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特集 生体肝移植をめぐる諸問題

2 生体肝移植ドナーをめぐる諸問題
(2) 生体肝移植ドナーにおける脂肪肝

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Key words：生体肝移植ドナー，NASH(非アルコール性脂肪性肝炎)，インスリン抵抗性，HOMA-IR

要旨

ドナーの安全性確保のためにさまざまな検討が術前になされるが，本稿ではNASH(non-alcoholic steatohepatitis，非アルコール性脂肪性肝炎)の除外の重要性を強調したい．既往歴の聴取に際しては糖尿病，高脂血症，肥満，高血圧，高尿酸血症などインスリン抵抗性に起因する病態の把握，家族歴については，cryptogenic cirrhosisや原因不明の肝障害の有無について聴取する．エコー，CTで脂肪肝と診断された場合，HOMA-IRにてインスリン抵抗性の有無を判断し，NASHが疑われれば，肝生検にて除外する必要がある．無計画な激しい減量は，炎症や線維化を進行させる可能性があるので注意が必要である．

はじめに

「ひとつのもの(ドナーの全肝)から，ふたつのもの(グラフト肝を通じてレシピエントの回復，残肝によるドナーの安全性の確保)を」という二律背反に陥りかねない命題を満たそうとしている生体部分肝移植において，大前提となっているのがドナーの安全性確保である．

ドナーの安全性確保のために，さまざまな要因が検討され，必要に応じ，ドナーから除外されることとなる．検討される項目には，心理的・社会的および精神科的な面からみたドナーの状態，耐術性からみた全身状態，残肝の大きさ，血管系の解剖，胆道系の解剖といった手術手技に関係したものが挙げられる．さらに，肝炎，線維化の除外といった，肝臓の質そのものに関する項目も検討される．

本稿では，右葉グラフトを念頭に，肝臓の質に関係して脂肪肝を取り上げ，NASH(non-alcoholic steatohepatitis，非アルコール性脂肪性肝炎)の除外の重要性を強調したい．なお，脂肪肝グラフトのレシピエントにおける影響については本稿では触れない．

Ⅰ．脂肪肝の評価

この項のポイント
- 詳細な既往歴，家族歴，飲酒歴およびBMIの把握に始まる．
- routineの肝生検についてはコンセンサスが得られていない．

術前ドナー評価における脂肪肝の評価は，ま

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ずドナー候補の既往歴，飲酒歴，家族歴の把握，さらにドナー候補の身長および体重からBMI(body mass index)を算出することから始まり，この段階でドナーとして適格か否か，「入り口」である程度振り分けることが可能である．常時大量飲酒しているドナー候補は飲酒節制に取り組むことが最大の課題となる．また，日本においてはBMIが25以上で肥満であるとされ，以後の検査で脂肪肝が見出されることが多い．ただ，BMIが25未満であっても，脂肪肝が見出されることがあり，後に述べるようにNASHを伴っている可能性も考慮されるべきである．既往歴および家族歴の重要性についても，改めてNASHの項で触れる．

次いで，採血による肝機能評価，HOMA-IR(Homeostasis Model Analysis for Insulin Resistance，後述)，腹部エコー検査へと進め，当院ではこの段階までをドナー候補者の一次検査として施行している．アルコール性でない，いわゆる過栄養性脂肪肝はAST＜ALT，コリンエステラーゼ高値をとることが多い．腹部エコーでは，血管系の評価，占居性病変の除外とあわせて，肝実質のエコーレベル上昇による肝腎コントラスト，肝内脈管の不明瞭化により脂肪肝を診断するのは通常と変わらない．

二次検査の一つとして単純および造影ダイナミックMDCT(multidetector-row CT)を施行する．ここで血管解剖，血管区域ごとの肝容積把握と同時に，単純CTにて脂肪肝の有無，その程度を把握している．われわれは，肝臓と脾臓のCT値の比をL/S ratioとし，その値が1.1～1.2以上であれば，組織学的にもmacrovesicular steatosisは30%以下であり，ドナーとしてグラフト提供可能であるとしている[1]．NASH除外を必要とするときや自己免疫性肝炎，原発性胆汁性肝硬変(PBC)，Alagille症候群などを否定するとき以外には肝生検を行っていない．ドナー候補の脂肪肝評価における肝生検の必要性については，コンセンサスが得られていない．海外でも「画像診断では脂肪肝の評価は不完全で，線維化や炎症が肝生検でのみとらえられることがある」という立場から，ドナー評価に肝生検が必須とする考え方から，「大多数の場合において非侵襲的検査で肝疾患が除外できるためroutineの肝生検は不要である」とする立場までさまざまである[2),3)]．われわれの立場は後者に近い．

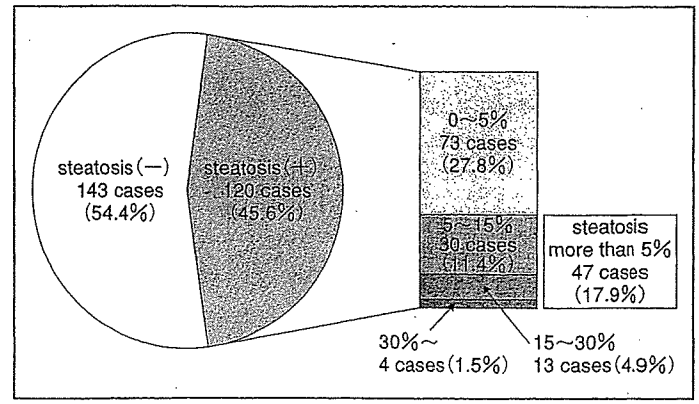


図1　脂肪肝の頻度と程度—右葉ドナー 263例

図1は，京都大学移植外科でのドナーゼロバイオプシー(右葉ドナー，手術時採取)263例における脂肪肝の程度の内訳である．5%以上の脂肪肝を17.9%で認めるが，30%以上の脂肪肝は全体の1.5%(4例)を占めるにすぎない．しかしながら，うち3例で軽微～中等度のNASHを認めている．

Ⅱ．脂肪肝(NAFLD)への対応

この項のポイント
- 計画的減量にて脂肪肝が軽快し，肝移植ドナーになれることがある．

NASH以外の脂肪肝，いわゆるNAFLD(non alcoholic fatty liver disease)を認めた場合の対応は，以下のとおりである．L/S比が1.2未満であり，組織学的に30%以上の脂肪肝を示唆するときは，ほかの全身性疾患が除外できれば減量ののち，1，2カ月後にCT，エ

コーにて再評価している．具体的には標準体重当り1日25～30 kcalの食事にて1カ月に0.5～2 kgの体重減少を目標に，糖尿病内科の協力のもと計画的に行っている．緊急移植の場合には，当該ドナーは断念せざるをえない．

Ⅲ．ドナー死亡例

この項のポイント
- 緊急移植，NASH，ドナー残肝が過小であったことが，死亡につながった可能性がある．

2002年の時点でいままでに述べてきた方針で，800例を超える生体部分肝移植を施行してきていたが，ドナーについてはmortalityにつながる問題は発生しなかった．しかし，同年8月に生体部分肝移植後8年目に肝不全から脳症を発症したレシピエントに対して，母親をドナーとして，緊急再移植(右葉，ABO不適合)を施行した．その後，ドナーが肝不全となり，救命のため肝移植を6カ月目に受けたが，9カ月目に亡くなるという事態に陥った[4]．肝不全に至ったきっかけとしては，結果的に小さめの残肝(25.9%)に加えて，ドナーがNASHであった可能性があげられた．また当該ドナーはBMIが29.3と肥満であり，高血圧に対して服薬を受けていたが，明らかな糖尿病の既往や家族歴は認めなかった．ゼロバイオプシーと移植(6カ月目)の際の摘出肝組織を図2，図3に示す．図2aはドナー手術時のHE染色であるが，20～30%のmacrovesicular steatosisを認め，この時点では線維化ないしは肝炎を指摘されなかった．図2bは移植後6カ月目に施行した同一標本の鍍銀染色であるが，軽度の線維化を認め，この時点でNASH(stage 2，grade 1)の可能性が指摘された．図3a，bは6カ月目の移植の際の摘出肝であるが，小葉の改築を伴う高度の脂肪肝を認め，NASHに引き続いて起こった肝硬変(NASH stage 4，grade 3)とされた．

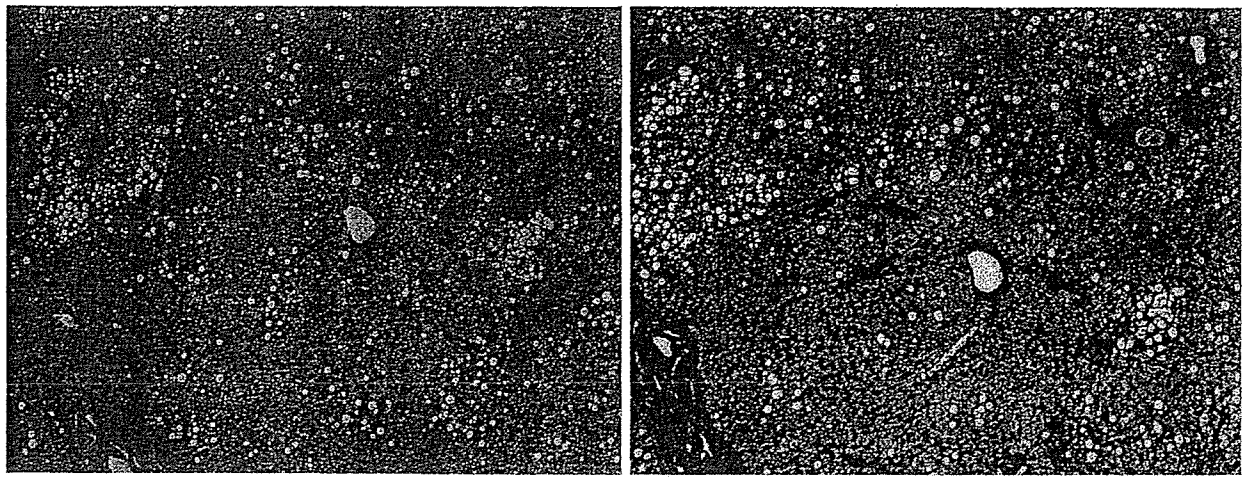
Ⅳ．NASHとは

この項のポイント
- NASH発症の背景にはインスリン抵抗性があり，肝硬変へ進行する可能性がある．

ここで，NASHについて簡単に触れるが，詳細は成書を参照されたい．NASHは1980年にLudwigらが提唱した疾患概念で，「アルコール摂取歴がないにもかかわらず，病理組織学的にアルコール性肝障害に類似した所見を認め，かつウイルス性肝炎などほかの疾患を除外できたもの」と定義されている[5),6)]．

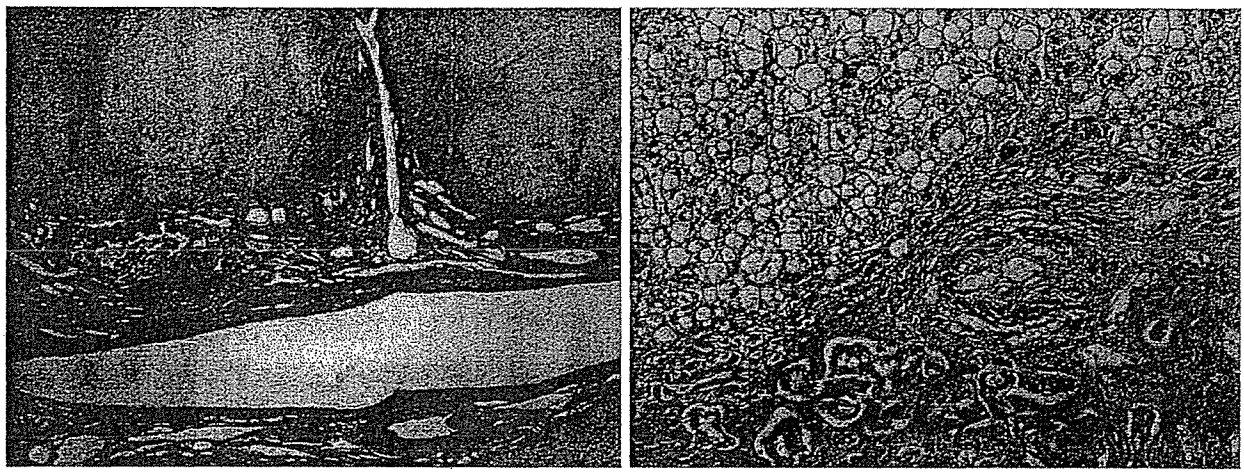
日本におけるNASHの頻度は不明である．危険因子として糖尿病，高脂血症，肥満，高血圧，高尿酸血症などが挙げられ，家族内集積を認めることもあるとされる．臨床検査所見としては5～9割の症例で軽度から中等度のトランスアミナーゼ上昇を認めるが，正常例もみられる．インスリン抵抗性はNASH発症の背景としてきわめて重要な位置を占めるとされ，HOMA-IR〔空腹時血糖値(mg/dl)×空腹時インスリン濃度(μU/ml)÷405〕がその指標として簡便かつ有用である．HOMA-IR>1.64をインスリン抵抗性ありと判断する[7)]．確定診断には肝生検が有用であるが，その病理診断は，定量性，再現性に問題があるため難しいとされてきた．しかし，BruntらがNASHのスコアリングシステムを提唱しており，有用とされている[8)]．

肝硬変への移行率は不明であるが，約10～20年の経過で肝硬変に至る可能性が指摘されている．Bruntらの指標を用いて，先の263例の肝生検をretrospective reviewしたところ，30%以上の脂肪肝を認めた4例のうち，



a：macrovesicular steatosis 30%(HE 染色)　　b：中心静脈周囲を主体とした線維化(鍍銀染色)

図2　ドナー死亡症例ゼロバイオプシー



a：高度の脂肪肝，小葉改築(+)　　b：細胆管増生，胆汁うっ滞

図3　ドナー死亡症例摘出肝標本

既述のごとく3例でNASHが認められた．1例は死亡例であり，あと2例で軽度のNASHを認めた．それら2例のドナーの肝機能の回復はほかのドナーに比べて遜色なく，元気に社会復帰している．またこれらのレシピエントも軽快退院している．

V．ドナー評価で，どういうときにNASHを疑い除外すべきか

この項のポイント
- 脂肪肝およびHOMA-IRの異常高値でNASHを鑑別にあげ，確定診断は肝生検による．

生体移植においてドナーの安全性確保は絶対条件である．われわれがドナー死亡例の経験から学び，同じ問題を二度と起こさないためにドナー評価の際に改善した点を以下に記す．

本人既往の聴取を徹底し，糖尿病，高脂血症，肥満，高血圧，高尿酸血症などインスリン抵抗性に起因する可能性がある病態の把握に漏れがないようにしている．また，家族歴については，これらの既往に加えて，cryptogenic cirrhosisや原因不明の肝障害の有無について聴取する．CTによる脂肪肝の評価はL/S>1.1を指標にしていたが，名古屋大学移植外科ではこれをさらに厳しくし，L/S>1.2としている．この段階でHOMA-IRにてインスリン抵抗性の有無を判断し，NASHが疑われれば，肝生検を施行し，NASHの診断がついた時点でドナー候補から外れる．HOMA-IRで

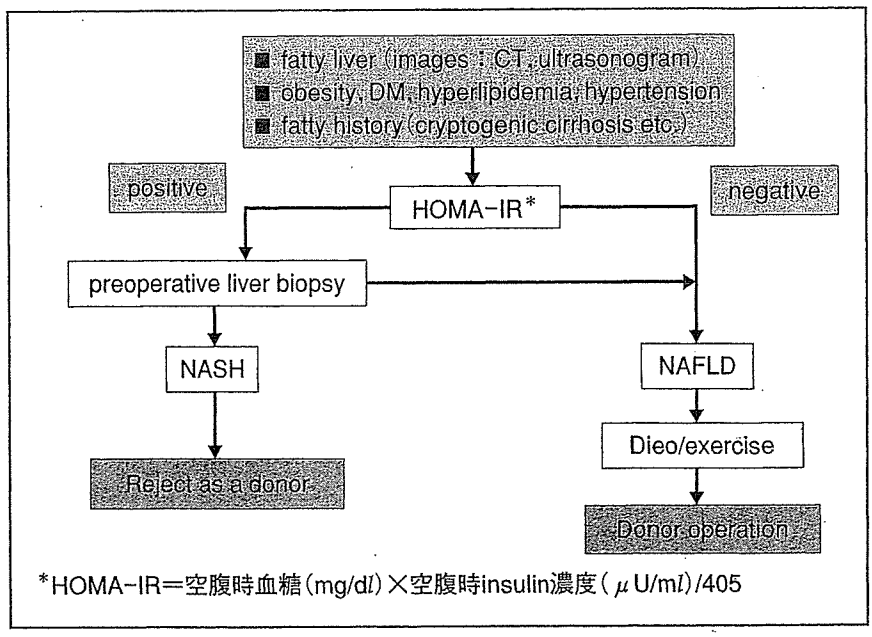


図4　NASH ドナー除外のためのアルゴリズム

NASH が否定的ないしは肝生検で NASH が除外できたときには，減量指導をすすめ，L/S>1.2 となった時点でドナーとしてグラフトを提供していただくこととしている．

図4に NASH ドナー除外のためのアルゴリズムを示す．強調するべきは，無計画な激しい減量は，炎症や線維化を進行させるとされ，NASH の場合はいうまでもなく，NAFLD の場合でも避けるべきで，コントロールされた体重減量が必要と考える．

Ⅵ．今後の課題

この項のポイント
●ドナー候補における NASH の診断プロセスについて評価し，安全性をさらに高める必要がある．

繰り返しになるが，ドナーの安全性の確保は生体移植の大前提である．死亡例の経験に際し，ドナー残肝容積が過小であった可能性が指摘された．この問題に対しては，以前は free hand でスライスごとに行っていた肝容積評価を，ドイツ ブレーメンの MeVis の協力のもと，亜区域血管ごとの容積を算出するようにし，安全性を高める努力を続けている．

NASH の除外については，既述の手順で，数名の NASH ドナーを発見しドナー候補から外れていただいている．十分鋭敏に NASH をとらえられていると感じているが，今後 prospective に検証していく必要がある．当院ではまだ採用していないが，ほかのマーカー(低マグネシウム血症，血清フェリチン，トランスフェリン)についても検討の余地があるであろう[9),10)]．また，緊急移植に際しては，より慎重なドナー評価が必要であろう．

国際移植学会主催の生体ドナーフォーラム(2005 年 9 月，バンクーバー，カナダ)に各国の情報を持ち寄った結果，現時点で左外側区域ないしは左葉で 3 例(死亡率推計 0.1%)，右葉で 11 例(死亡率推計 0.5%)のドナー死亡が明らかとなった．アジアだけをとると右葉，左葉合わせて 4,000～5,000 例で 2 例(ほか 1 例は東アジアで術後 3 カ月目の十二指腸潰瘍穿孔)が死亡(死亡率推計 0.05%)している．他の死亡症例については 2006 年の World Transplant Congress(ボストン)にてフォーラムの総括と合わせて報告される予定である．

最後に，本稿が脂肪肝からみたドナーの安全性確保にお役に立てれば幸いです．NASH の

除外診断基準など，よりよいステップがあればご教示いただきたく思います．(藤本メールアドレス yfjmt@med.nagoya-u.ac.jp)

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Summary

Fatty Change in Donors for Living Donor Liver Transplantation

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With living-donor transplantation, donor safety is of vital importance. Since one donor death was reported, in Japan, with the donor probably having NASH(non alcoholic steatohepatitis), those with NASH should be ruled out as donor candidates for living-donor liver transplantation. Thorough evaluation of the medical history of potential donors is mandatory in cases involving diabetes mellitus, hypertension, obesity, hyperlipidemia, or hyperuricemia, in which there are indications of insulin intolerance. In addition, a family history of cryptogenic liver cirrhosis may be attributable to a previous NASH condition and should be seriously considered, since NASH is thought to have familial or inherited components. If a potential donor is diagnosed as having steatosis by use of ultrasounds and/or CAT scans(Liver/Spleen ratio with plain CT less than 1.2 is compatible with macrovesicular steatosis over 30%), insulin intolerance should be ruled out through HOMA-IR(Homeostasis Model Analysis for Insulin Resistance). If insulin intolerance is suspected, liver biopsy should be undertaken to exclude NASH. NAFLD(non alcoholic fatty liver disease) or NASH conditions can be improved by gradual, controlled diet. However, if the patient loses body weight too quickly, there may be a progression toward fibrosis.

Key words：donor for living-donor liver transplantation, NASH(non alcoholic steatohepatitis), insulin intolerance, HOMA-IR(Homeostasis Model Analysis for Insulin Resistance)

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ORIGINAL ARTICLE

Fatal Graft-Versus-Host Disease after Living Donor Liver Transplantation: Differential Impact of Donor-Dominant One-Way HLA Matching

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Graft-versus-host disease (GVHD) is an uncommon but potentially devastating complication following liver transplantation. Recently, it was shown that use of a human leukocyte antigen (HLA)-homozygous donor leading to one-way HLA matching significantly increases the risk of GVHD after living donor liver transplantation (LDLT). However, the precise impact of HLA matching between donor and recipient on the risk of GVHD is not yet clear. We surveyed instances of fatal GVHD following LDLT in Japan and reviewed all 8 cases in detail, especially with respect to HLA matching. Serological typing showed that 7 of those cases had donor-dominant one-way HLA matching in the 3 loci of HLA-A, -B, and -DR, while one had donor-dominant one-way HLA matching in the 2 loci of HLA-A and -DR and identical alleles in the B locus. However, DNA typing revealed that the latter case had 1-way HLA matching in the 3 loci. Further, we analyzed HLA typing of 906 donor-recipient pairs who underwent LDLT. There were 5 cases with donor-dominant one-way matching in 2 loci and 2 with donor-dominant one-way matching in 1 locus. All of those cases except 1, who died from an unrelated cause, are alive without an obvious presentation of GVHD. In conclusion, our results suggest that the total number of loci with donor-dominant one-way HLA matching is important for determining the risk of fatal GVHD following LDLT, and that DNA typing of HLA alleles is indispensable in some cases to identify the true risk of donor-dominant 1-way HLA matching. *Liver Transpl 12:140–145, 2006.*

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Graft-vs.-host disease (GVHD) is an uncommon but devastating complication following liver transplantation that results from the engraftment of T lymphocytes associated with the liver graft. It is characterized by fever, skin rash, diarrhea, or pancytopenia, which usually occurs 2 to 6 weeks after the procedure.[1-3] One-way matching between a human leukocyte antigen (HLA)-homozygous donor and a haploidentical recipient is a recognized risk factor for GVHD after transplantation.[4,5] Although such a combination of donor and recipient HLA is extremely rare in case of cadaver donor liver transplantation, complete donor-dominant 1-way HLA matching between donor and recipient is a realistic possibility in living donor liver transplantation (LDLT) cases, because most of those donors are genetically related to the respective recipients. Recently, it was shown that use of an HLA-homozygous donor resulting in donor-dominant one-way HLA matching significantly increases the risk of developing GVHD after LDLT, and some have insisted that such donors should be completely excluded.[6,7] However, the precise impact of HLA matching between donor and recipient on the incidence of GVHD has not been clarified. In the present study, we reviewed all reported cases of fatal GVHD after LDLT in Japan and focused on the number of loci with HLA matching between donor and recipient.

PATIENTS AND METHODS

In this retrospective study, we investigated the incidence of fatal GVHD following LDLT in Japan at the time of writing and reviewed all the cases, including

Abbreviations: GVHD, graft-vs.-host disease; HLA, human leukocyte antigen; LDLT, living donor liver transplantation.

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ours, and analyzed the ages of the recipient and donor, donor relation, original disease, initial symptom, Grucksberg stage (peak),[8] onset and course, and HLA type of each recipient and donor. Serologic HLA typing had been performed in all donors and recipients for HLA-A, -B, and -DR according to microlymphotoxicity testing using well-standardized alloantisera.[9,10] A polymerase chain reaction technique was subsequently applied if indicated, using DNA samples of the recipients and donors extracted from peripheral blood lymphocytes or preserved lymphocytes, which were typed for HLA-A, -B, and -DR using a polymerase chain reaction sequence-specific primer[11-13] or restriction fragment length polymorphism method.[14,15] In addition, we collected HLA typing results for 906 donor-recipient pairs who underwent LDLT at Nagoya University Hospital and Kyoto University Hospital from October 1990 to March 2004. The diagnosis of GVHD was based on clinical signs characterized by fever, skin rash, diarrhea, or pancytopenia, and supported by pathologic findings of a skin biopsy consistent with GVHD or the presence of a donor-derived chimerism in peripheral blood.[16]

RESULTS

A total of 8 cases of fatal GVHD after LDLT have been identified in Japan, of whom 2 were infants (Table 1). These patients were transplanted from October 1996 to April 2003. The donor age ranged from 20 to 62 yr old, and recipient age in the 6 adult cases ranged from 37 to 62 yr old. Four of the donors were a son, 3 were mothers, and 1 was a sister of the respective recipients. During the donor selection process, 2 donors with a known potential risk of GVHD were selected because of a lack of alternative candidates. Most of the initial symptoms of GVHD were skin rash with or without fever. The median number of days before appearance of the initial symptom was 40 (range, 14-114), and the median number of days after transplantation to death was 130 (range, 36-540). Regarding peak Gluckberg clinical grade, 4 of the cases were grade 3 and 2 were grade 4.

As for HLA matching between recipient and donor, the serological technique showed that all cases except 1 (case 8) had donor-dominant one-way HLA matching in 3 loci of HLA-A, -B, and -DR, while case 8 had donor-dominant one-way HLA matching in 2 loci of HLA-A and -DR with the B locus neutral because of the homozygote status of the recipient. Following the onset and diagnosis of GVHD, HLA of both the donor and the recipient in the latter case was retyped using the PCR technique, which revealed donor-dominant one-way HLA matching in all 3 loci due to a minor heterogeneity in locus B of the recipient. Consequently, all 8 cases of fatal GVHD after LRLT identified in Japan had donor-dominant one-way HLA matching in 3 loci of HLA-A, -B, and -DR.

Nine cases with donor-dominant one-way matching in 3 loci were identified among 906 donor-recipient pairs who underwent LDLT (Table 2). Among these 9 pairs, fatal GVHD occurred in 4 cases that were included in 8 fatal GVHD cases reviewed in this report. Among the other 5 cases with donor-dominant HLA matching in 3 loci, 3 are alive for a median 61 months without obvious GVHD. Twenty-six cases with donor-dominant 1-way matching in 2 loci were identified among them. Of those, the remaining HLA locus was identical in 5 (both homozygote in 4 and both heterozygote in 1) and 1 haplotype mismatch in 21 (Table 2). There were no cases with donor-dominant one-way matching in 2 loci and 2 mismatch in the other locus. Further, 171 cases with donor-dominant one-way matching in 1 locus were also identified, of which 2 cases were identical in the other 2 loci, 41 were identical in the other 1 locus, and 128 were not identical in the other 2 loci. Five cases with donor-dominant one-way matching in 2 loci and an identical combination in the other locus, and 2 cases with donor-dominant one-way HLA matching in 1 locus and an identical combination in the other 2 loci were identified among the 906 donor-recipient pairs who underwent LDLT. All of these cases were alive at the time of writing without clinical presentation of GVHD except for 2 patients who died (cases 3 and 5), 1 because of an intracerebral hemorrhage and 1 from idiopathic thrombocytopenic purpura and autoimmune hemolytic anemia, of whom neither had clinical signs of skin rash, diarrhea, or fever (Table 3). Although these HLA combinations also seem to contribute theoretically to GVHD, none received the diagnosis of GVHD; this suggests that the number of vectors directing GVHD contribute to the risk of fatal GVHD.

DISCUSSION

GVHD following liver transplantation is difficult to control and devastating in most cases. Clinical symptoms of GVHD after liver transplantation typically become overt between 2 and 8 weeks after transplantation,[1] which is consistent with all cases except 1 in our review. That patient developed GVHD 114 days following transplantation, which was presented first as a skin rash. We could not find any appropriate explanation for the relative delay of the onset in this case. Nemoto et al.[6] reported that case as chronic GVHD after liver transplantation, and it was controlled with administrations of methylprednisolone and tacrolimus, though we later confirmed by personal communication that the patient died from infection due to GVHD. The median onset time of transfusion-associated GVHD is about 10 days[17]; thus, GVHD following liver transplantation can occur late as compared with transfusion-associated GVHD.

As for the donor relationship, in 4 of the 8 cases of fatal GVHD after LDLT the donors were a son, while in 3 they were the mother, and in 1 the sister. This finding demonstrates that GVHD derived from one-way HLA matching can occur even between siblings.

The clinical symptoms of GVHD are generally characterized by fever, skin rash, diarrhea, and/or pancytopenia. Unlike GVHD that follows allogeneic bone marrow or stem cell transplantation, where the biliary epithelium is one of the major targets that results in

TABLE 1. Clinical Characteristics, Donor and Recipient HLA, Initial Symptom, and Clinical Course of the Cases with Fatal GVHD After LDLT

Case no.	Age	HLA			Original disease	Donor relationship	Onset (POD)	Initial symptons	Other symptons	Death (POD)	Glucksberg clinical grade (peak)
		A locus	*B* locus	*DR* (*DRB1**)							
1											
Donor	35	24,–	52,–	*1502,–	PBC	Sister	24	Skin rash	Diarrhea, fever	36	Grade 3
Recipient	37	24,31	52,62	*0901,*1502							
2											
Donor	27	*2402,–	*5201,–	*1502,–	HCC	Son	38	Skin rash, fever	Diarrhea, pancytopenia	149	Grade 3
Recipient	59	*2402,*3101	*5201,*3501	*1502,*0803							
3											
Donor	20	33,–	44,–	6,–	HCC, alcohol	Son	35	Fever, diarrhea	Skin rash, leukopenia	61	Grade 4
Recipient	48	33,24	44,62	6,4							
4											
Donor	31	24,–	52,–	15,–	HCC, HCV	Son	42	Skin rash	Fever	139	Grade 2
Recipient	62	24,31	52,51	15,14							
5											
Donor	32	A3301/03,–	*4402,–	*1301-02,–	PBC	Son	114	Skin rash	Fever	540	Grade 2
Recipient	50	*3301/03, *2601-07	*4402,*4006	*1301-02,*09012							
6											
Donor	30	24,–	52,–	*1502,–	BA	Mother	14	Skin rash, fever	Diarrhea, pancytopenia	43	Grade 4
Recipient	8M	24,2	52,61	*1502,*0802							
7											
Donor	26	2,–	44,–	13,–	BA	Mother	23	Skin rash	Fever, diarrehea	217	Grade 3
Recipient	6M	2,23	44,54	13,04							
8											
Donor	62	*3303,–	44,-(*4403,–)	*1302,–	Alcohol	Mother	24	Skin rash	Fever, diarrhea	36	Grade 3
Recipient	38	*3303,*0301	44,-(*4403,*4402)	*1302,*0403							

Abbreviations: HLA, human leukocyte antigen; LDLT, living donor liver transplantation; PBC, primary biliary cirrhosis; HCC, hepatocellular carcinoma; BA, biliary atresia; POD, postoperative day.

TABLE 2. Donor-Recipient HLA in Cases With Donor-Dominant 1-way Matching

Relation of HLA matching between donor and recipient	Number of cases		GVHD incidence
Donor-dominant 1-way HLA matching at 3 loci	9		4 (44%)
Donor-dominant 1-way HLA matching at 2 loci	26		0
The other locus			
Homo-homo identical		4	
Hetero-hetero identical		1	
1-mismatch		21	
2-mismatch		0	
Donor-dominant 1-way HLA matching at 1 locus	171		0
The other 2 loci			
Identical in both loci		2	
Identical in one locus		41	
Not identical in both loci		128	

TABLE 3. Cases With Donor-Dominant 1-Way Matching at 2 Loci or 1 Locus and Identical at the Other Locus

Case no.	Age	HLA			Original disease	Donor relationship	Outcome (follow-up)
		A locus	*B* locus	*DR* (*DRB1**)			
1							
Donor	34	24,–	62,–	4,–	BA	Father	Alive (13 yr)
Recipient	2	24,–	62,51	4,9			
2							
Donor	33	24,–	61,–	10,–	BA	Father	Alive (8 yr)
Recipient	1	24,–	61,35	10,9			
3							
Donor	42	11,–	39,–	8,–	BA	Father	Death (36 days)
Recipient	9	11,–	39,35	8,4			
4							
Donor	41	24,33	44,–	13,–	BA	Mother	Alive (7 yr)
Recipient	16	24,33	44,52	13,15			
5							
Donor	27	24,– (*2402,–)	7,– (*0702,–)	1,– (*0101,–)	HCC	Son	Death (5 months)
Recipient	57	24,– (*2402,–)	7,52 (*0702,*5201)	1,15 (*0101,*1502)			
6							
Donor	56	33,–	44,–	13,–	FHF	Father	Alive (2 yr)
Recipient	23	33,–	44,–	13,8			
7							
Donor	46	24,–	55,62	4,–	Caroli's disease	Father	Alive (2 yr)
Recipient	16	24,–	55,62	4,9			

Abbreviations: HLA, human leukocyte antigen; LDLT, living donor liver transplantation; BA, biliary atresia; HCC, hepatocellular carcinoma; FHF, fulminant hepatic failure; POD, postoperative day.

abnormal liver function,[5] the transplanted liver is not a target of GVHD, as both the liver and the immunocompetent cells responsible have the same donor origin.[2,16] Taylor et al.[2] noted that the outcome of GVHD is closely related to its clinical pattern, with prognosis particularly poor in those patients who were presented with a fever, as 29 of 30 (97%) reported adult cases died following presentation with fever, while patients with a skin rash alone survived. All of the presented patients with fatal GVHD developed not only a skin rash, but also a fever as the initial symptom or later. Therefore, fever accompanying skin rash seems to be an important prognostic sign in GVHD following LDLT irrespective of whether it is the initial symptom or not. And each was rated as greater than Glucksberg grade 2. Glucksberg et al.[8] reported that there were no significant differences between grades 0 and 1, grades 2 and 3, and grades 3 and 4 for survival, whereas the difference between grades 1 and 2 was highly significant. However, evaluation of the severity of GVHD following liver transplantation based on Glucksberg grade may be difficult, because liver function is usually normal in those patients.

Close HLA matching between donor and recipient is one of the risk factors for the development of GVHD.[1,2] Several authors have reported that use of a graft from an HLA-homozygous donor with 1-way donor-recipient HLA matching led to an extremely high risk of developing GVHD in LDLT.[7,18,19] However, none have analyzed the differential impact of the number of loci with one-way HLA matching on the risk of fatal GVHD following LDLT. Homozygosity at all HLA loci is not as rare as might be expected from a mathematical calculation of all haplotypes.[20] One study found that 1.6% of Caucasian blood donors demonstrate this condition.[21] This is in contrast with the data from the Japanese Red Cross Society showing that approximately 3.2% of blood donors in Japan have the condition. While the probability of one-way HLA matching between nonrelatives is 1 in 800, it is 1 in 100, 1 in 190, and 1 in 180 in combinations of parent-child, sibling-sibling, and grandparent-grandchild, respectively.[22] Therefore, the risk of encountering donor-dominant one-way HLA matching may be extremely high in an LDLT setting as compared to with a cadaver donor.[7] Kiuchi et al.[19] analyzed a large series of LDLT cases and reported that one-way HLA matching in 2 or more loci prone to GVHD was 3.9% (1.4% in 3 loci), and 1 in 4 cases with complete one-way HLA matching in 3 loci died from GVHD. Therefore, the risk of GVHD after liver transplantation seems to be high in Japan, though several cases have also been reported in the United States despite the theoretically low risk.[1,18]

We investigated HLA matching between the recipient and donor in all cases of fatal GVHD after LDLT in Japan. Undiagnosed GVHD may have occurred, because the early symptoms are often nonspecific and often self-limited, making it difficult to distinguish from an infectious disease or drug reaction. DNA typing demonstrated that all of the cases had donor-dominant one-way HLA matching in the 3 loci of HLA-A, -B, and -DR. Fatal GVHD has not occurred in any cases with donor-dominant one-way matching in 2 loci or those with 1-way matching in 1 locus, despite that such 1-way matching theoretically contributes to GVHD, because the host defense system of the recipient is unable to recognize and eliminate donor cells, and donor cells recognize the unshared haplotypes as foreign and react against them.

Our results suggest that the risk of fatal GVHD following LDLT may depend on the number of loci with donor-dominant one-way HLA matching. This is very important information for donor selection and can help avoid unnecessary donor exclusion. However, additional investigations of whether fatal GVHD can occur in cases with donor-dominant one-way matching in 1 or 2 loci must be performed carefully, as well as discussion of cases that did not develop GVHD despite donor-dominant one-way matching in 3 loci.

In conclusion, homozygous donor with one-way donor-dominant HLA matching at 3 loci should be excluded if possible, because of the very high risk of developing fatal GVHD. However, in those with donor-dominant one-way HLA matching at 2 or fewer loci, the evidence for exclusion is not sufficient. Therefore, when such a donor is the only candidate for LDLT, it seems acceptable, following fully informed consent to the theoretical risk of GVHD. In addition, DNA typing of HLA alleles is strongly recommended for combinations carrying a suspected risk of GVHD.

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Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients

Background: Although the prevention of immunologic reactions with sufficient immunosuppression prolongs graft and patient survival rates, the large interindividual variation in tacrolimus pharmacokinetics interferes with treatment. In this study we have examined whether intestinal MDR1 (ABCB1) is a potential biomarker predicting the occurrence of acute cellular rejection, as well as a factor to predict absorption of tacrolimus, after living-donor liver transplantation.

Methods: By use of tissue specimens of intestinal mucosa (n 164) obtained at surgery, the messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and cytochrome P450 (CYP) 3A4 was quantified.

Results: The probability of acute cellular rejection during the first 10 days after surgery was significantly associated with the average trough concentration of tacrolimus between postoperative days 2 and 4 (45.1% for <7 ng/mL versus 22.9% for >7 ng/mL,*P* .0040). High levels of MDR1 were associated with an episode of acute cellular rejection before postoperative day 10 (odds ratio, 2.306 [95% confidence interval, 1.058-5.028]) and with a poor survival rate during the first postoperative year (odds ratio, 7.413 [95% confidence interval, 1.567-36.073]). The mRNA expression level of MDR1 was inversely correlated with the tacrolimus concentration–oral dose ratio during the initial 4 days after surgery in patients with a graft-to-recipient weight ratio greater than 1.5 (r 0.6798, P< .0001) and those with a graft-to-recipient weight ratio of less than 1.5 (r 0.7180, P< .0001).

Conclusion: The enterocyte MDR1 mRNA level was suggested to be a risk factor for acute cellular rejection and death after surgery. Therefore obtaining a sufficient tacrolimus blood level via this molecular information–based initial dosage adjustment may enable the episode of acute cellular rejection after liver transplantation to be reduced. (Clin Pharmacol Ther 2006;79:90–102.)

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Living-donor liver transplantation and subsequent immunosuppressive therapy are well acknowledged to provide excellent results and are usually used in coordination with a cadaveric organ transplant program.[1,2] In countries where cadaveric donors are limited, living-donor liver transplantation is often the only treatment option for patients with end-stage liver disease.[3] Because loss of the graft liver will lead to death, postoperative immunosuppressive therapy is essential to protect the grafted liver from immunologic reactions. As acute cellular rejection occurs mostly within 6 weeks of a transplant,[4] high-dose steroid pulse therapy or anti-CD3 monoclonal antibody treatment is required to save the graft liver.[5,6] Subclinical rejection, where cytologic or histologic signs of rejection exist in the absence of clinical dysfunction of the graft, is particularly frequent (incidence of around 59%) between days 5 and 14 after

liver transplantation.[6] Twenty-five percent of patients with subclinical rejection are treated with high-dose steroid injections. Therefore early exposure to immunosuppressants could reduce the frequency of acute cellular rejection including subclinical rejection. However, these antirejection treatments lead to overimmunosuppression and an infectious state, which are closely associated with death.[7] Although there is a need to protect patients from opportunistic infections including enterobacterium, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus after antirejection treatment, some anti-infectious treatments with antibiotics, antifungal agents, and antiviral drugs are accompanied by drug-induced hepatic and renal dysfunction.[8,9] In addition, high-dose steroid injections should be avoided in patients carrying the hepatitis B or C virus, because steroidal drugs allow the amplification of these viruses in the graft liver and accelerate the recurrence of virus-related hepatitis and cirrhosis.[10,11] Therefore acute cellular rejection should be avoided to prevent further complications, especially immediately after transplantation.

The calcineurin inhibitor tacrolimus (FK-506) has been used as a primary immunosuppressive agent in orthotopic liver transplantation.[12] Therapeutic drug monitoring has facilitated maintaining the blood concentration of tacrolimus within a narrow therapeutic range (between 10 and 20 ng/mL) to prevent side effects such as nephrotoxicity, neurotoxicity, and life-threatening infection.[13,14] However, the bioavailability of orally administered tacrolimus is variable, ranging from 4% to 89% (with a mean value of about 25%),[15,16] and a dosage regimen for the drug immediately after transplantation has yet to be established. This can be attributed to several factors, including poor absorption or extensive first-pass metabolism in the intestine and liver. Therefore a rational dosage regimen for tacrolimus should be determined as early as possible, focusing on its pharmacokinetic interindividual variability.

Tacrolimus is principally metabolized by cytochrome P450 (CYP) 3A subfamilies in the liver. The contribution of active secretion by P-glycoprotein (the product of the *MDR1/ABCB1* gene) and the metabolism by CYP3A expressed in enterocytes are acknowledged as factors influencing the bioavailability of tacrolimus.[17] We reported that the intraindividual variation in the concentration/dose (C/D) ratio of tacrolimus was closely related to the variation in the enterocyte messenger ribonucleic acid (mRNA) expression level of MDR1, but not CYP3A4, in recipients of living-donor small-bowel transplantation.[18,19] Similar results were obtained in patients after living-donor liver transplantation during the initial 7 days after surgery.[20,21] Because of the small number of cases in our past reports, we could not analyze the relationship between the intestinal mRNA expression level of MDR1 and endpoints such as acute cellular rejection. It is necessary to clarify the clinical significance of the intestinal expression level of MDR1 in patients after living-donor liver transplantation to establish the clinical usefulness of adjusting the initial dosage of tacrolimus.

In this study we examined whether the intestinal expression level of MDR1 mRNA could be a molecular marker for acute cellular rejection episodes in patients after living-donor liver transplantation with more enrolled patients, as well as the potential contribution of the molecular information to initial dose setting.

METHODS

Patients and mucosal specimens. The study included 164 patients, having first provided written informed consent, who were enrolled consecutively between November 1998 and December 2004 in whom tissue specimens had been obtained at surgery. The donor was a parent in 119 cases, a spouse in 14, a sibling in 12, an offspring in 12, a grandmother in 3, an uncle in 2, an aunt in 1, and a father-in-law in 1. The demographics of the recipients are listed in Table I. The clinical samples of the upper jejunum were obtained from a part of the Roux-en-Y limb for biliary reconstruction or from a part of the mucosal specimen around the bile drainage tube at living-donor liver transplantation.[22] This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University, Kyoto, Japan; each adult patient and each parent of small children provided written informed consent.

Dosage regimen of tacrolimus, analysis of blood samples, and criteria for acute cellular rejection. The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids.[23] To cover the immediate postoperative period (the day of living-donor liver transplantation, day 0, and postoperative day 1), induction of immunosuppressive therapy was started from the day before the operation, except in cases of hepatic encephalopathy and severe infection. Tacrolimus was administered orally at a dose of 0.075 mg/kg body weight every 12 hours from the evening of day 1.[13,23] The target for the post-transplantation whole-blood trough concentration of tacrolimus was 10 to 12 ng/mL during the first 2 weeks. Steroid treatment was started at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 $mg \cdot kg^{-1} \cdot d^{-1}$ to 0.3 $mg \cdot kg^{-1} \cdot d^{-1}$ during the first 2 weeks after surgery.

Table I. Demographic characteristics of recipients (N 164)

Age (y)	0.3-67 (median, 3.2)
Adults (15 y) (n 54)	15.0-67 (median, 46)
Children (15 years) (n 110)	0.3-13.7 (median, 1.2)
Body weight (kg)	4.3-92.1 (median, 13.5)
Graft-to-recipient weight ratio (%)	0.63-5.6 (median, 1.84)
Gender (male/female)	70/94
Graft lobe (left/right)	113/51
ABO blood group match (identical/compatible/incompatible)	99/37/28
Preoperative condition (home-bound/hospitalized/intensive care unit–bound)	71/85/8
Primary disease*	
Biliary atresia	89 (21)
Cirrhosis	
Hepatitis B virus	9 (1)
Hepatitis C virus	12 (3)
Primary biliary cirrhosis	7 (2)
Unknown	3 (0)
After liver transplantation	11 (3)
Primary sclerosing cholangitis	8 (4)
Fulminant hepatic failure	4 (2)
Other†	21 (6)

*The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.

†The primary disease was Byler disease in 4 cases (2), Alagille syndrome in 3 (0), Wilson disease in 2 (2), hepatoblastoma in 3 (0), polycystic liver disease in 2 (1), biliary dilation in 2 (0), multiple hepatocellular carcinoma in 1 (0), citrullinemia in 1 (1), hypertyrosinemia in 1 (0), Budd-Chiari syndrome in 1 (0), and portal vein deficiency in 1 (0). The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.

The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 hours after the evening dosage every day, by use of a semiautomated microparticle enzyme immunoassay (IMX; Dainabot, Tokyo, Japan).[24]

Acute cellular rejection was principally diagnosed with liver biopsy specimens, and the histologic diagnosis was performed according to criteria based on the Banff schema.[25] All episodes of rejection were treated with a high-dose steroid bolus injection.

Evaluation of intestinal expression levels of MDR1 and CYP3A4. Biopsy specimens from intestinal mucosa were homogenized in RLT buffer (Qiagen, Hilden, Germany), and total RNA was isolated with MagNA-Pure LC RNA Isolation kit II (Roche) and reverse-transcribed as described previously.[26] The isolated total RNA (500 ng/40 L reaction mixture) was reverse-transcribed by Superscript II reverse transcriptase (Invitrogen, Carlsbad, Calif) with random primers (100 ng/reaction) and digested by RNase H (Invitrogen). After dilution of the single-stranded deoxyribonucleic acid (DNA) mixture with 60 L of sterile water (final volume, 100 L), 5- L aliquots were used for a subsequent real-time polymerase chain reaction (PCR) (final volume, 20 L) with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, Calif). The primer/probe set used for glyceraldehyde 3-phosphate dehydrogenase, as an internal control, was predeveloped TaqMan Assay Reagents (Applied Biosystems), and the reaction was performed according to the manufacturer's instructions. The primer/probe set specific for MDR1 and CYP3A4 was as described previously.[26] Each PCR fragment of the target sequences was generated with specific primer/probe sets as described, ligated into the pCR-Script Cloning Vector (Stratagene, La Jolla, Calif), and confirmed to have the exact sequences of the cloned amplicons by the chain-termination method by use of a fluorescence 373A DNA sequencer (Applied Biosystems). After measurement of the concentrations of the purified plasmid DNA by spectrophotometry, the corresponding concentrations (in moles per microliter) were calculated and serial dilutions of respective plasmid DNA were used as standards for calibration curves. The starting mRNA concentration of MDR1 or CYP3A4 was established by determining the fractional PCR threshold cycle number at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the experimental fractional PCR threshold cycle value with a standard curve generated by use of known amounts of standard plasmid DNA.

Statistical analyses. Normally distributed values were presented as mean SD. Values that were not

normally distributed were presented as the median and range. Logarithmic transformation of the mRNA levels of MDR1 and CYP3A4 was performed to improve normality before statistical analyses were performed. The nonpaired Student *t* test was used to compare groups with respect to normally distributed variables. If different variances between 2 samples were found with the F test, an unpaired *t* test with Welch correction was performed. The Mann-Whitney *U* test was used to compare groups without normality. The calculated mRNA expression levels of MDR1 and CYP3A4 in each intestinal specimen were categorized as high or low, if the quantified value for the mRNA in question exceeded or fell below the median value for all specimens, respectively. Statistical tests were 2-tailed, and significance was defined as P .05.

The outcome measure studied was immunologic events and survival, defined as the time from living-donor liver transplantation to the first episode of acute cellular rejection during the initial 10 days after surgery and to death during the first year after surgery, respectively. The patients without complications until at least postoperative day 10 were categorized as the event-free group. The patients who were diagnosed with acute cellular rejection by liver biopsy before postoperative day 10 were categorized as the acute cellular rejection group. The probability analysis was performed according to the method of Kaplan and Meier, and the outcome was compared among the subgroups by use of a 2-tailed log-rank test for univariate comparisons. An odds ratio was calculated for the risk. Statistical analyses were performed by use of the statistical software package StatView (version 5.0; Abacus Concepts, Berkeley, Calif).

RESULTS

Patients. Table I shows the demographics and primary diseases of living-donor liver transplant recipients whose mucosal samples we studied. Of the recipients who had acute cellular rejection, 28, 11, and 3 had an ABO blood type that was identical, compatible, and incompatible with that of their donor, respectively. Moreover, 18, 21, and 3 recipients with acute cellular rejection were home-bound, hospitalized, and intensive care unit (ICU)–bound, respectively, before surgery. Of the recipients with acute cellular rejection, 29 and 13 had a graft from the left lobe and right lobe, respectively. Steroid-pulse therapy was used in 32 patients without acute cellular rejection during the first 10 days after surgery, and 11 post–liver transplant patients were treated with immunosuppressants until immediately before the second transplantation. Therefore these 43 patients were excluded from the analyses for the probability of acute cellular rejection but not from the analyses on gene expression and tacrolimus pharmacokinetics, and the analyses for acute cellular rejection were performed with the findings of the other 121 recipients, including 82 event-free patients and 39 acute cellular rejection patients. The survival analysis was performed with these 121 recipients, including 13 patients who died within 1 year after transplantation.

Acute cellular rejection and postoperative tacrolimus trough level. By comparing the daily trough concentration of tacrolimus between the event-free group (n 82) and the acute cellular rejection group (n 39), it was found that the trough concentration at postoperative days 3 (P .0075) and 4 (P .0022) was significantly lower in the acute cellular rejection group (Fig 1). These results suggest that the blood level of tacrolimus immediately after living-donor liver transplantation was associated with the occurrence of acute cellular rejection until postoperative day 10. Then, we examined the relationship between the average trough concentration of tacrolimus between postoperative days 2 and 4 and the complications of patients, because the tacrolimus was usually administered to recipients in the ICU during the first 3 days after liver transplantation. At first, we categorized the patients by the average trough concentrations of tacrolimus between postoperative days 2 and 4. Because a low dosage of tacrolimus was administered to patients at risk of infection or renal impairment from the preoperative status to avoid any further deterioration in condition, the categorization was started from 5 ng/mL, which is considered the lower limit of the initial average concentration of tacrolimus. As shown in Fig 2, the frequency of acute cellular rejection tended to be high in patients with relatively lower tacrolimus blood levels, between 5 and 7 ng/mL. The other complications frequently occurred in the patients whose average tacrolimus trough levels were below 5 ng/mL. The frequency of acute cellular rejection compared with the event-free group tended to be lower in the patients whose average tacrolimus trough levels were maintained above 7 ng/mL. Next, we examined the probability of acute cellular rejection in the recipients dividing the average trough concentration of tacrolimus at 7 ng/mL between postoperative day 2 and 4 (Fig 3). Kaplan-Meier analysis demonstrated that the average trough concentration of tacrolimus immediately after living-donor liver transplantation was significantly associated with acute cellular rejection (P .0040). The resultant odds ratio was 2.772 (95% confidence interval [CI], 1.265-6.075) for the patients whose mean trough level of tacrolimus was

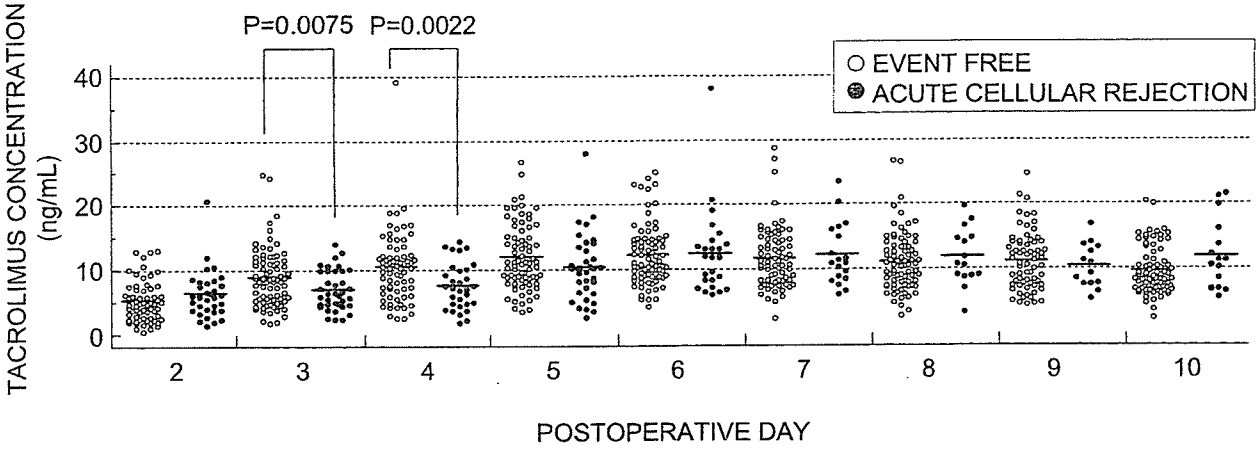

Fig 1. Daily trough levels of tacrolimus in living-donor liver transplant patients. Trough concentrations of tacrolimus in 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: event-free (*open circles*) and acute cellular rejection (*solid circles*). A statistical analysis was performed with the unpaired *t* test after Welch correction. *P* values of less than .05 are shown.

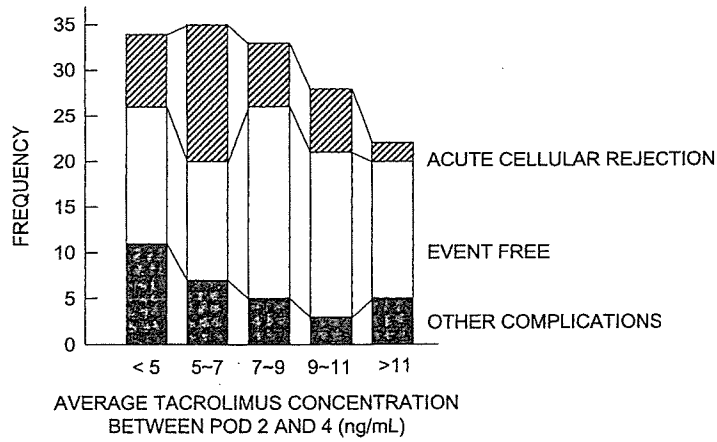

Fig 2. Frequency of complications after living-donor liver transplantation with respect to tacrolimus trough level between postoperative days (POD) 2 and 4. Frequencies of an event-free clinical course, acute cellular rejection, and the need for high-dose steroid treatment for other complications are shown as *open, hatched,* and *solid columns,* respectively. The patients were classified on the basis of the average trough concentration of tacrolimus between postoperative days 2 and 4.

below 7 ng/mL between postoperative days 2 and 4 (Table II).

Association between intestinal mRNA level of MDR1 or CYP3A4 and acute cellular rejection. We previously reported that patients with high levels of enterocyte MDR1, but not CYP3A4, required about 2-fold higher oral dosages of tacrolimus than patients with low levels of MDR1.[20] On the basis of the previous findings, we have re-examined the expression profile of the intestinal mRNA level of MDR1 and CYP3A4 to re-evaluate the influences of these factors on the risk for acute cellular rejection, as well as the interindividual variation of postoperative tacrolimus pharmacokinetics. In Fig 4, *A* and *B*, the logarithmically transformed distribution of the intestinal expression level of MDR1 and CYP3A4 at living-donor liver transplantation is shown. The median value of MDR1 and CYP3A4 was 0.242 amol/ g (range, 0.01-6.51

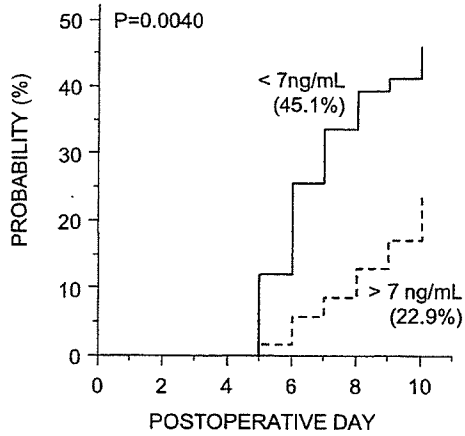

Fig 3. Probability of acute cellular rejection episodes in initial 10 days after living-donor liver transplantation. Kaplan-Meier curves show the probability of acute cellular rejection with respect to the average trough concentration of tacrolimus between postoperative days 2 and 4 (7 ng/mL or 7 ng/mL). *P* values were determined with the log-rank test.

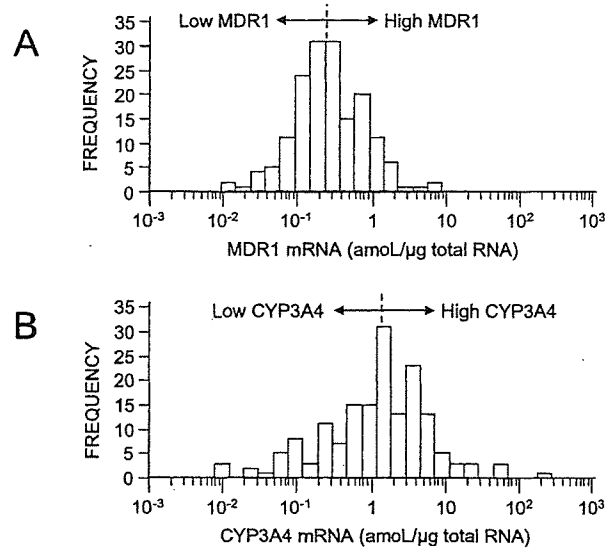

Fig 4. Histograms of messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and CYP3A4 at living-donor liver transplantation. Distribution of mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in intestinal mucosa, both logarithmically transformed to improve normality, are illustrated as histograms for 164 recipients after living-donor liver transplantation. The *dotted lines* denote the median value. RNA, Ribonucleic acid.

amol/ g) of total RNA and 1.278 amol/ g (range, 0.002-185.5 amol/ g) of total RNA, respectively. After dividing the samples by each median value, we examined the probability of acute cellular rejection based on the expression of MDR1 or CYP3A4 (high or low). As illustrated in Fig 5, *A*, a high level of intestinal MDR1 expression was associated with the probability of acute cellular rejection (42.1% in high-MDR1 group versus 23.4% in low-MDR1 group, *P* .0265). The resultant odds ratio was 2.376 (95% CI, 1.087-5.191) for the patients with a high level of intestinal MDR1 mRNA at living-donor liver transplantation (Table II). However, there was no significant association between the intestinal CYP3A4 mRNA level and the probability of acute cellular rejection (*P* .9211) (Fig 5, *B*). The odds ratio showed that a high level of CYP3A4 mRNA at liver transplantation was not a risk factor for the occurrence of postoperative acute cellular rejection (Table II). Moreover, the mRNA expression level of mucosal MDR1 in the patients with acute cellular rejection was weakly but significantly higher compared with those in the event-free group (*P* .0476) (Fig 5, *C*).

Furthermore, the impact of mRNA expression levels of absorptive barriers on patient survival was also examined. According to the method of Kaplan-Meier and subsequent log-rank statistics, the high-level expression of both MDR1 mRNA (Fig 6, *A*) and CYP3A4 (Fig 6, *B*) was significantly associated with patient survival. The odds ratio of the intestinal expression level of MDR1 mRNA at surgery was 7.413 (95% CI, 1.567-36.073), whereas that of CYP3A4 was 3.590 (95% CI, 0.936-13.769).

Table II. Risk factors associated with acute cellular rejection until postoperative day 10

Factors	*Odds ratio*	*95% CI*
Mean trough level of tacrolimus 7 ng/mL between postoperative days 2 and 4	2.772	1.265-6.075
High level of intestinal MDR1 mRNA at surgery	2.376	1.087-5.191
High level of intestinal CYP3A4 mRNA at surgery	1.026	0.485-2.168

CI, Confidence interval; mRNA, messenger ribonucleic acid.

Dosage adjustment based on expression level of intestinal MDR1. To obtain more information about the effect of the intestinal expression level of MDR1 on the pharmacokinetics of tacrolimus, as well as the risk of acute cellular rejection, we compared the daily oral dosage and trough level of tacrolimus between the high- and low-MDR1 groups (Fig 7). The oral dosages

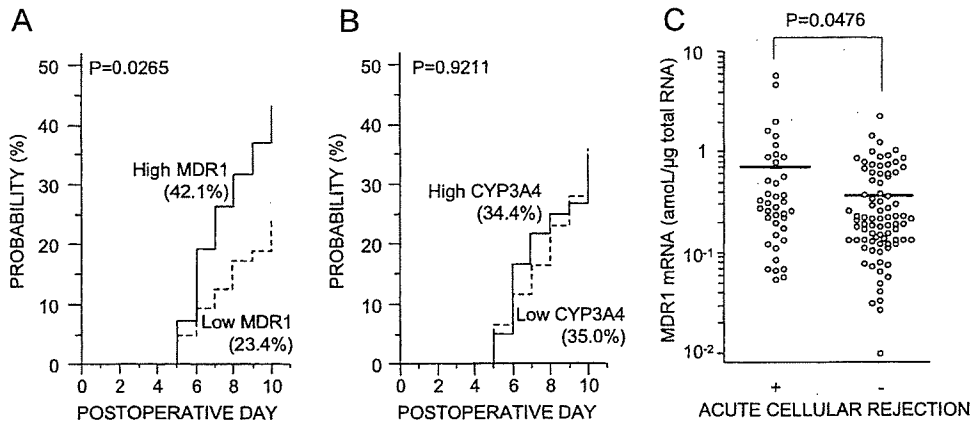

Fig 5. mRNA expression levels of MDR1 and CYP3A4 and acute cellular rejection episodes in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time polymerase chain reaction (PCR) analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. **C**, The mRNA expression levels of MDR1 in mucosa were shown with ( ) or without ( ) acute cellular rejection during 10 days postoperatively. The *P* value was determined with the Mann-Whitney *U* test.

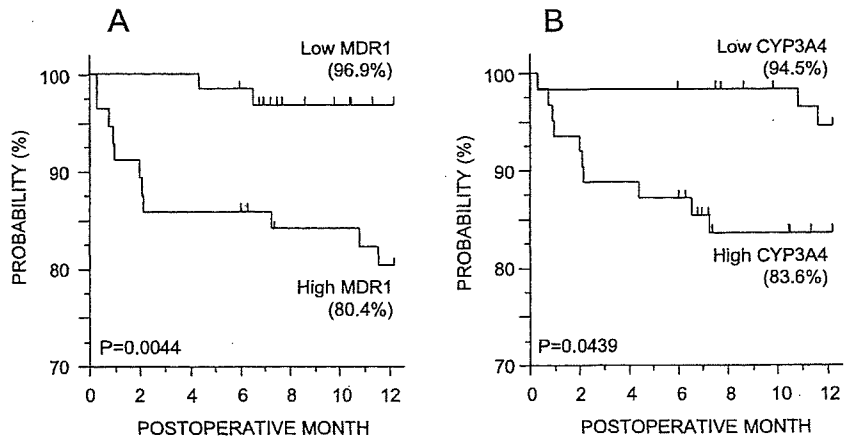

Fig 6. mRNA expression levels of MDR1 and CYP3A4 and cumulative survival rate in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time PCR analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. *Tick marks* indicate the length of follow-up of individual patients who survived.

of tacrolimus were significantly higher in patients categorized in the high-MDR1 group than those in the low-MDR1 group from postoperative day 3 (Fig 7, *A*). However, the daily trough levels of tacrolimus between postoperative days 2 and 10 were comparable between the 2 groups (Fig 7, *B*). The odds ratio was 2.283 (95% CI, 1.058-4.926) for patients whose average trough concentration of tacrolimus between postoperative days

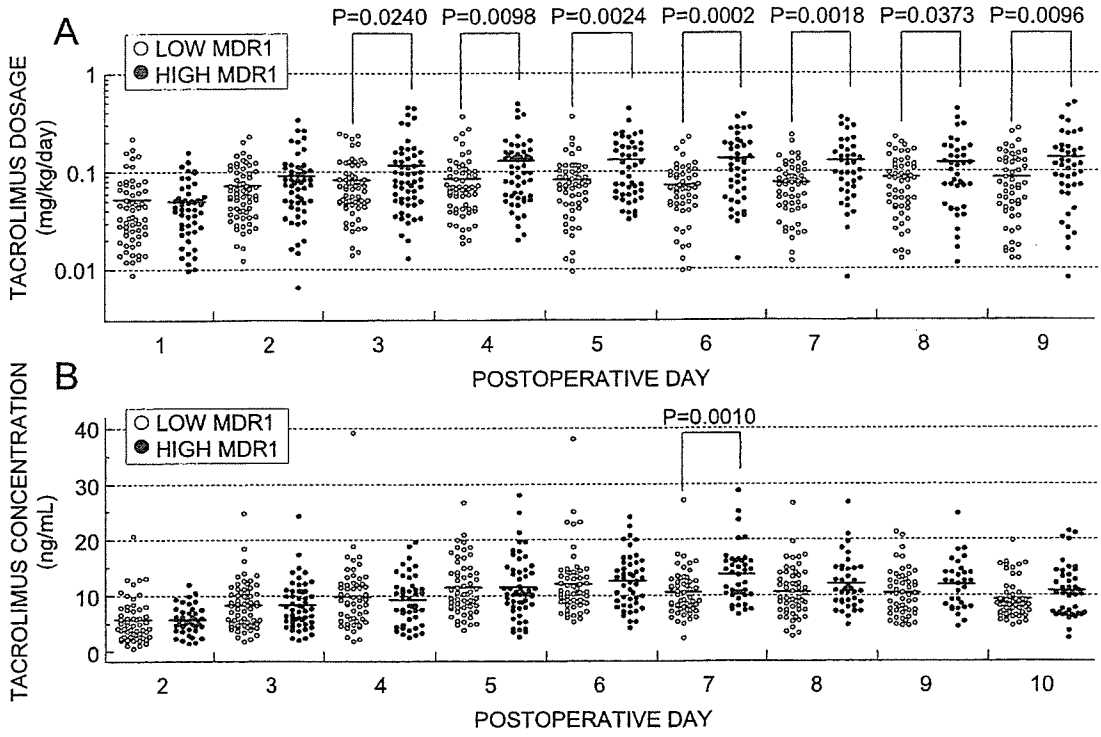

Fig 7. Postoperative oral dosage and trough concentration of tacrolimus in recipients after living-donor liver transplantation. Daily oral dosages (**A**) and trough concentrations (**B**) of tacrolimus for 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: low MDR1 (*open circles*) and high MDR1 (*solid circles*). Low MDR1 and high MDR1 indicate whether the expression level of MDR1 mRNA in individual mucosa was lower or higher than the median value for all intestinal samples. Statistical analysis was performed by use of the unpaired *t* test after Welch correction. *P* values of less than .05 were shown.

2 and 4 was under 7 ng/mL and 2.306 (95% CI, 1.058-5.028) for patients whose intestinal expression level of MDR1 at surgery was greater than 0.242 (Table II).

Correlation between mRNA level of MDR1 and tacrolimus C/D ratio. In this study the average trough concentration of tacrolimus immediately after living-donor liver transplantation and the intestinal expression level of MDR1 were identified as factors useful for predicting the risk of acute cellular rejection immediately after transplantation (Figs 3 and 5 and Table II). If the mRNA level of MDR1 at operation is a potential pharmacokinetic factor, control of the tacrolimus blood concentration will be easier, and the frequency of episodes of acute cellular rejection may be reduced. On the basis of this hypothesis, we performed a correlation analysis of the molecular data on intestinal absorptive barriers and tacrolimus pharmacokinetics to examine whether the MDR1 mRNA level at operation could be a pharmacokinetic factor for individualized initial dosage adjustment. As shown in Fig 8, the mRNA expression level of MDR1 (r 0.5672, P .0001), but not of CYP3A4 (r 0.0490, P .5466), was inversely correlated with the C/D ratio of tacrolimus between postoperative days 2 and 4. Although the mass of graft liver from the living donor was limited in the adult patients, the graft liver was relatively sufficient or large in the pediatric patients. Therefore it is also important to evaluate the engrafted liver mass as the graft-to-recipient weight ratio (Graft liver mass [in kilograms]/Recipient body weight [in kilograms] at surgery 100 [percent]).[2] Furthermore, when the patients were divided into 2 groups based on the graft-to-recipient weight ratio (1.5) (Fig 9, *A*), the coefficient of the correlation between the intestinal mRNA level of MDR1 and tacrolimus C/D ratio improved to 0.6798 (P .0001) and 0.7180 (P