotyping of IL-17 in transplant recipients before transplantation may provide a 197A-positive recipient an opportunity to avoid the risk of acute GVHD by favoring a BM or cord blood, and an HLA-matched graft rather than a PBSC or HLA-mismatched graft. However, single polymorphisms in one cytokine gene are unlikely to determine the majority of acute GVHD. Future development of predictive strategies including multiple sets of genes will be required.

Conflict of interest

The authors declare no conflict of interest.

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