Transplant International

ORIGINAL ARTICLE

Impacts of single nucleotide polymorphisms in Fc gamma receptor IIA (*rs1801274*) on lung transplant outcomes among Japanese lung transplant recipients

Hidenao Kayawake[1], Toyofumi F. Chen-Yoshikawa[1,2], Satona Tanaka[1], Yuka Tanaka[3], Hideki Ohdan[3], Yojiro Yutaka[1], Yoshito Yamada[1], Akihiro Ohsumi[1], Daisuke Nakajima[1], Masatsugu Hamaji[1], Hiroto Egawa[4] & Hiroshi Date[1]

1 Department of Thoracic Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
2 Department of Thoracic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan
3 Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
4 Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan

Correspondence
Toyofumi F. Chen-Yoshikawa, MD, PhD, Department of Thoracic Surgery, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan.
Tel.: +81-75-751-4975;
fax: +81-86-751-4974;
and
Department of Thoracic Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.
Tel.: +81-52-744-2375;
fax: +81-52-744-2383;
e-mail: fengshic@kuhp.kyoto-u.ac.jp

SUMMARY

This study aimed to analyze the influences of single nucleotide polymorphisms (SNPs) in Fc gamma receptor IIA (FCGR2A) on postoperative outcomes after lung transplantation (LTx). We enrolled 191 lung transplant recipients [80 undergoing living-donor lobar lung transplants (LDLLTs) and 111 undergoing deceased-donor lung transplants (DDLTs)] in this study. We identified SNPs in FCGR2A (131 histidine [H] or arginine [R]; rs1801274) and reviewed the infectious complication-free survival after ICU discharge. The SNPs in FCGR2A comprised H/H ($n = 53$), H/R ($n = 24$), and R/R ($n = 3$) in LDLLT and H/H ($n = 67$), H/R ($n = 42$), and R/R ($n = 2$) in DDLT. Recipients with H/H (H/H group) and those with H/R or R/R (R group) were compared in the analyses of infectious complications. In multivariate analyses, the R group of SNPs in FCGR2A was associated with pneumonia-free survival {HR: 2.52 [95% confidence interval (CI): 1.35–4.71], $P = 0.004$}, fungal infection-free survival [HR: 2.50 (95% CI: 1.07–5.84), $P = 0.035$], and cytomegalovirus infection-free survival [HR: 2.24 (95% CI: 1.07–4.69), $P = 0.032$] in LDLLT, but it was not associated with infectious complication-free survival in DDLT. Therefore, in LDLLT, more attention to infectious complications might need to be paid for LTx recipients with H/R or R/R than for those with H/H.

Transplant International 2021; 34: 2192–2204

Key words
Fc gamma receptor IIA, infectious complications, lung transplantation, single nucleotide polymorphism

Received: 24 November 2020; Revision requested: 17 June 2021; Accepted: 9 July 2021; Published online: 19 September 2021

Introduction

Lung transplantation (LTx) is the last resort to save the lives of patients with end-stage pulmonary diseases [1,2]. Although the prognoses of LTx recipients have improved over time, they are still worse than those of patients receiving other solid organ transplants [3]. There remain some mid-to-long-term issues to

doi:10.1111/tri.13974

overcome after LTx, such as infections [4], de novo malignancy [5], and chronic lung allograft dysfunction (CLAD) [6,7].

Single nucleotide polymorphisms (SNPs) represent a form of genetic variants among individuals that may affect clinical courses. Indeed, results of recent reports indicate that SNPs that affect immune regulation, including complement C3, dectin-1, and PTPN22, appear to have an impact on post-transplant prognoses [8–12].

Fc gamma receptors (FCGRs) play an important role in maintaining balance in the immune system by regulating the production of antibodies in humoral immunity, in addition to modulating the activity of effector cells in innate immunity [13–15]. Three classes of FCGRs in humans (FCGR1, FCGR2, and FCGR3) have been recognized. FCGRs comprise several activating receptors and one inhibitory receptor. Among them, the Fc gamma receptor IIA (FCGR2A) gene codes for the activating type CD32 receptor, which is expressed on a variety of immune cells, including neutrophils, eosinophils, and macrophages [16]. Either histidine [H] or arginine [R] at position 131 was placed by a SNP in the gene coding FCGR2A (*rs1801274*). Specifically, FCGR2A [131H] has been reported to have a higher affinity for IgG1 and IgG2 [13–16].

Although there are some reports about the influences of SNPs in FCGRs on postoperative outcomes after solid organ transplantation, such as liver and kidney transplantation [17–20], only a few reports about LTx have been published to date [21,22]. In this study, we assessed the impact of SNPs in FCGR2A (*rs1801274*) on postoperative outcomes after LTx by performing a retrospective review of LTx recipients.

Patients and methods

Between 2008 and 2018, 201 lung transplants [88 living-donor lobar lung transplants (LDLLTs) and 113 deceased-donor lung transplants (DDLTs)] were performed at Kyoto University Hospital. In all, 191 LTx recipients (80 LDLLTs and 111 DDLTs) were enrolled in this study after excluding five patients who died before discharge from the intensive care unit (ICU) as well as four patients who had undergone re-transplantation; one more patient without a preoperative DNA sample was also excluded (Fig. 1). First, identification of SNPs in FCGR2A [131 H/R] (rs1801274) was performed in 80 LDLLT recipients and 111 DDLT recipients. Second, the influences of these SNPs on postoperative outcomes after LTx were evaluated. The infectious complications were reviewed, and the overall survival (OS), CLAD-free survival, and de novo donor-specific anti-HLA antibody (dnDSA)-free survival were calculated. In this study, infectious complications included pneumonia requiring antibiotic treatment, fungal infection, and cytomegalovirus infection after ICU discharge. These analyses were performed separately for LDLLT and DDLT recipients.

The observation period for the infectious complication-free survival after ICU discharge was the interval between the date of ICU discharge and the date of infectious complications, last follow-up, or death. The observation period of OS was defined as the interval between the date of LTx and the date of the last follow-up or death. The observation period of CLAD-free survival was defined as the date of LTx to the date of last follow-up, CLAD development, or death. CLAD was diagnosed based on the definition in the consensus report [23]. The observation period for dnDSA-free survival was defined as the interval between LTx and the last follow-up, dnDSA development, or death. Follow-ups were censored at the end of December 2019. The median observation period of OS was 1295 days (range: 98–4226 days). This study was approved by the Kyoto University Institutional Review Board (G1174).

Analysis of SNPs in FCGR2A [131 H/R]

The details of the analysis of SNPs in FCGR2A are described elsewhere [17,18]. The remaining genomic DNA of LTx recipients initially prepared for preoperative HLA typing was used for analysis. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) was used to genotype the SNPs in FCGR2A [131 H/R] (rs1801274). The analysis results obtained from PCR-RFLP were confirmed using a fully automated SNP detection system (I-density®, ARKRAY Inc., Kyoto, Japan). These analyses were performed at Hiroshima University with blinding of the clinical data of LTx recipients.

Perioperative management including prophylaxis of infections and immunosuppressive protocol

Perioperative prophylaxis of antibiotics is determined after discussion with the infection control team (ICT) and takes the sputum culture of donors and recipients into consideration. In summary, cefazolin is chosen in LDLLT cases with negative cultures, and cefozopran is selected in other cases. If a donor or a recipient has an antibiotic-resistant bacterial infection, such as

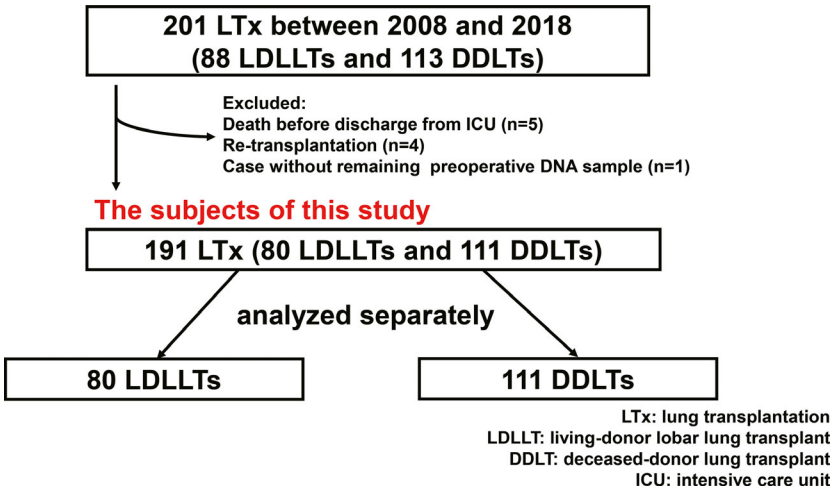

Figure 1 Between 2008 and 2018, 201 recipients received lung transplantation (LTx) at Kyoto University Hospital [88 received living-donor lobar lung transplants (LDLLTs) and 113 received deceased-donor lung transplants (DDLTs)]. Ten cases were excluded due to death before intensive care unit (ICU) discharge (n = 5), re-transplantation (n = 4), or no remaining preoperative DNA sample (n = 1). The remaining 191 LTx recipients (80 LDLLTs and 111 DDLTs) were enrolled in this study. The analyses in this study were performed separately for LDLLT and DDLT recipients. DDLT, deceased-donor lung transplants; ICU, intensive care unit; LDLLT, living-donor lobar lung transplants; LTx, lung transplantation.

methicillin-resistant *Staphylococcus aureus*, suitable antibiotics are selected for each case. The termination of antibiotic administration is determined after discussion with the ICT approximately one week after LTx. Thereafter, prophylactic use of antibiotics is essentially not performed.

As prophylaxis against fungal infections, the administration of micafungin is performed perioperatively and then switched to oral administration of itraconazole. As prophylaxis against cytomegalovirus infections, the administration of ganciclovir is usually started a week after LTx and followed by the oral administration of valganciclovir until at most one year after LTx. When the continued administration of these drugs is difficult due to issues like severe adverse effects, reduction or withdrawal of these drugs is considered.

Postoperative maintenance of immunosuppressive agents is mainly performed using triple therapy with calcineurin inhibitors (tacrolimus or cyclosporine), antimetabolite agents (mycophenolate mofetil or azathioprine), and prednisolone, as previously reported [24,25]. Blood trough levels of tacrolimus are maintained between 10 and 20 ng/ml postoperatively, and after 6 months post-LTx, the levels are maintained between 8 and 12 ng/ml.

Diagnoses of infectious complications

Pneumonia is defined by the presence of certain symptoms, such as fever, fatigue, existence of inflammation detected by laboratory tests, and abnormal shadow in the lung field on a chest computed tomography (CT) scan and requires hospitalization with the administration of antibiotics. Identification of the pathogen is not essential for diagnosis in this study.

The diagnosis of fungal infection is made with positive fungal cultures in culture tests, including sputum and biopsy via bronchoscopy, and requires a change in treatment from itraconazole to antimycotic drugs based on the results of the fungal cultures.

Cytomegalovirus infection comprises cytomegalovirus viremia and/or tissue invasive disease detected by biopsies and requires treatment with ganciclovir. Cytomegalovirus viremia was determined when more than 4/50 000 cells positive for the cytomegalovirus pp65 antigen were detected as we previously reported [26].

Statistical analyses

Descriptive statistics were obtained using EZR software version 1.33, a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [27]. Continuous variables were expressed as medians with ranges while categorical variables were expressed as percentages. The Mann–Whitney *U*-test and Fisher's exact test were used for two-group analyses. Actuarial survival rates were calculated using the Kaplan–Meier method, and groups were compared using the log-rank test. Multivariate analyses of predictive factors for survival analyses were performed using Cox proportional-

Transplant International 2021; 34: 2192–2204

hazards models. The following variables were included in the multivariate analyses for pneumonia-free survival and fungal infection-free survival: SNPs in FCGR2A [H/H group: (H/H) or R group: (H/R) or (R/R)], age (>55 years or ≤55 years), sex, and type of LTx. Since no recipients who underwent single LDLLT developed fungal infection, SNPs in FCGR2A, age, and sex were included in the multivariate analysis to determine the fungal infection-free survival in LDLLT. The CMV mismatch status between donors and recipients was added to multivariate analyses as a predictive factor for CMV infection-free survival and dnDSA-free survival and as a prognostic factor for OS and CLAD-free survival. Statistical significance was defined as $P < 0.05$.

Results

Patient characteristics in LDLLT recipients

The median age of LDLLT recipients was 41 years (range: 6–64 years); 44 recipients in this group were female and 36 were male. The most frequent indication for LTx was pulmonary complications after hematopoietic stem cell transplantation ($n = 35$, 43.8%), followed by interstitial pneumonia ($n = 34$, 42.5%). The SNPs in FCGR2A consisted of H/H ($n = 53$, 66.2%), H/R ($n = 24$, 30.3%), and R/R ($n = 3$, 3.8%). There were no significant differences in age, sex, operative methods, total ischemic time, CMV mismatch status, or indications for LTx between recipients with H/H (H/H group, $n = 53$) and those with H/R or R/R (R group, $n = 27$), except for duration of ICU stay ($P = 0.002$; Table 1).

Patient characteristics in DDLT recipients

The median age of DDLT recipients was 46 years (range: 8–62 years); 44 recipients in this group were female and 67 were male. The most frequent indication for LTx was interstitial pneumonia ($n = 50$, 45.0%). The SNPs in FCGR2A consisted of H/H ($n = 67$, 60.4%), H/R ($n = 42$, 37.8%), and R/R ($n = 2$, 1.8%). There were no significant differences in age, sex, operative methods, total ischemic time, CMV mismatch status, and indications for LTx between the H/H group ($n = 67$) and the R group ($n = 44$; Table 2).

Infectious complications after ICU discharge

Among LDLLT recipients, pneumonia-free survival after ICU discharge was significantly better in the H/H group than in the R group {5-year survival rate: 56.6% [95% confidence interval (CI): 41.2–69.5%] in the H/H group and 29.6% (95% CI: 12.4–49.3%) in the R group, $P = 0.012$, Fig. 2a}. Although the difference was not statistically significant, fungal infection-free survival after ICU discharge in the H/H group was better than that in the R group ($P = 0.055$, Fig. 3a). Furthermore, CMV infection-free survival after ICU discharge was also significantly better in the H/H group than in the R group [$P = 0.025$ (Fig. 4a)]. In contrast, pneumonia-free survival after ICU discharge in the H/H group of DDLT recipients was comparable to that in the R group [5-year survival rate: 37.9% (95% CI: 24.8–50.9%) in the H/H group and 32.3% (95% CI: 18.0–47.4%) in the R group, $P = 0.129$, Fig. 2b]. Similarly, fungal infection-free survival and CMV infection-free survival after ICU discharge were not significantly different between the two groups [$P = 0.137$ (Fig. 3b) and $P = 0.406$ (Fig. 4b), respectively].

In multivariate analyses, the R group of SNPs in FCGR2A was identified as a significant predictive factor for pneumonia [HR: 2.52 (95% CI: 1.35–4.71), $P = 0.004$, Table 3], fungal infection [HR: 2.50 (95% CI: 1.07–5.84), $P = 0.035$, Table 4], and CMV infection-free survival after ICU discharge [HR: 2.24 (95% CI: 1.07–4.69], $P = 0.032$, Table 5) in LDLLT. However, it was not identified as a significant predictive factor for pneumonia (Table S1), fungal infection (Table S2), or CMV infection-free survival after ICU discharge (Table S3) in DDLT.

Prognosis and de novo DSA development

Among LDLLT recipients, the actuarial 5-year OS rate in the H/H group was 85.3% (95% CI: 71.4–92.8%), which was comparable with that of the R group [74.7% (95% CI: 51.1–90.9%); $P = 0.941$; Fig. 5a]. Similarly, the actuarial 5-year CLAD-free survival rate was comparable between the H/H and R groups [73.1% (95% CI: 56.9–83.9%) in the H/H group vs. 55.2% (95% CI: 33.4–33.4%) in the R group; $P = 0.377$; Fig. 6a]. In DDLT, the actuarial 5-year OS was similar between the H/H and R groups [64.0% (95% CI: 47.7%) in the H/H group vs. 62.3% (95% CI: 39.6–78.5%) in the R group; $P = 0.963$; Fig. 5b]. Moreover, the actuarial 5-year CLAD-free survival rate in the H/H group was 57.5% (95% CI: 41.6–70.6%), which was not significantly different from that of the R group [46.6% (95% CI: 23.9–66.6%); $P = 0.779$; Fig. 6b]. Regarding dnDSA-free survival, there were no significant differences between LDLLT and DDLT recipients ($P = 0.65$ and $P = 0.771$, respectively).

Table 1. Overall characteristics and comparisons between the two groups of patients in LDLLT.

Variables (median with range)	All LDLLT recipients (n = 80)	H/H group (n = 53)	R group (n = 27)	P value
Age (years)	41 (6–64)	43 (6–64)	35 (6–64)	0.895
Sex (%)				
Female	44 (55.0%)	29 (54.7%)	15 (55.6%)	1
Male	36 (45.0%)	24 (45.3%)	12 (44.4%)	
Body mass index (kg/m^2)	17.2 (10.1–25.9)	16.8 (10.1–25.8)	18.1 (12.1–25.9)	0.114
Distribution of SNPs in FCGR2A (%)				
H/H	53 (66.2%)			
H/R	24 (30.0%)			
R/R	3 (3.8%)			
Indications for LDLLT (%)				
Interstitial pneumonia	34 (42.5%)	24 (45.3%)	10 (37.0%)	0.356
PC after HSCT	35 (43.8%)	24 (45.3%)	11 (40.7%)	
IPAH	5 (6.2%)	3 (5.7%)	2 (7.4%)	
Others	6 (7.5%)	2 (3.8%)	4 (14.8%)	
Operative methods (%)				
Single LDLLT	10 (12.5%)	6 (11.3%)	4 (14.8%)	0.726
Bilateral LDLLT	70 (87.5%)	47 (88.7%)	23 (85.2%)	
Total ischemic time (min)	148 (80–252)	151 (80–252)	143 (89–250)	0.47
Preoperative WBC (/μl)	7450 (2300–23 920)	7350 (2300–23 920)	8420 (3250–18 190)	0.632
Preoperative CRP (mg/dl)	0.3 (0.0–14.0)	0.3 (0.0–14.0)	0.4 (0.0–2.8)	0.678
CMV mismatch status (%)				
Positive	14 (17.5%)	7 (13.2%)	7 (25.9%)	0.323
Negative	65 (81.2%)	45 (84.9%)	20 (74.1%)	
Unknown	1 (1.2%)	1 (1.9%)	0 (0.0%)	
Having preformed DSA (%)				
Yes	2 (2.5%)	2 (3.8%)	0 (0.0%)	0.547
No	78 (97.5%)	51 (96.2%)	27 (100.0%)	
Immunosuppressive agents (%)				
Calcineurin inhibitors				
Tacrolimus	54 (67.5%)	38 (71.7%)	16 (59.3%)	0.316
Cyclosporine	26 (32.5%)	15 (28.3%)	11 (40.7%)	
Antimetabolite agents				
Mycophenolate mofetil	70 (87.5%)	49 (92.5%)	21 (77.8%)	0.116
Azathioprine	8 (10.0%)	3 (5.7%)	5 (18.5%)	
None	2 (2.5%)	1 (1.9%)	1 (3.7%)	
Steroids				
Prednisolone	80 (100.0%)			
Duration of ICU stays (days)	12 (4–33)	11 (4–28)	14 (7–33)	0.002
Developing de novo DSA (%)				
Yes	6 (7.5%)	3 (5.7%)	3 (11.1%)	0.4
No	74 (92.5%)	50 (94.3%)	24 (88.9%)	

CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DSA, donor-specific anti-HLA antibody; FCGR2A, Fc gamma receptor IIA; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; IPAH, idiopathic pulmonary arterial hypertension; LAM, lymphangiomyomatosis; LDLLT, living-donor lobar lung transplant; PC, pulmonary complications; SNP, single nucleotide polymorphism; WBC, white blood cell.

In LDLLT, multivariate analyses revealed that SNPs in FCGR2A were not significant predictors of prognosis. In contrast, younger age was identified as a good prognostic factor for both OS [HR: 0.21 (95% CI: 0.07–0.62); Table 6] and CLAD-free survival [HR: 0.33 (95% CI: 0.15–0.70); Table 7], while male sex was identified as a negative prognostic factor for OS [HR: 3.19 (95% CI: 1.02–10.00), P = 0.046]. However, no factors were found to be significant predictive factors for survival among DDLT recipients (Tables S4 and S5).

Discussion

In this study, several important results were obtained. First, the R group of SNPs in FCGR2A was identified as a significant predictive factor for pneumonia-, fungal

Transplant International 2021; 34: 2192–2204

Table 2. Overall characteristics and comparisons between the two groups of patients in DDLT.

Variables (median with range)	All DDLT recipients (n = 111)	H/H group (n = 67)	R group (n = = 44)	P value
Age (years)	46 (8–62)	44 (8–62)	48 (19–60)	0.229
Sex (%)				
Female	44 (39.6%)	26 (38.8%)	18 (40.9%)	0.845
Male	67 (60.4%)	41 (61.2%)	26 (59.1%)	
Body mass index (kg/m^2)	18.7 (11.4–30.5)	18.4 (12.2–30.5)	19.6 (11.4–28.5)	0.119
Distribution of SNPs in FCGR2A (%)				
H/H	67 (60.4%)			
H/R	42 (37.8%)			
R/R	2 (1.8%)			
Indications for LDLLT (%)				
Interstitial pneumonia	50 (45.0%)	29 (43.3%)	21 (47.7%)	0.612
PC after HSCT	9 (8.1%)	3 (4.5%)	6 (13.6%)	
IPAH	10 (9.0%)	6 (9.0%)	4 (9.1%)	
LAM	11 (9.9%)	6 (9.0%)	5 (11.4%)	
COPD	12 (10.8%)	10 (14.9%)	2 (4.5%)	
Others	19 (17.1%)	13 (19.4%)	6 (13.6%)	
Operative methods (%)				
Single DDLT	57 (51.4%)	32 (47.8%)	25 (56.8%)	0.438
Bilateral DDLT	54 (48.6%)	35 (52.2%)	19 (43.2%)	
Total ischemic time (min)	477 (242–780)	470 (274–718)	484 (242–780)	0.575
Preoperative WBC (/μl)	8320 (3200–21 900)	8440 (3200–21 900)	7805 (4200–14 600)	0.557
Preoperative CRP (mg/dl)	0.1 (0.0–7.7)	0.1 (0.0–7.0)	0.2 (0.0–4.5)	0.968
CMV mismatch status (%)				
Positive	15 (13.5%)	11 (16.4%)	4 (9.1%)	0.065
Negative	93 (83.8%)	56 (83.6%)	37 (84.1%)	
Unknown	3 (2.7%)	0 (0.0%)	3 (6.8%)	
Having preformed DSA (%)				
Yes	4 (3.6%)	3 (4.5%)	1 (2.3%)	1
No	107 (96.4%)	64 (95.5%)	43 (97.7%)	
Immunosuppressive agents (%)				
Calcineurin inhibitors				
Tacrolimus	90 (81.1%)	57 (85.1%)	33 (75.0%)	0.219
Cyclosporine	21 (18.9%)	10 (14.9%)	11 (25.0%)	
Antimetabolite agents				
Mycophenolate mofetil	111 (100%)			
Steroids				
Prednisolone	111 (100%)			
ICU stay (days)	10 (3–88)	10 (3–57)	19 (4–88)	0.627
Developing de novo DSA (%)				
Yes	18 (16.2%)	11 (16.4%)	7 (15.9%)	1
No	93 (83.8%)	56 (83.6%)	37 (84.1%)	

CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DDLT, deceased-donor lung transplant; DSA, donor-specific anti-HLA antibody; FCGR2A, Fc gamma receptor IIA; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; IPAH, idiopathic pulmonary arterial hypertension; LAM, lymphangiomyomatosis; PC, pulmonary complications; SNP, single nucleotide polymorphism; WBC, white blood cell.

infection-, and CMV infection-free survival after ICU discharge in LDLLT. Also, SNPs in FCGR2A were not identified as significant predictive factors for infectious complications in DDLT. Additionally, despite the difference in the frequencies of infectious complications after ICU discharge among LDLLT recipients, there were no significant differences in prognosis between the H/H group and the R group.

This is the first study in Japan to assess the distribution of SNPs in FCGR2A among LTx recipients. In this study, we observed that more than 60% of LTx recipients had SNPs of FCGR2A [131 H/H], whereas less

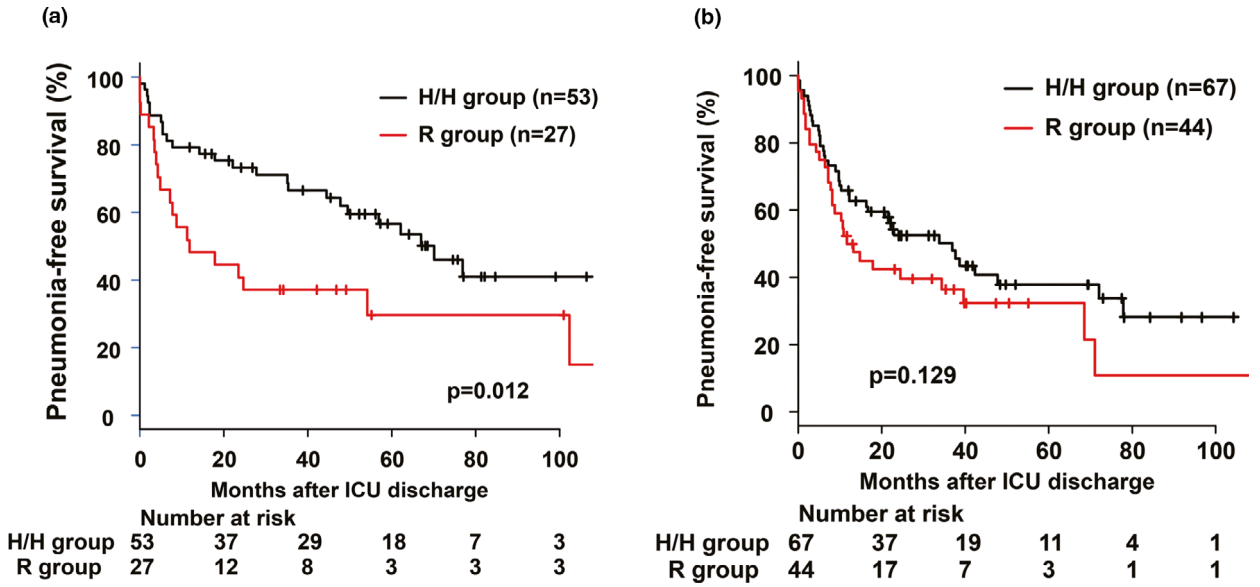

Figure 2 (a) Among LDLLT recipients, the 5-year pneumonia-free survival after ICU discharge in the H/H group was 56.6% [95% confidence interval (CI): 41.2–69.5%], which was significantly better than that in the R group [29.6% (95% CI: 12.4–49.3%), $P = 0.012$]. (b) In DDLT, pneumonia-free survival after ICU discharge in the H/H group was comparable with that in the R group [5-year survival: 37.9% (95% CI: 24.8–50.9%) in the H/H group and 32.3% (95% CI: 18.0–47.4%) in the R group, $P = 0.129$]. CI, confidence interval; DDLT, deceased-donor lung transplant; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant.

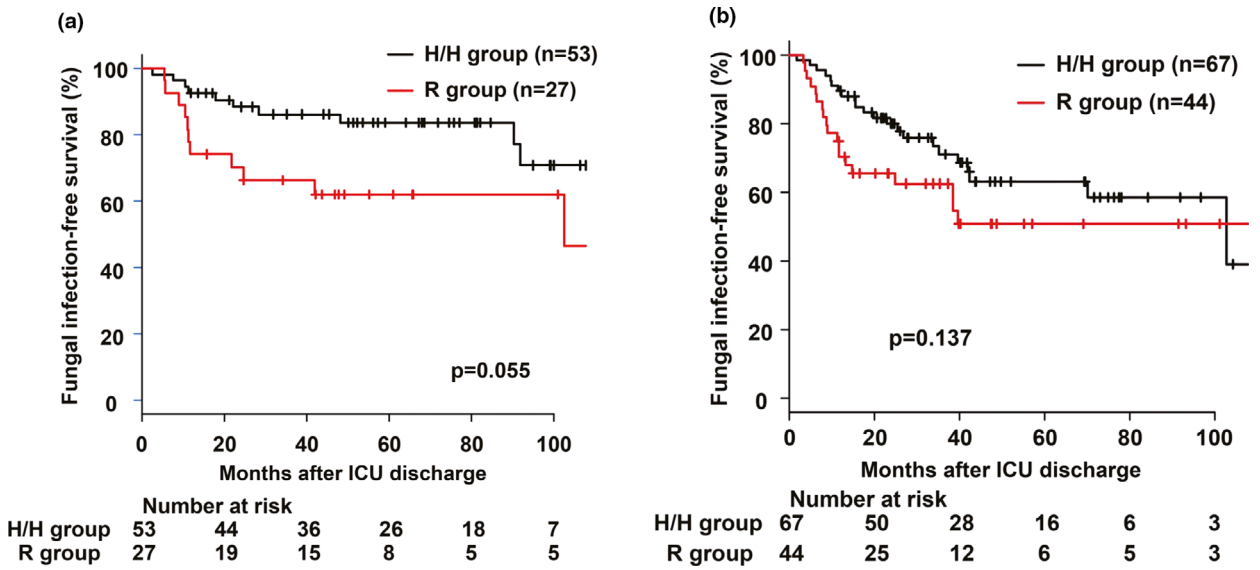

Figure 3 (a) In LDLLT, fungal infection-free survival after ICU discharge in the H/H group was non-significantly better than that in the R group [5-year survival: 83.6% (95% CI: 69.6–91.5%) in the H/H group and 61.9% (95% CI: 40.5–77.4%) in the R group, $P = 0.055$]. (b) Fungal infection-free survival after ICU discharge was not significantly different between the two groups in DDLT (5-year survival: 63.0% [95% CI: 47.8–74.9%] in the H/H group and 50.8% [95% CI: 33.0–66.0%] in the R group, $P = 0.137$). CI, confidence interval; DDLT, deceased-donor lung transplant; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant.

than 5% of LTx recipients had SNPs of FCGR2A [131 R/R] in both LDLLT and DDLT. These distributions are compatible with Japanese recipients undergoing other solid organ transplantations [17,18] and healthy Japanese individuals [28–30]. However, ethnic differences in the distribution of SNPs have also been reported [28]. Compared to French LTx recipients [22], the proportion of Japanese LTx recipients with SNPs of FCGR2A [131 H/H] was higher and the proportion with FCGR2A [131 R/R] was lower. In contrast, there were

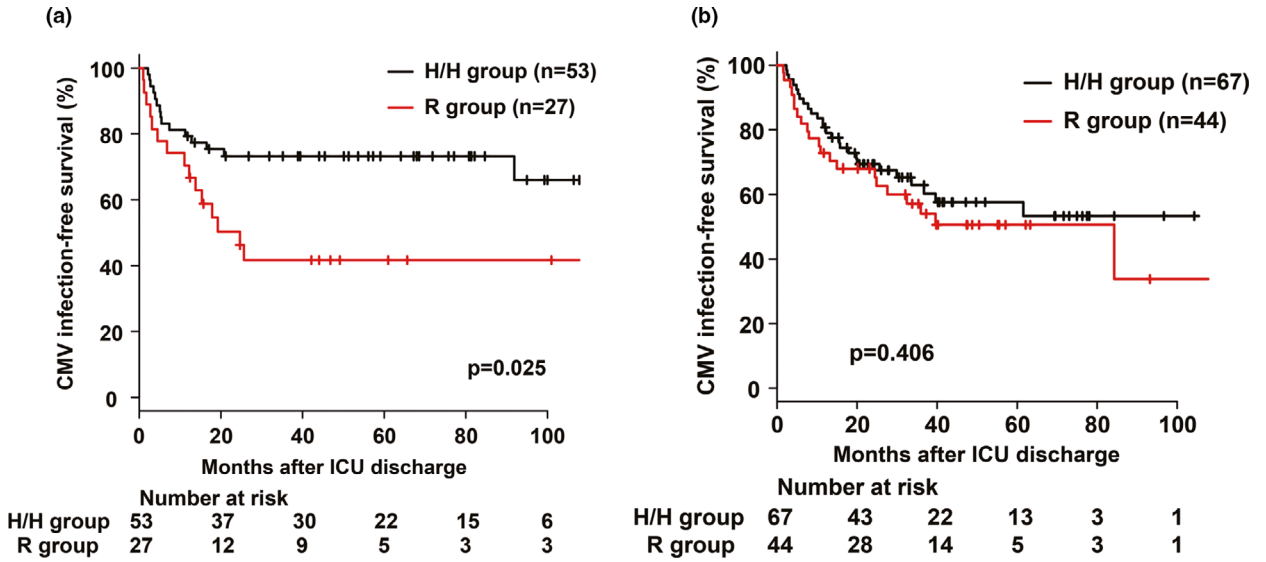

Figure 4 (a) In LDLLT, CMV infection-free survival after ICU discharge in the H/H group was significantly better than that in the R group [5-year survival: 73.3% (95% CI: 59.1–83.2%) in the H/H group and 41.6% (95% CI: 22.4–59.8%) in the R group, $P = 0.025$). (b) In DDLT, CMV infection-free survival after ICU discharge was not significantly different between the two groups in DDLT [5-year survival: 57.6% (95% CI: 43.1–69.6%) in the H/H group and 50.5% (95% CI: 33.8–65.0%) in the R group, $P = 0.406$]. CI, confidence interval; CMV, cytomegalovirus; DDLT, deceased-donor lung transplant; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant.

Table 3. Multivariate analysis for pneumonia-free survival after ICU discharge in LDLLT.

	Multivariate analysis for pneumonia-free survival after ICU discharge		
Variables	Hazard ratio	95% Confidence interval	*P* value
Age			
>55	1		0.661
≤55	0.86	0.43–1.71	
Sex			
Female	1		0.038
Male	1.92	1.04–3.56	
SNPs in FCGR2A			
H/H group	1		0.004
R group	2.52	1.35–4.71	
Operative method			
Single LDLLT	1		0.167
Bilateral LDLLT	2.13	0.73–6.20	

FCGR2A, Fc gamma receptor IIA; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant; SNP, single nucleotide polymorphism.

no differences in the distribution of SNPs in FCGR2A between male and female recipients in this study, which was different from the results of a study conducted in France [22].

In this study, the influence of SNPs in FCGR2A on postoperative outcomes was analyzed separately between LDLLT and DDLT recipients due, in part, to the fact that LDLLT recipients typically receive grafts with no injury, while DDLT recipients receive grafts that have some influence of brain death and intubation. Also, graft size is different between LDLLT and DDLT recipients [2].

Possible predictive factors in multivariate analyses were determined based on a previous report of infectious complications after LTx [31]. The present study showed that, in LDLLT, the R group of SNPs in FCGR2A was identified as a significant predictive factor for pneumonia-, fungal infection-, and CMV infection-free survival; however, it was not identified as a significant predictor in DDLT. We hypothesize that this difference between LDLLT and DDLT recipients is due to the following reasons. Since the implanted grafts in LDLLT are essentially clear and have no injury, the postoperative outcomes largely depend on recipient factors and characteristics. On the other hand, the quality of implanted grafts in DDLT varies, and the grafts often have injuries, which exert some influence on postoperative outcomes. Furthermore, since more than half of DDLT recipients receive a single LTx, postoperative outcomes, including infectious complications, seem to be affected by the remaining native lungs [32–34].

FCGR2A [H/R and R/R] are reported to have a lower affinity for IgG2, which is the subclass of IgGs thought to play an important role in the immune response to

Table 4. Multivariate analysis for fungal infection-free survival after ICU discharge in LDLLT.

	Multivariate analysis for fungal infection-free survival after ICU discharge		
Variables	Hazard ratio	95% Confidence interval	*P* value
Age			
>55	1		0.023
≤55	0.35	0.14–0.86	
Sex			
Female	1		0.104
Male	2.12	0.86–5.25	
SNPs in FCGR2A			
H/H group	1		0.035
R group	2.50	1.07–5.84	

FCGR2A, Fc gamma receptor IIA; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant; SNP, single nucleotide polymorphism.

Table 5. Multivariate analysis for CMV infection-free survival after ICU discharge in LDLLT.

	Multivariate analysis for CMV infection-free survival after ICU discharge		
Variables	Hazard ratio	95% Confidence interval	*P* value
Age			
>55	1		0.081
≤55	0.49	0.22–1.09	
Sex			
Female	1		0.467
Male	1.32	0.62–2.81	
SNPs in FCGR2A			
H/H group	1		0.032
R group	2.24	1.07–4.69	
Operative method			
Single LDLLT	1		0.103
Bilateral LDLLT	5.53	0.71–43.4	
CMV mismatch status			
Negative	1		0.595
Positive or unknown	1.33	0.47–3.79	

CMV, cytomegalovirus; FCGR2A, Fc gamma receptor IIA; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant; SNP, single nucleotide polymorphism.

antigens from bacteria, than FCGR2A [H/H] [35,36]. Therefore, the phagocytosis of bacteria receiving IgG2 opsonization by polymorphonuclear leukocytes with FCGR2A [H/R or R/R] is less efficient than that of polymorphonuclear leukocytes with FCGR2A [H/H] [14,15,17,35,36]. Previous reports have suggested that SNPs in FCGR2A are associated with the severity of community-acquired pneumonia [35], susceptibility to recurrent respiratory tract infections [36], and severity of pneumococcal pneumonia [37]. The results among LDLLT recipients in this study are compatible with the findings of the previous reports. This is also the first study to highlight the association between SNPs in FCGR2A (*rs1801274*) and the frequency of infectious complications after LTx. In contrast, it has previously been reported that, after liver transplantation, an incidence of bloodstream infections was associated with SNPs in FCGR IIIA (FCGR3A) [17]. In addition, a significantly higher incidence of urinary tract infections was reported to correlate with FCGR3A SNPs after kidney transplantation [18]. In both papers, it was described that the combination of FCGR2A and FCGR3A SNPs might better predict each post-transplant infection. The differences from the findings of this study may reflect the organ-specific innate or humoral immune response after transplantation.

Due to the routine prophylactic administration of micafungin followed by the prophylactic oral administration of itraconazole, the entire frequency of fungal infection was kept relatively low at our institution. Nonetheless, the R group of SNPs in FCGR2A was identified as a significant predictive factor for fungal infection-free survival in LDLLT, but it was not identified as a significant predictor in DDLT. It has previously been indicated that FCGR2A is associated with protection from Aspergillus infection [38]. Thus, the indication obtained from this study that SNPs in FCGR2A might influence the prevention of fungal infection seems reasonable. However, differences in the incidence of fungal infections with respect to SNPs in FCGR2A among kidney transplant recipients were not observed in a previous study [18]. It was hypothesized that the difference in results between LTx and kidney transplant may be due to the strength of immunosuppression since the trough level of calcineurin inhibitor in LTx was higher than that used in kidney transplant. In fact, the frequency of fungal infection in this study was higher than that in a kidney transplant study [18]. Similarly, the R group of SNPs in FCGR2A was detected as a significant predictive factor for CMV infection-free survival in LDLLT but not in DDLT. Regarding CMV infection, the association between CMV infection and SNPs in FCGR3A was previously reported in a study on cardiac transplantation [39]. In contrast, some other studies on solid organ transplantation reported no significant association between SNPs in FCGR2A and CMV infection [17,18]. These

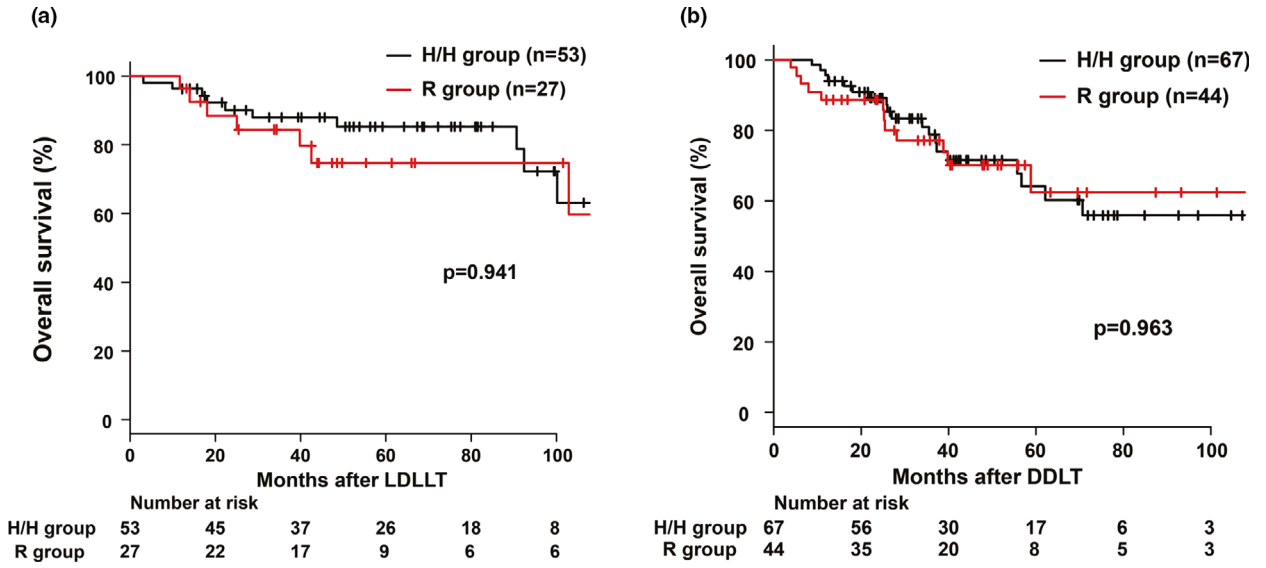

Figure 5 (a) In LDLLT, the actuarial 5-year overall survival (OS) in the H/H group was 85.3% (95% CI: 71.4–92.8%), which was comparable with that of the R group [74.7% (95% CI: 51.8–87.9%); *P* = 0.941). (b) The actuarial 5-year OS in DDLT was comparable between the two groups [64.0% (95% CI: 47.0–76.8%) in the H/H group vs. 62.3% (95% CI: 39.6–78.5%) in the R group, respectively; *P* = 0.963]. CI, confidence interval; DDLT, deceased-donor lung transplant; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant; OS, overall survival.

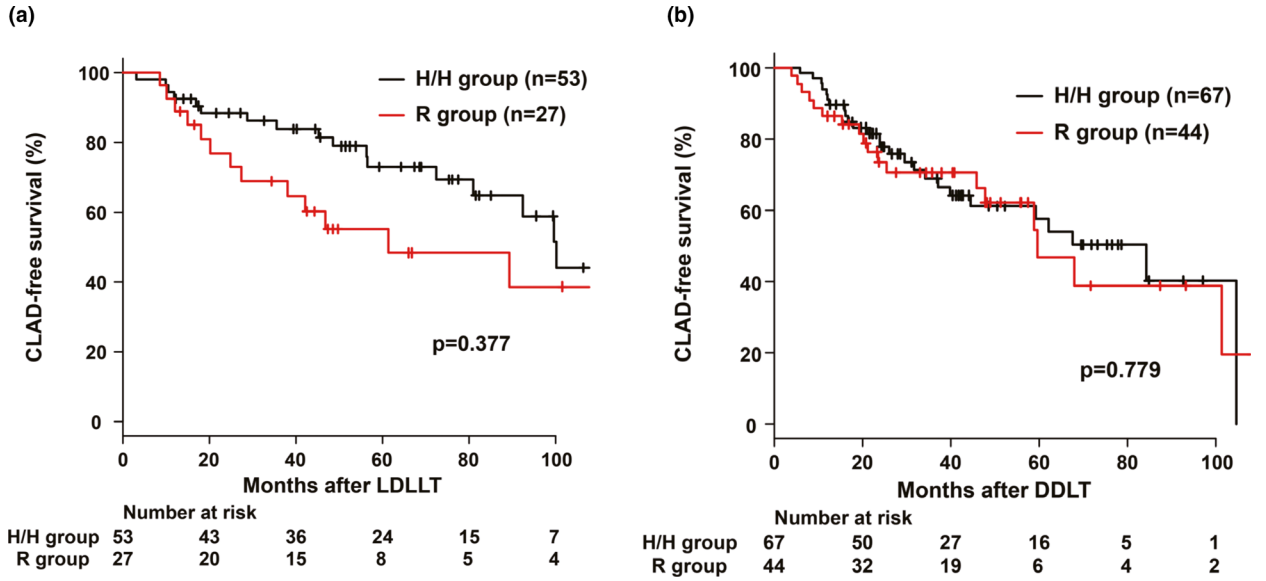

Figure 6 (a) In LDLLT, the actuarial 5-year CLAD-free survival rate was not significantly different between the H/H and R groups [73.1% (95% CI: 56.9–83.9%) in the H/H group vs. 55.2% (95% CI: 33.5–72.4%) in the R group, respectively; *P* = 0.377]. (b) In DDLT, the actuarial 5-year CLAD-free survival rate in the H/H group was 57.5% (95% CI: 41.6–70.6%), which was comparable with that of the R group [46.6% (95% CI: 23.9–66.6%); *P* = 0.779]. CI, confidence interval; CLAD, chronic lung allograft syndrome; DDLT, deceased-donor lung transplant; ICU, intensive care unit; LDLLT, living-donor lobar lung transplant.

discrepancies might be derived from differences in the strategies for the prevention of CMV infection among transplant centers.

The SNPs in FCGR2A were not identified as predictive factors for prognoses in LDLLT or DDLT; however, they were identified as predictive factors for infectious complications in LDLLT. This discrepancy in LDLLT may be due, in part, to early detection of infectious complications and appropriate postoperative management. In contrast, SNPs in FCGR2A were reported to be associated with CLAD-free survival but not OS in a previous paper [22]. One potential explanation for this

Table 6. Multivariate analysis for overall survival in LDLLT.

Variables	Multivariate analysis for overall survival		
	Hazard ratio	95% Confidence interval	*P* value
Age			
>55	1		0.005
≤55	0.21	0.07–0.62	
Sex			
Female	1		0.046
Male	3.19	1.02–10.00	
SNPs in FCGR2A			
H/H group	1		0.524
R group	1.39	0.51–3.78	
Operative method			
Single LDLLT	1		0.404
Bilateral LDLLT	2.57	0.28–23.59	
CMV mismatch status			
Negative	1		0.334
Positive or unknown	0.36	0.05–2.83	

CMV, cytomegalovirus; FCGR2A, Fc gamma receptor IIA; LDLLT, living-donor lobar lung transplant; SNP, single nucleotide polymorphism.

Table 7. Multivariate analysis for CLAD-free survival in LDLLT.

Variables	Multivariate analysis for CLAD-free survival		
	Hazard ratio	95% confidence interval	*P* value
Age			
>55	1		0.004
≤55	0.33	0.15–0.70	
Sex			
Female	1		0.056
Male	2.08	0.98–4.41	
SNPs in FCGR2A			
H/H group	1		0.068
R group	2.03	0.95–4.33	
Operative method			
Single LDLLT	1		0.092
Bilateral LDLLT	3.76	0.81–17.51	
CMV mismatch status			
Negative	1		0.914
Positive or unknown	0.95	0.36–2.52	

CMV, cytomegalovirus; FCGR2A, Fc gamma receptor IIA; LDLLT, living-donor lobar lung transplant; SNP, single nucleotide polymorphism.

discrepancy is that, compared to other countries, more LDLLTs and single DDLTs are performed in Japan due to a severe donor shortage, and the HLA mismatch between donors and recipients in Japan appears smaller than that of other countries [24,40]. Nevertheless, infection is known as one of the main causes of death after LTx in mid-term and long-term follow-up; therefore, postoperative careful follow-ups should be performed to detect potential signs of infection in LTx recipients with H/R and R/R.

It has been reported that dnDSAs are associated with worse outcomes after LTx [41,42]. In this study, SNPs in FCGR2A were not significant predictors of dnDSA-free survival in LDLLT or DDLT. The underlying cause of these results remains unclear; however, the results may be related to the fact that the frequency of dnDSA in LDLLT was significantly lower than that in DDLT; furthermore, dnDSAs in DDLT were detected earlier than those in LDLLT [43]. Nevertheless, it is necessary to continue to perform postoperative monitoring of anti-HLA antibodies, since the dnDSA development may increase with the longer postoperative observation period.

This study has several limitations. First, this was a retrospective, nonrandomized, single institutional study. Second, this study included a relatively large number of recipients undergoing LDLLT because of the severe donor shortage in Japan. Thus, the characteristics of recipients in this study may differ from other studies [44].

In conclusion, although there were no significant differences in prognoses after LTx among SNPs in FCGR2A, a SNP in FCGR2A (H/R or R/R) was detected as a significant predictive factor for pneumonia, fungal infection, and CMV infection after ICU discharge among LDLLT recipients. Therefore, LDLLT recipients with these FCGR2A SNPs presenting infectious complications may require careful monitoring.

Authorship

HK, TC-Y, ST, YT, HO, HE and HD: participated in the research design. HK, TC-Y, ST and HD: participated in the data collection and analysis. YT and HO: participated in the analysis of single nucleotide polymorphisms in Fc gamma receptor IIA. HK, TC-Y, ST, YY, YY, AO, DN, MH and HD: participated in performing lung transplantations and following the patients. All authors participated in the writing and approval of the paper.

Transplant International 2021; 34: 2192–2204

Funding

This study was funded by Japan Agency for Medical Research and Development (No. 18ek0510024h0002).

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are grateful to the coordinators for LTx, anesthesiologists, cardiac surgeons, pulmonologists, clinical engineers, and laboratory technologists from Kyoto University Hospital for their support of the LTx program.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Multivariate analysis for pneumonia-free survival after ICU discharge in DDLT.

Table S2. Multivariate analysis for fungal infection-free survival after ICU discharge in DDLT.

Table S3. Multivariate analysis for CMV infection-free survival after ICU discharge in DDLT.

Table S4. Multivariate analysis for overall survival in DDLT.

Table S5. Multivariate analysis for CLAD-free survival in DDLT.

REFERENCES

1. Kulkarni HS, Cherikh WS, Chambers DC, *et al*. Bronchiolitis obliterans syndrome-free survival after lung transplantation: an International Society for Heart and Lung Transplantation Thoracic Transplant Registry analysis. *J Heart Lung Transplant* 2019; **38**: 5.
2. Kayawake H, Chen-Yoshikawa TF, Hamaji M, *et al*. Acquired recipient pulmonary function is better than lost donor pulmonary function in living-donor lobar lung transplantation. *J Thorac Cardiovasc Surg* 2019; **158**: 1710.
3. Chambers DC, Cherikh WS, Harhay MO, *et al*. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-sixth adult lung and heart-lung transplantation report-2019; focus theme: donor and recipient size match. *J Heart Lung Transplant* 2019; **38**: 1042.
4. Yu H, Bian T, Yu Z, *et al*. Bilateral lung transplantation provides better long-term survival and pulmonary function than single lung transplantation: a systematic review and meta-analysis. *Transplantation* 2019; **103**: 2634.
5. Tanaka S, Chen-Yoshikawa TF, Yamada T, *et al*. Malignancies after living-donor and cadaveric lung transplantations in Japanese patients. *Surg Today* 2016; **46**: 1415.
6. Saito M, Chen-Yoshikawa TF, Nakamoto Y, *et al*. Unilateral chronic lung allograft dysfunction assessed by biphasic computed tomographic volumetry in bilateral living-donor lobar lung transplantation. *Transplant Direct* 2018; **4**: e398.
7. Verleden SE, Todd JL, Sato M, *et al*. Impact of CLAD phenotype on survival after lung retransplantation: a multicenter study. *Am J Transplant* 2015; **15**: 2223.
8. Oetting WS, Schladt DP, Dorr CR, *et al*. Analysis of 75 candidate SNPs associated with acute rejection in kidney transplant recipients: validation of rs2910164 in microRNA MIR146A. *Transplantation* 2019; **103**: 1591.
9. Verma S, Tanaka Y, Shimizu S, Tanimine N, Ohdan H. Significant association between FOXP3 gene polymorphism and steroid-resistant acute rejection in living donor liver transplantation. *Hepatol Commun* 2017; **1**: 406.
10. Kardol-Hoefnagel T, Budding K, van de Graaf EA, *et al*. A single nucleotide C3 polymorphism associates with clinical outcome after lung transplantation. *Front Immunol* 2019; **10**: 2245.
11. Calabrese DR, Wang P, Chong T, *et al*. Dectin-1 genetic deficiency predicts chronic lung allograft dysfunction and death. *JCI Insight* 2019; **4**: e133083.
12. Budding K, van Setten J, van de Graaf EA, *et al*. The autoimmune-associated single nucleotide polymorphism within PTPN22 correlates with clinical outcome after lung transplantation. *Front Immunol* 2019; **9**: 3105.
13. Nimmerjahn F, Ravetch JV. Fc gamma receptors as regulators of immune responses. *Nat Rev Immunol* 2008; **8**: 34.
14. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 2012; **119**: 5640.
15. Li X, Gibson AW, Kimberly RP. Human FcR polymorphism and disease. *Curr Top Microbiol Immunol* 2014; **382**: 275.
16. Rosales C. Fcγ receptor heterogeneity in leukocyte functional responses. *Front Immunol* 2017; **8**: 280.
17. Shimizu S, Tanaka Y, Tazawa H, *et al*. Fc-gamma receptor polymorphisms predispose patients to infectious complications after liver transplantation. *Am J Transplant* 2016; **16**: 625.
18. Das LK, Ide K, Tanaka A, *et al*. Fc-gamma receptor 3A polymorphism predicts the incidence of urinary tract infection in kidney-transplant recipients. *Hum Immunol* 2017; **78**: 357.
19. Castro-Dopico T, Clatworthy MR. Fcγ receptors in solid organ transplantation. *Curr Transplant Rep* 2016; **3**: 284.
20. Ivan E, Colovai AI. Human Fc receptors: critical targets in the treatment of autoimmune diseases and transplant rejections. *Hum Immunol* 2006; **67**: 479.
21. Ruttens D, Verleden SE, Goeminne PC, *et al*. Genetic variation in immunoglobulin G receptor affects survival after lung transplantation. *Am J Transplant* 2014; **14**: 1672.
22. Paul P, Pedini P, Lyonnet L, *et al*. FCGR3A and FCGR2A genotypes differentially impact allograft rejection and patients' survival after lung transplant. *Front Immunol* 2019; **10**: 1208.
23. Verleden GM, Glanville AR, Lease ED, *et al*. Chronic lung allograft

dysfunction: definition, diagnostic criteria, and approaches to treatment – a consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* 2019; **38**: 493.
24. Kayawake H, Chen-Yoshikawa TF, Aoyama A, *et al*. Surgical management of bronchial stumps in lobar lung transplantation. *J Thorac Cardiovasc Surg* 2018; **156**: 451.
25. Yamanashi K, Chen-Yoshikawa TF, Hamaji M, *et al*. Outcomes of combination therapy including rituximab for antibody-mediated rejection after lung transplantation. *Gen Thorac Cardiovasc Surg* 2020; **68**: 142.
26. Ohata K, Chen-Yoshikawa TF, Takahashi K, *et al*. Cytomegalovirus infection in living-donor and cadaveric lung transplantations. *Interact Cardiovasc Thorac Surg* 2017; **25**: 710.
27. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452.
28. Kyogoku C, Dijstelbloem HM, Tsuchiya N, *et al*. Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum* 2002; **46**: 1242.
29. Moriya H, Saito K, Helsby N, *et al*. Single-nucleotide polymorphisms and copy number variations of the FCGR2A and FCGR3A genes in healthy Japanese subjects. *Biomed Rep* 2014; **2**: 265.
30. Iwasaki M, Shimada N, Kasuga Y, *et al*. Fragment c gamma receptor gene polymorphisms and breast cancer risk in case-control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians. *Breast Cancer Res Treat* 2011; **126**: 497.
31. Sarmiento E, Cifrian J, Calahorra L, *et al*. Monitoring of early humoral immunity to identify lung recipients at risk for development of serious infections: a multicenter prospective study. *J Heart Lung Transplant* 2018; **37**: 1001.
32. Miyoshi R, Chen-Yoshikawa TF, Hijiya K, *et al*. Significance of single lung transplantation in the current situation of severe donor shortage in Japan. *Gen Thorac Cardiovasc Surg* 2016; **64**: 93.
33. Venuta F, Boehler A, Rendina EA, *et al*. Complications in the native lung after single lung transplantation. *Eur J Cardiothorac Surg* 1999; **16**: 54.
34. King CS, Khandhar S, Burton N, *et al*. Native lung complications in single-lung transplant recipients and the role of pneumonectomy. *J Heart Lung Transplant* 2009; **28**: 851.
35. Endeman H, Cornips MC, Grutters JC, *et al*. The Fcgamma receptor IIA-R/R131 genotype is associated with severe sepsis in community-acquired pneumonia. *Clin Vaccine Immunol* 2009; **16**: 1087.
36. Sanders LA, van de Winkel JG, Rijkers GT, *et al*. Fc gamma receptor IIa (CD32) heterogeneity in patients with recurrent bacterial respiratory tract infections. *J Infect Dis* 1994; **170**: 854.
37. Yee AM, Phan HM, Zuniga R, Salmon JE, Musher DM. Association between FcgammaRIIa-R131 allotype and bacteremic pneumococcal pneumonia. *Clin Infect Dis* 2000; **30**: 25.
38. Moalli F, Doni A, Deban L, *et al*. Role of complement and Fc{gamma} receptors in the protective activity of the long pentraxin PTX3 against Aspergillus fumigatus. *Blood* 2010; **116**: 5170.
39. Paul P, Picard C, Sampol E, *et al*. Genetic and functional profiling of CD16-dependent natural killer activation identifies patients at higher risk of cardiac allograft vasculopathy. *Circulation* 2018; **137**: 1049.
40. Date H, Sato M, Aoyama A, *et al*. Living-donor lobar lung transplantation provides similar survival to cadaveric lung transplantation even for very ill patients. *Eur J Cardiothorac Surg* 2015; **47**: 967.
41. Le Pavec J, Suberbielle C, Lamrani L, *et al*. De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. *J Heart Lung Transplant* 2016; **35**: 1067.
42. Visentin J, Chartier A, Massara L, *et al*. Lung intragraft donor-specific antibodies as a risk factor for graft loss. *J Heart Lung Transplant* 2016; **35**: 1418.
43. Gochi F, Chen-Yoshikawa TF, Kayawake H, *et al*. Comparison of de novo donor-specific antibodies between living and cadaveric lung transplantation. *J Heart Lung Transplant* 2021; **40**: 607.
44. Kayawake H, Chen-Yoshikawa TF, Gochi F, *et al*. Postoperative outcomes of lung transplant recipients with preformed donor-specific antibodies. *Interact Cardiovasc Thorac Surg* 2021; **32**: 616.