Figure 1. Establishment of human iPSCs from $CD34^+$ cord blood cells.
(A) The iPSC induction protocol. Frozen $CD34^+$ cells were thawed and cultured in αMEM supplemented with FCS and cytokines. The cells were then transfected with the episomal vector mixture, followed by additional culture for 2 to 5 days, and were plated on 6 well plates covered with feeder cells. The culture medium was gradually changed to ESC medium supplemented with bFGF and a Rock inhibitor. The iPSC colonies were counted and picked up for expansion around days 17 to 26. (B and C) Phase contrast images of an iPSC colony and established cells. Bar = 1 mm in B and 50 μm in C. (D – F) Teratoma formation. iPSCs derived from $CD34^+$ cord blood cells (clone 604B-1) were transplanted into immunodeficient mice. After eight weeks, tumors were sectioned and stained with haematoxylin and eosin. Shown are neural tissues (D), gut-like epithelial tissues (E), and cartilage (F). Scale bar = 100 μm.

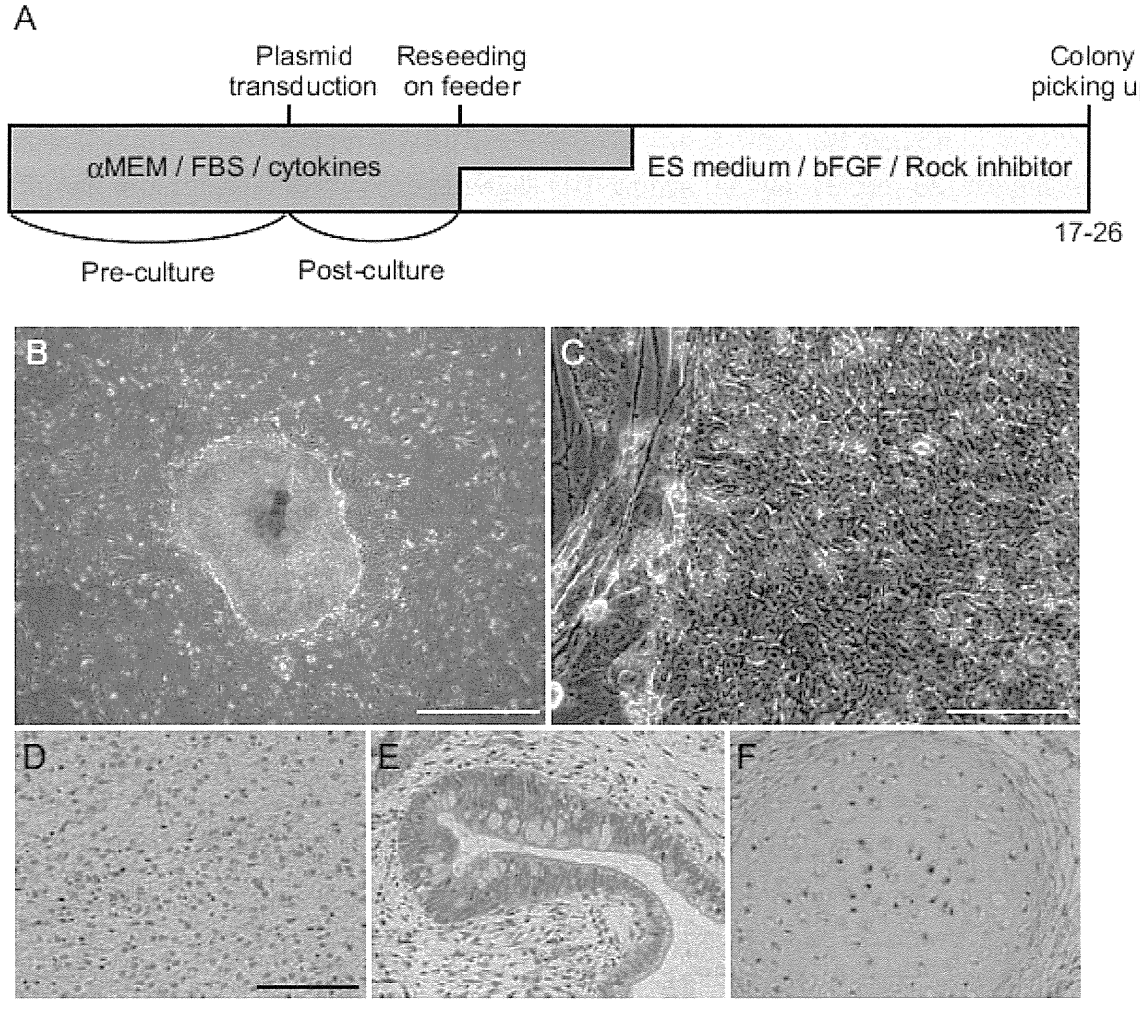

Figure 2. Establishment of human iPSCs from PMNCs.

(A) The iPSC induction protocol. On day 0, PMNCs were isolated by gradient centrifusion, transfected with the episomal vector mixture, and were plated on 6 well plates covered with MEF feeder cells. The cells were cultured in medium specific for different cell types. For example, the culture medium containing IL-2 and anti-CD3 and CD28 antibodies was used for T cell stimulation. On day 2, the medium was diluted with the same volume of ESC medium supplemented with bFGF and a Rock inhibitor, followed by complete replacement on day 4. iPSC colonies were counted and picked up for expansion around days 16 to 25. (B and C) Phase contrast images of an iPSC colony (B) and an established iPSC (C). Bar = 1 mm in B and 50 μm in C. (D – F) Teratoma formation. iPSCs (clone 604B1) were transplanted into immunodeficient mice. After eight weeks, tumors were sectioned and stained with haematoxylin and eosin. Shown are neural tissues (D), gut-like epithelial tissues (E), and cartilage (F). Scale bar = 100 μm. (G) The results of the Southern blot analyses of the TRB locus. Genomic DNA (6 μg) was extracted from human ES cells (KhES3) and iPSCs, digested with Hind III, and analyzed for V(D)J rearrangements by a Southern blot analysis using a probe for the TRB locus. The open arrowheads indicate bands derived from the germline allele. The iPSCs were established in medium for non-T cells (NTm) or for T cells (Tm) from two different donors. The iPS clones (585A1, 585B1, 604A1, and 604B1) showed genomic rearrangement in the TRB locus.

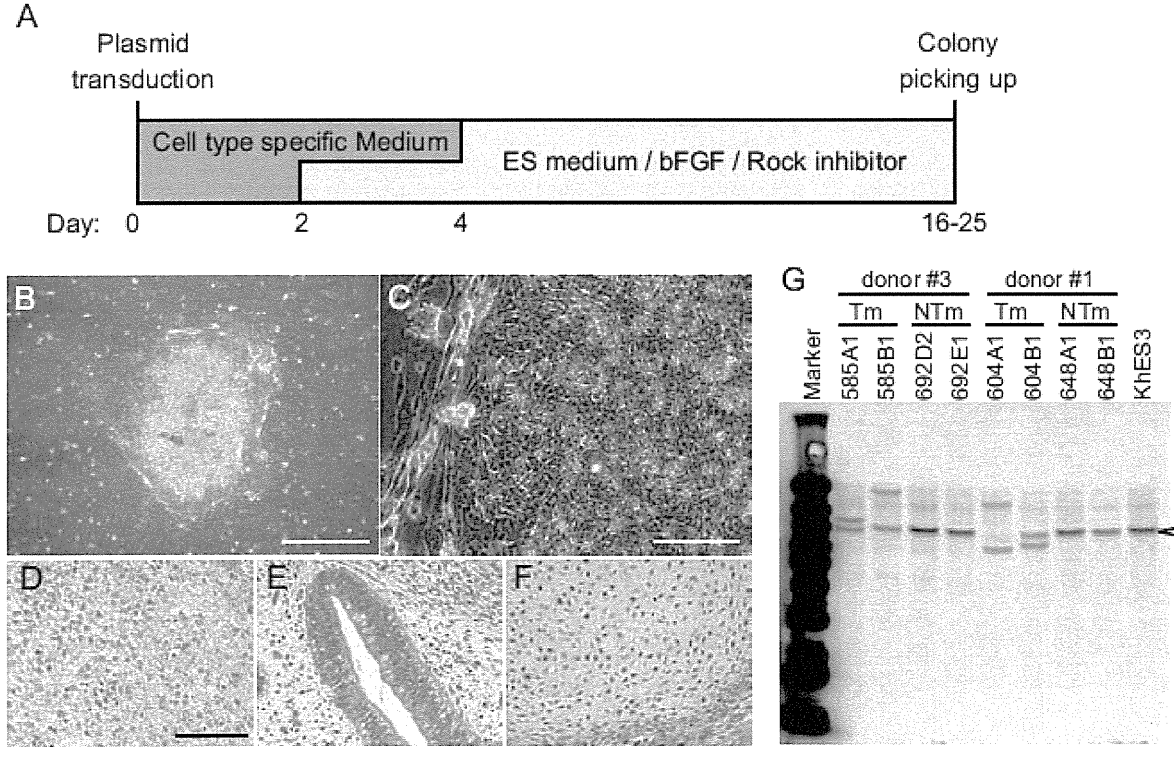

Figure 3. Improvement of the reprogramming efficiency by extra EBNA1
(A and B) The efficiency of iPSC generation from PMNCs cultured in the medium for T cells (A) and non-T cells (B) with GLIS1. The data are shown as the means ± standard deviation of ESC-like colony numbers obtained from at least 3 independent experiments. (C) The copy numbers of episomal vectors that remained in the iPSC clones. The numbers in parentheses indicate the passage numbers of each clone. Also shown are the estimated numbers of cells analyzed for each clone. The PMNCs collected 4 days after electroporation of the Y4 (Y4-elepo) or Y4 plus EBNA1 vector (Y4E-elepo) combination were analyzed as positive controls. The tissue origin (cord blood (CB), PMNC, and fibroblast (HDF)), and vectors used for iPSC induction are shown below. (D and E) The efficiency of iPSC generation from the PMNCs from 7 donors in the medium for T cells (D) or non-T cells (E) with the additional EBNA1 vector. The donor number, age, and sex are indicated. M, male; F, female.

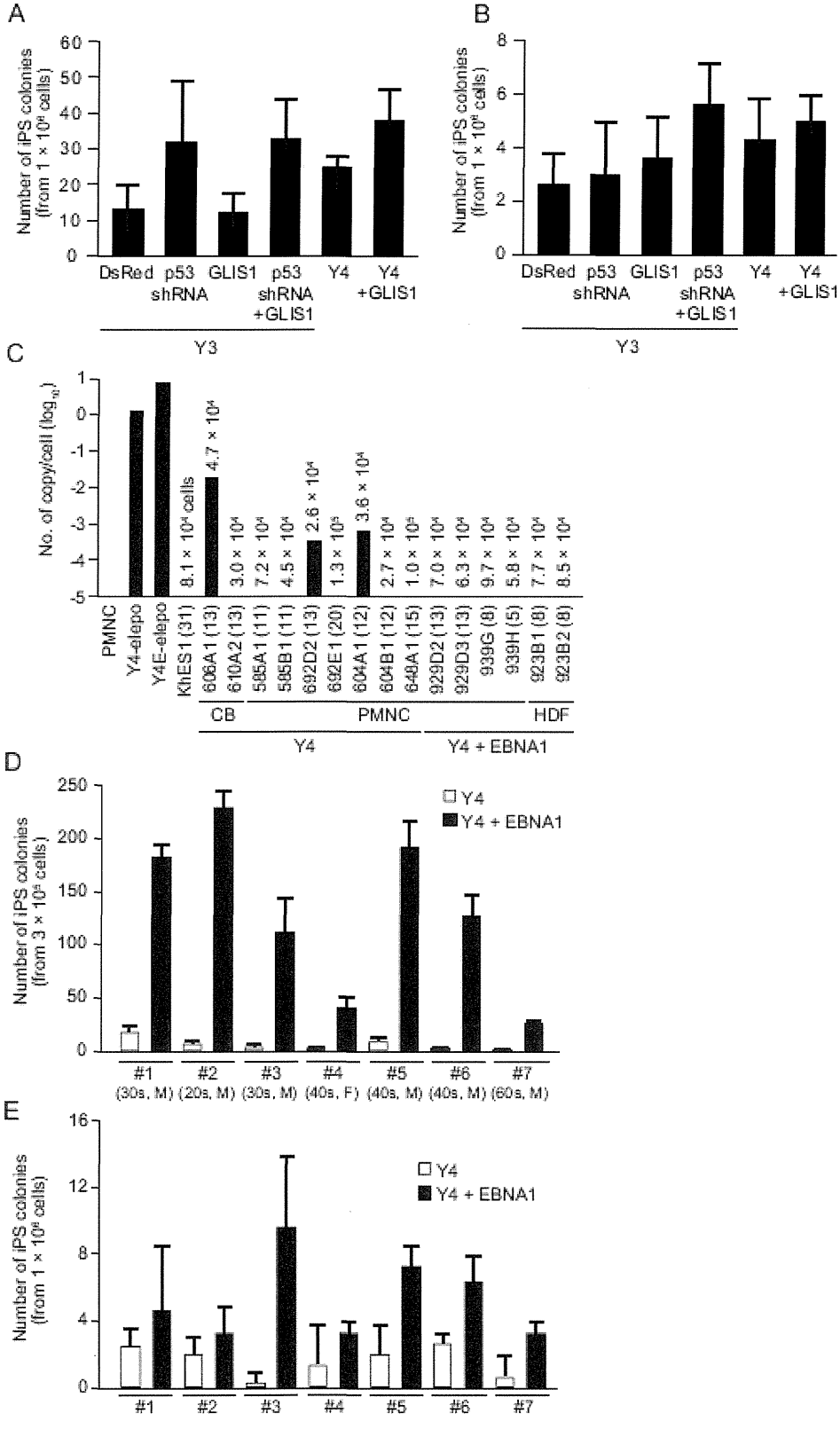
A
Number of iPS colonies (from 1 × 10⁴ cells)
DsRed
p53 shRNA
GLIS1
p53 shRNA +GLIS1
Y4
Y4 +GLIS1
Y3
B
Number of iPS colonies (from 1 × 10⁴ cells)
DsRed
p53 shRNA
GLIS1
p53 shRNA +GLIS1
Y4
Y4 +GLIS1
Y3
C
No. of copy/cell (log10)
PMNC
Y4-elepo
Y4E-elepo
KhES1 (31)
606A1 (13)
610A2 (13)
585A1 (11)
585B1 (11)
692D2 (13)
692E1 (20)
604A1 (12)
604B1 (12)
648A1 (15)
929D2 (13)
929D3 (13)
939G (8)
939H (5)
923B1 (8)
923B2 (8)
8.1 × 10⁴ cells
4.7 × 10⁴
3.0 × 10⁴
7.2 × 10⁴
4.5 × 10⁴
2.6 × 10⁴
1.3 × 10⁵
3.6 × 10⁴
2.7 × 10⁴
1.0 × 10⁵
7.0 × 10⁴
6.3 × 10⁴
9.7 × 10⁴
5.8 × 10⁴
7.7 × 10⁴
8.5 × 10⁴
CB
PMNC
HDF
Y4
Y4 + EBNA1
D
Number of iPS colonies (from 3 × 10⁵ cells)
Y4
Y4 + EBNA1
#1 (30s, M)
#2 (20s, M)
#3 (30s, M)
#4 (40s, F)
#5 (40s, M)
#6 (40s, M)
#7 (60s, M)
E
Number of iPS colonies (from 1 × 10⁴ cells)
Y4
Y4 + EBNA1
#1
#2
#3
#4
#5
#6
#7

Table 1. iPSC induction efficiency from PMNCs obtained from donor #1

		Plasmid mixtures and conditions																														
Medium[a]	Cell number ($\times 10^5$)	C1 (n=3)						T1 (n=3)						T2 (n=6)						Y3 (n=3)						Y3 + EBNA1 (n=3)						Y4 (n=6)						Y4 + EBNA1 (n=3)						Y4 + EBNA1 with frozen PMNCs (n=3)					
NTm	10	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				0.7	±	0.6				1.3	±	1.2				2.5	±	1.0				4.7	±	3.8				9.7	±	2.1		
	3	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				0.7	±	0.6				0.7	±	0.8				4.3	±	2.1				2.3	±	1.2		
Tm	10	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				15.7	±	8.1				51.7	±	64.4				42.2	±	8.4				ND[b]						ND[b]				
	3	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				6.0	±	3.5				9.0	±	9.6				18.8	±	6.1				184.0	±	11.3				241.0	±	77.7		
	1	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				2.7	±	2.1				5.3	±	3.1				9.5	±	2.7				82.7	±	11.6				101.0	±	26.5		
	0.3	0.0	±	0.0				0.0	±	0.0				0.0	±	0.0				0.3	±	0.6				0.7	±	1.2				1.2	±	0.8				19.3	±	9.6				31.3	±	12.9		

Note; [a]NTm, medium for non-T cell populations; Tm, medium for T cells. [b]ND, not determined because too many colonies had formed.

Table 2. iPS induction efficiency from 6 other donors.

Factor	Medium[a]	Cell number ($\times 10^5$)	iPS colony number (n=3) Donor#2 (20s, M)	Donor#3 (30s, M)	Donor#4 (40s, F)	Donor#5 (40s, M)	Donor#6 (40s, M)	Donor#7 (60s, M)
Y4	NTm	10	2.0 ± 1.0	0.3 ± 0.6	1.3 ± 2.3	2.0 ± 1.7	2.7 ± 0.6	0.7 ± 1.2
		3	0.7 ± 0.6	0.7 ± 0.6	0.0 ± 0.0	0.3 ± 0.6	1.0 ± 1.0	0.3 ± 0.6
	Tm	10	15.3 ± 1.5	6.3 ± 0.6	4.0 ± 1.7	17.3 ± 3.1	10.3 ± 5.7	5.0 ± 1.7
		3	7.7 ± 2.3	4.0 ± 2.0	3.3 ± 0.6	9.3 ± 3.5	2.7 ± 0.6	0.3 ± 0.6
		1	3.3 ± 2.1	1.3 ± 1.5	0.7 ± 0.6	6.0 ± 3.6	3.3 ± 1.5	2.3 ± 1.2
		0.3	1.0 ± 1.0	0.3 ± 0.6	0.7 ± 1.2	1.7 ± 2.1	0.0 ± 0.0	0.3 ± 0.6
Y4+EBNA1	NTm	10	3.3 ± 1.5	9.7 ± 4.2	3.3 ± 0.6	7.3 ± 1.2	6.3 ± 1.5	3.3 ± 0.6
		3	2.7 ± 0.6	4.0 ± 2.0	0.3 ± 0.6	2.7 ± 0.6	3.3 ± 3.2	1.0 ± 1.0
	Tm	10	ND[b]	ND[b]	116.7 ± 9.5	ND[b]	ND[b]	113.0 ± 19.1
		3	230.3 ± 15.2	113.0 ± 32.7	41.0 ± 10.4	192.7 ± 25.1	127.7 ± 19.4	27.0 ± 1.7
		1	100.3 ± 8.0	42.3 ± 15.5	21.3 ± 9.5	62.3 ± 19.5	47.3 ± 7.8	16.0 ± 7.0
		0.3	36.7 ± 22.9	10.7 ± 1.5	3.3 ± 2.1	20.0 ± 4.6	11.3 ± 2.5	6.7 ± 0.6

Note; [a]NTm, medium for non-T cell populations; Tm, medium for T cells. [b]ND, not determined because too many colonies formed. The donor age and sex are indicated. M, male; F, female.

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ORIGINAL ARTICLE

Prevalence and characteristics of unilateral knee osteoarthritis in a community sample of elderly Japanese: do fractures around the knee affect the pathogenesis of unilateral knee osteoarthritis?

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Abstract
Background The purpose of this study was to investigate the prevalence and characteristics of unilateral knee osteoarthritis (KOA), to investigate what percent of contralateral healthy knees in patients with unilateral KOA progress to KOA, and to investigate whether knee fractures influence unilateral KOA.
Methods Studies were performed every two years from 1997 to 2009 in Miyagawa village, for a total of seven studies. A total of 1239 village inhabitants aged ≥65 years participated in these studies at least once. KOA was defined as a Kellgren–Lawrence (K/L) grade ≥2. Based on the knee X-ray at the first examination, participants were divided into three groups: no KOA (N group), unilateral KOA (U group), and bilateral KOA (B group). The U group was divided into two subgroups: K/L grade II-I combination (II-I group), and the U group without the II-I combination (G>2 group). To investigate whether knee fractures influence unilateral KOA, the fracture history was considered.
Results The percentages of participants classified into the N, B, and U groups (II-I and G>2 group) were 68.4, 21.6, and 10.0 % (7.8 and 2.1 %), respectively. Most of the U group had the II-I combination (78.7 %). The percentages of knee fractures in the N, B, II-I, and G>2 groups were 3.3, 5.3, 6.3, and 38.5 %, respectively. Overall, 49.2 % of the U group proceeded to bilateral KOA over an average of 5.3 years.
Conclusions The prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 %, respectively. Overall, 49.2 % of the participants with unilateral KOA developed KOA in the contralateral knee over an average of 5.3 years. If bilateral KOA advanced simultaneously, the II-I group was considered to represent the midpoint of progression to bilateral KOA. Bilateral KOA advanced simultaneously except in cases with a history of knee trauma, such as fractures.

Introduction

Osteoarthritis of the knee (KOA) is the most common form of arthritis leading to pain and loss of function in older adults [1]. It is well known that Japan has an aging population, and thus the prevalence of Japanese patients with KOA is increasing [2]. Several studies have described the prevalence of KOA as well as various risk factors and their associations with KOA [3–10]. However, in most previous studies, information on only one knee—the knee with the worst grade—was recorded. There have been only a few studies where each knee grade was recorded, meaning that the natural history of the contralateral knee is poorly understood [11, 12]. In clinical practice, patients with unilateral knee pain are frequently encountered. If only the knee with the worst grade is X-rayed, then the condition of the contralateral knee can only be assumed. For clinicians to treat KOA, it is important to know the prevalences of unilateral and bilateral KOA in order to satisfy both the

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physical and psychological needs of the patients. The possibility and predicted speed of KOA progression in the contralateral knee is important, as it may influence therapeutic approaches. A further reason for obtaining the estimated rates of progression is to estimate sample sizes when planning clinical trials. To the best of our knowledge, only one study [11] has estimated the rate of incidence of KOA in the contralateral knee in a population-based cohort study.

The purpose of this study was to investigate the prevalence and characteristics of unilateral KOA, to investigate what percent of the contralateral healthy knees in the unilateral KOA group progressed to KOA, and to investigate whether knee fractures influence unilateral KOA.

Materials and methods

The Miyagawa cohort study, a population-based study, began in 1997 in Miyagawa, a mountain village located in the center of Mie Prefecture, Japan. Participants were self-recruited, community-dwelling volunteers who were ≥65 years old. Studies were performed every two years from 1997 to 2009 at Houtoku Hospital in the village, for a total of seven studies. The population of the village was 4196 in 1997, when 1463 of the residents met the age criterion. The population dropped to 3490 in 2010, at which time 1553 individuals met the age criterion. A total of 1239 inhabitants (786 women and 453 men) participated in these studies at least once. The Committee for the Ethics of Human Research of Mie University approved the study protocol, and all participants provided their written informed consent before study enrollment. Using data from the Miyagawa cohort study, individuals who were found at a baseline screening examination to have unilateral KOA were investigated.

Baseline data obtained from standard questionnaires administered by orthopedic surgeons included information regarding age, sex, medical history, knee fracture history, and knee pain. Knee fractures were defined as including the patella, distal femur, and proximal tibia (such as the tibial plateau). Knee pain was determined from the question, "Have you experienced knee pain lasting for over one month during the past year?" Knee pain was recorded as absent, unilateral, or bilateral. Height and weight were measured, and the body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

All participants had anteroposterior (AP) radiographs of both knees in the fully extended position with the same equipment. These radiographs were scored blind to clinical details according to the Kellgren–Lawrence (K/L) grading system [13] that uses the following grades: 0, normal; 1, possible osteophytes only; 2, definite osteophytes and possible joint space narrowing; 3, moderate osteophytes and/or definite joint space narrowing; and 4, large osteophytes, severe joint space narrowing, and/or bony sclerosis. Confirmed radiographic KOA was defined as a K/L grade of ≥2. All knee radiographs were independently evaluated by three orthopedists, and the final score was reached by consensus among two or three of the orthopedists, but the median score was accepted when the opinions of all three differed. Ankylosing spondylitis was not identified in any of the participants. Knees that had undergone total knee replacements (TKA) were defined as having KOA.

The following factors were examined. Firstly, based on the first knee X-ray examination for each participant, the participants were classified into three groups: no OA (N group), unilateral KOA (U group), and bilateral KOA (B group). Moreover, the U group was classified into two subgroups: K/L grade II-I combination (II-I group), and the U group without the II-I combination (G>2 group; participants in this group had one knee grade that differed by two or more grades from that of the other knee). These groups were compared in terms of physical characteristics such as age, sex, height, weight, BMI, and knee pain. Secondly, to investigate whether knee fractures influenced unilateral KOA, the percent of participants with a knee fracture history was examined in each group. Knee fractures were defined as patellar fracture, tibial plateau fracture, and/or distal femur fracture. Thirdly, the natural history of the contralateral (healthy) side knee was examined in the U group. The subjects of this series were participants in the U group who had participated in the examinations at least twice. Changes in the contralateral (healthy) side knee K/L grade U group over 2–12 years were recorded, and we determined whether the KOA had changed (K/L grade ≥2). Moreover, the incidence of KOA was compared between those with and without a history of knee fracture.

Statistical analysis

Mean ± standard deviations (SD) were calculated for variables unless otherwise noted. Associations among the physical characteristics among the groups were determined by the unpaired *t* test. The relationships between KOA and knee fractures were analyzed using age, sex and BMI-adjusted logistic regression analyses with the Bonferroni correction. The change in the contralateral (healthy) side knee of the U group was analyzed by Kaplan–Meier analysis with the log-rank test. The significance level for entry into the model was 0.05. All data were analyzed using the PASW Statistics (version 18) software package (SPSS, Chicago, IL, USA).

Table 1 Characteristics of the participants with no knee osteoarthritis, bilateral knee osteoarthritis, and unilateral knee osteoarthritis

	N group (n = 837)	B group (n = 264)	U group (n = 122)	
			II-I group (n = 96)	G>2 group (n = 26)
Age	71.0 ± 6.6	73.5 ± 7.5*	72.4 ± 6.9	73.8 ± 5.5**
Sex (female/male)	480/357	210/54*	70/26*	15/11††
Height (cm)	152.1 ± 8.3	149.6 ± 8.5*	149.1 ± 8.3*	151.9 ± 9.7
Weight (kg)	53.2 ± 19.2	55.6 ± 10.1**	52.9 ± 9.0††	55.7 ± 11.8
BMI (kg/m^2)	22.9 ± 8.5	24.8 ± 3.6*	23.7 ± 3.2††	23.9 ± 3.2
Knee pain (−/+/++)	589/147/100	79/69/116*	39/32/25*†	11/9/6**

Knee pain defined as: −, absent; +, unilateral; ++, bilateral

N group no knee osteoarthritis group, *B group* bilateral knee osteoarthritis group, *U group* unilateral knee osteoarthritis group, *BMI* body mass index

*$p < 0.01$ versus N group, **$p < 0.05$ versus N group, †$p < 0.01$ versus B group, ††$p < 0.05$ versus B group

Table 2 Distribution of Kellgren–Lawrence grades at baseline in the unilateral knee osteoarthritis group

K/L grade	II-0	II-I	III-0	III-I	IV-0	IV-I	TKA-0	TKA-I
Participants	8	96	2	13	0	2	0	1
%	6.6	78.7	1.6	10.7	0.0	1.6	0.0	0.8

OA osteoarthritis, *K/L grade* Kellgren–Lawrence grade, *TKA* total knee arthroplasty

Results

Of the 1239 participants who attended at least one of the seven examinations associated with this study, 16 patients with rheumatoid arthritis were excluded, and a total of 1223 villagers fulfilled the study criteria.

Table 1 shows the physical characteristics of the four groups. The percentages of participants who were classified into the N, B, and U groups (II-I and G>2 groups) were 68.4, 21.6, and 10.0 % (7.8 and 2.1 %), respectively. The B group differed significantly from the N group in terms of age, sex, height, weight, BMI, and knee pain. The II-I group differed significantly from the N group in terms of sex, height, and knee pain. The G>2 group differed significantly from the N group in terms of age and knee pain. The II-I group differed significantly from the B group in terms of weight, BMI, and knee pain. There was a significant difference in sex between the G>2 and B groups.

Table 2 shows the distribution of K/L grades at baseline for the U group. Most of the U group had the II-I combination (78.7 %), followed by the III-I combination (10.7 %), and then the II-0 combination (6.6 %). Only one participant had TKA (TKA-I combination).

Figure 1 shows the relationship between knee fracture and group. There were 28, 14, 6, and 10 participants with a knee fracture history in the N, B, II-I, and G>2 groups, respectively. The percentages of knee fractures were 3.3, 5.3, 6.3, and 38.5 % in the N, B, II-I, and G>2 groups, respectively. The G>2 group experienced more knee fractures than the N, B, and II-I groups ($p < 0.01$).

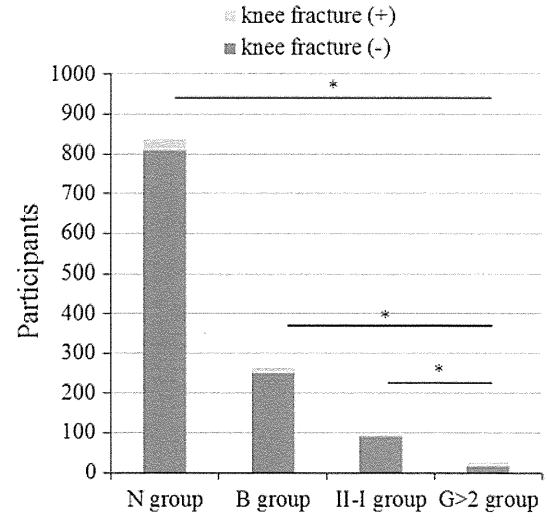

Fig. 1 Relationship between knee fracture and knee osteoarthritis. *N group* no knee osteoarthritis group, *B group* bilateral knee osteoarthritis group, *U group* unilateral knee osteoarthritis group. *$p < 0.01$ versus G>2 group

Table 3 shows the natural K/L grade history of the knees in the U group over 2–12 years (average, 5.3 ± 3.2 years). A total of 65 participants in the U group participated in the examination at least twice. The percentages of participants in whom the contralateral knee had changed to KOA (K/L grade ≥2) were 33.3 % (1/3), 54.7 % (29/53), 0 % (0/2), 40 % (2/5), 0 % (0/1), and 0 % (0/1) in the II-0, II-I, III-0, III-I, IV-0, and TKA-I groups, respectively. Thus, 32 of 65 participants (49.2 %) proceeded to bilateral KOA. Figure 2 shows the change in the contralateral (healthy) knee in the

Table 3 Natural history of bilateral knees in the unilateral knee osteoarthritis group

Baseline K/L grade combination	Follow-up K/L grade combination										Total
	II-0	II-I	II-II	III-I	III-II	III-III	IV-0	IV-I	IV-IV	TKA-I	
II-0	1	1			1						3
	(4.0)	(2.0)			(12.0)						(6.0)
II-I		21	17	3	4	7			1		53
		(4.4)	(5.3)	(6.0)	(5.5)	(8.0)			(12.0)		(5.5)
III-0				1			1				2
				(4.0)			(2.0)				(3.0)
III-I				2	1	1		1			5
				(5.0)	(4.0)	(8.0)		(2.0)			(4.8)
IV-0											0
IV-I								1			1
								(2.0)			(2.0)
TKA-0											0
TKA-I										1	1
										(2.0)	(2.0)

The numbers in parentheses are the average numbers of follow-up years

K/L grade Kellgren–Lawrence grade, *OA* osteoarthritis, *TKA* total knee arthroplasty

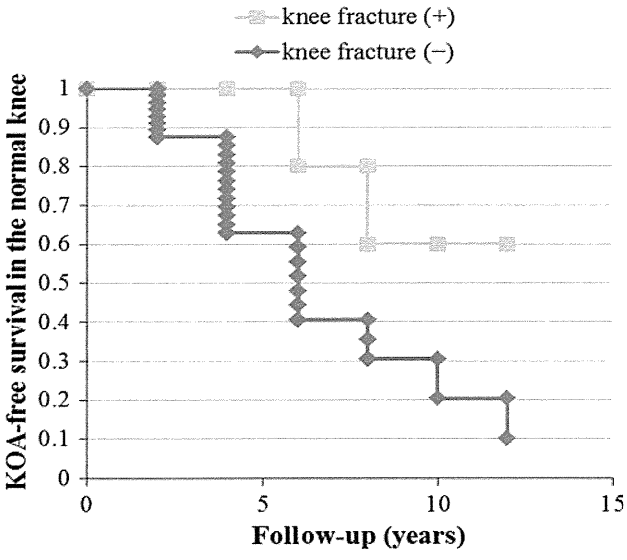

Fig. 2 The prognosis for the opposite healthy side in the unilateral knee group. The censored data in this Kaplan–Meier analysis are the normal knees (Kellgren–Lawrence grade 0 or 1). There was no significant difference between knee fracture (+) and knee fracture (−) based on the log-rank test

U group (normal knee or KOA) on Kaplan–Meier analysis and compares KOA between those with and without a history of knee fracture. There was no significant difference between those with and without a history of knee fracture based on the log-rank test.

Discussion

The present study found that the prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 % in older Japanese villagers. The present data also show that, in participants with unilateral KOA, a high percentage (49.2 %) developed OA in the contralateral healthy knee within an average of 5.3 years (range 2–12 years). A history of knee fractures had a stronger influence on the unilateral KOA group (except for those with the II-I combination) than the no KOA and bilateral KOA groups.

Davis et al. [14] reported that bilateral KOA was more than twice as prevalent as unilateral KOA in the National Health and Nutrition Examination Survey I sample. The present data showed that the B group was twice as large as the U group, so the present findings support their data. Spector et al. [11] reported that 34 % of women with unilateral KOA progressed to contralateral OA within two years. Spector et al. [14] also reported that 92 % of patients with unilateral KOA developed bilateral KOA over 11 years. The present data showed that 49.2 % participants with unilateral KOA developed bilateral KOA within an average of 5.3 years (range 2–12 years). This rate is similar to previously reported rates of 34 % for 2 years [11] and 92 % for 11 years [15]. McMahon et al. [16] reported that, in their TKA series, the percentages of bilateral KOA and unilateral KOA were 88.8 and 11.2 %, respectively. They also reported that the ten-year risk of undergoing TKA in their study population was 37.2 %, excluding

patients who underwent a contralateral TKA at the time of index surgery or within six months. Moreover, Hochberg et al. [17] reported that the presence of contralateral KOA was a risk factor for the development of definite KOA in their longitudinal study [adjusted odds ratio (OR) 6.04]. Sayre et al. [18] reported that the OR for having a contralateral knee with K/L >2 was 20.1 compared to a knee without KOA in K/L >2 knees. A contralateral knee joint grade of K/L was strongly associated with a K/L grade in the other knee. These data suggest that, with unilateral KOA, the contralateral healthy knee is at a high risk of developing KOA.

Many cross-sectional studies and a longitudinal study have reported a relationship between knee injury and KOA [14, 19–21]. Rademakers et al. [22] reported that 31 % of patients with a history of tibial plateau fractures developed secondary KOA. Marsh et al. [23] performed a review concerning the importance of anatomic reduction with respect to articular fractures. They also underlined the fact that malalignment after treatment contributes to a poor outcome after tibial plateau fractures. Experimental and observational evidence suggest that knee injuries with irregularity of the articular surface secondary to tibial plateau and distal femoral fractures, as well as angular deformity following fracture of the femoral and tibial shaft, produce increased articular surface stress, thus increasing the risk of subsequent KOA. Davis et al. [14] reported that obesity was a stronger predictor of bilateral osteoarthritis than knee injury was, with an OR of 6.6 for obesity and an OR of 3.5 for right knee injury. However, knee injury was a stronger predictor of unilateral osteoarthritis than obesity was (ORs of 3.4 and 2.4 for obesity in the right and left knee, respectively, and ORs of 16.3 and 10.9 for knee injury in the right and left knee, respectively). They concluded that different pathogenetic processes may exist for unilateral and bilateral KOA.

In the present study, the most common distribution of K/L grades at baseline for the U group was the II-I combination (78.7 %). If bilateral KOA advanced simultaneously, this combination was considered to represent the midpoint of the progression to bilateral KOA. Therefore, the relationship between the U group without the II-I combination (G>2 group) and knee fractures was examined. The G>2 group had far more cases with a knee fracture history than the N, B, and II-I groups. These data suggest that, with no history of knee fracture, the bilateral knee would become worse (and develop KOA) almost simultaneously.

The present study had several potential limitations. Firstly, Miyagawa is a mountain village, and many inhabitants are typically engaged in forestry. Secondly, participants who could attend the hospital were generally healthier than nonparticipants. Thirdly, the knee X-rays were non-weight-bearing, so the K/L grade was underestimated. Therefore, the prevalence of KOA was lower than in other reports from Japan [2, 24]. Fourthly, other traumatic risks for KOA—knee ligament injuries [25, 26] and meniscus injuries [25–27]—were not considered in this study.

Conclusion

The prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 %, respectively, in a group of older Japanese villagers. Overall, 49.2 % of the participants with unilateral KOA developed KOA in the contralateral knee over an average of 5.3 years. A very strong association was found between unilateral KOA (except for the K/L grade II-I combination) and knee fracture. The results of the present study indicate that bilateral KOA advanced simultaneously, except in cases with a history of knee injury, such as fractures.

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Conflict of interest The authors state that they have no conflict of interest.

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Donor Age Affects Liver Regeneration during Early Period in the Graft Liver and Late Period in the Remnant Liver after Living Donor Liver Transplantation

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Abstract

Background The aim of the present study was to evaluate the influence of donor age on liver regeneration and surgical outcomes in recipients and donors.

Patients and methods Among 101 cases of adult-to-adult living donor liver transplantation (LDLT) between March 2002 and March 2011, according to donor age: younger (Y) <50 years of age or older (O) ≥50 years of age, the donors and recipients using right (R) or left (L) graft were divided into groups Y/R ($n = 51$) and O/R ($n = 17$), and groups Y/L ($n = 26$) and O/L ($n = 7$), respectively. Remnant liver volume (RemLV) and graft liver volume (GLV) were estimated by computed tomography (CT) volumetry. A disintegrin and metalloprotease with thrombospondin type I domain 13 (ADAMTS13) activities and von Willebrand factor (vWF) antigen levels were measured as factors reflecting thrombotic microangiopathy.

Results Among the donors, RemLV/total liver volume (TLV) was lower in group O/R than in group Y/R, although there were no significant differences by *t*-test with the Bonferroni correction (rough p value = 0.02 at 6 months and rough p value > 0.05 at 1, 3, and 12 months). Donor age (≥50 years) was independently correlated with impaired remnant liver regeneration at 6 months in right lobe LDLT ($p = 0.04$). Among the recipients, GLV/standard liver volume (SLV) was lower during the first month, although there were no significant differences between the two groups by *t*-test with the Bonferroni correction (rough p value = 0.03 at 1 week and rough p value >0.05 at 2 weeks and 1 and 3 months). Donor age (≥50 years) was independently correlated with impaired graft liver regeneration at 1 week in both right and left lobe LDLT ($p < 0.05$). ADAMTS13 activities were lower in group O/R than in group Y/R, although there were no significant differences by *t*-test with the Bonferroni correction (rough p value = 0.049 on postoperative days (POD) 1 and 28 and rough p value >0.05 on POD 7 and 14). vWF/ADAMTW13 ratios were higher on POD 14, although there were no significant differences between the two groups by *t*-test with the Bonferroni correction (rough p value = 0.044 on POD 14 and rough p value >0.05 on POD 1, 7, 14, and 28).

Conclusions The surgical outcomes using older donor livers for LDLT were comparable to those using younger donor livers. When using older donor livers, however, we should pay attention to the liver volume for recipients as well as donors, because older donor livers might have impaired regenerative ability.

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Introduction

To expand the donor pool for liver transplantation, the use of marginal donors such as steatotic liver, small-for-size graft, graft under prolonged ischemia [1], and older donors has gradually increased. Conflicting outcomes have been reported in the use of older donor liver grafts. In deceased

donor liver transplantation (DDLT), several reports have shown that older donor liver grafts present an increased risk for lower rates of graft and patient survival [2–5]. In these reports, the suggested cut-off ages for older donors range from 40 to 60 years. In contrast, others have reported acceptable outcomes after DDLT with the use of selected older donor grafts as long as they were in good condition [6–9].

In living donor liver transplantation (LDLT), according to the Japanese Liver Transplantation Registry in 2009, the percentages of the donors older than 50 and 60 years were 18.1 and 4%, respectively. It is expected that the number of older donors will increase in the future because of the continuing donor shortage [10]. This registry, however, reported that the 5-year survival rate of the recipients of older donor (age >50) grafts was less than 70%, which was inferior to that for younger donors. In contrast, several reports from a single center showed acceptable graft survival rates with the use of older donor grafts, although the incidence of small-for-size syndrome was higher [11] or graft liver regeneration was slightly impaired [12]. Although it is not well known what kinds of factors in older donors influence recipient outcomes, Selzner et al. [13] have indicated a relationship between increased tumor necrosis factor α (TNFα) release and age-related liver injury. In other studies, it was found that cell cycle disruption caused age-related regenerative defects [14, 15].

A disintegrin and metalloprotease with thrombospondin type I domain 13 (ADAMTS13) is a metalloproteinase that specifically cleaves the multimeric von Willebrand factor (vWF) [16–19]. This enzyme is almost entirely produced by stellate cells in the hepatic sinusoid and endothelial cells, and it has assumed great importance because deficiency of this enzyme results in thrombotic microangiopathy [20, 21]. ADAMTS13 activity significantly decreases after hepatectomy as a result of ischemic injury together with liver mass reduction, reflecting postoperative liver dysfunction [22]. We previously reported that the activity of this enzyme in patients after LDLT was significantly reduced from postoperative day (POD) 1–POD 21 [23]. Recently, Kokame et al. [24] revealed that ADAMTS13 activity tended to decrease with age, especially after age 60, and their report led us to hypothesize that production activity of this enzyme in the liver decreases with age. Therefore, it is expected that ADAMTS13 activity is more significantly decreased in recipients who have received graft livers from older donors than in those who have received grafts from younger donors, and if this is true, decreased activity of this enzyme in the liver may influence liver regeneration.

A major difference between DDLT and LDLT is the donor's safety. In LDLT, we have to have concern for not only the surgical outcome of the recipient but also postoperative course of the donor, especially in the case of older donors. A database analysis of the Japanese liver transplantation society revealed that postoperative complications in the donors occurred in 8.4% [25]. The Kyoto group [26] reported that older donors (age ≥60 years) had significantly prolonged length of hospital stay. However, they did not examine remnant liver regeneration in older donors.

The aim of the present study was to evaluate how donor age affects surgical outcomes and liver regeneration in both donors and recipients, paying special attention to ADAMTS13 activity after LDLT.

Materials and methods

Subjects

We reviewed the database of LDLT at Mie University Hospital, and identified 101 cases with adult-to-adult LDLT using right (R) or left (L) lobe grafts from March 2002 to March 2011. These 101 pairs of donor and recipient (R graft: 68 and L graft: 33) were the subjects of this study. According to donor age: younger (Y) <50 years of age or older (O) ≥50 years of age. The donors and recipients, using R or L grafts, were divided into groups Y/R ($n = 51$) and O/R ($n = 17$), and groups Y/L ($n = 26$) and O/L ($n = 7$), respectively. Additionally, only 3 donors were more than 60 years old.

Donor selection and management

Donor candidates were limited to blood relatives up to the third degree and the spouse or equivalent of the recipient if they manifested a strong desire to donate part of their liver of their own free will. In brief, our clinical criteria for living donors were as follows: healthy individuals between 18 and 65 years of age, no significant medical history, no abnormalities in blood examinations or cardiopulmonary function tests, and no history of viral hepatitis. Preoperative estimation of the graft and remnant liver volume was performed using three-dimensional reconstructed images from multi-detector computed tomography (CT) of the liver, aiming to obtain 0.8% or more graft/recipient weight ratio (GRWR), and 35% (30% between 2002 and 2005) or more remnant liver volume. Donors were usually admitted to the hospital on the day before LDLT, and postoperative management was carried out according to general surgical care. Laboratory tests were performed on PODs 1, 3, 5, 7, and 10. Donors were discharged from the hospital after confirmation of normal liver function and were seen in outpatient clinics 1, 3, 6, and 12 months after discharge to check laboratory tests and condition.

Surgery and postoperative care of the recipients

All LDLT procedures for both donors and recipients were performed according to our previously reported methods [27]. In donors, after the abdomen was opened, a liver specimen was collected to evaluate for hepatic steatosis. For recipients, immunosuppression drugs consisted of tacrolimus and low-dose corticosteroid administration. Serial Doppler ultrasound of the deep veins was performed postoperatively. The immunosuppression protocol and dose of immunosuppressive drugs did not differ between the recipients of older and younger donor groups. The recipients were assessed for changes in liver function test values and postoperative complications during and after the initial hospital stay.

CT volumetry of donor remnant liver and recipient graft liver

Volumetric studies of the liver grafts were conducted with a High-Speed Advantage QX-1 scanner (GE Medical Systems, Tokyo, Japan) and Aquilion 64 (Toshiba Medical Systems, Tokyo, Japan). Preoperative total liver volume (TLV) and postoperative remnant liver volumes (RemLV) of right liver donors were calculated by tracing the liver on each CT image at 1, 3, 6 months and 1 year after LDLT. Graft liver volumes (GLV) of all recipients were calculated at 1 and 2 weeks, and at 1 and 3 months after LDLT. Standard liver volume (SLV) was calculated according to the formula proposed by Urata et al. [28]:

$$\mathrm{SLV(ml)} = 706.2 \times \text{body surface area(BSA)}\left(\mathrm{m}^2\right) + 2.4.$$

Liver regeneration was assessed by RemLV/TLV and GLV/SLV ratios.

The CT value of liver to spleen ratio (L/S) was calculated from measurements of the mean value of CT density on the right and left lobe of the liver and on the anterior and posterior part of the spleen.

Evaluation of the degree of hepatic steatosis

All liver biopsy specimens were examined histologically. The specimens were classified into two groups based on the degree of macrovesicular steatosis observed: none (0% steatosis), minimal (≤10%), and mild (11–30%).

Analysis of ADAMTS13 and vWF

ADAMTS13 activities and vWF levels in the serum were measured in the 81 recipients between 2002 and 2005 in our institution, and the procedure and data were reported previously [23]. Because of a data shortage from recipients of left lobe LDLT, we analyzed the data of 47 recipients of R grafts: 34 from group Y/R and 13 from group O/R.

Statistical analyses

Categorical and continuous data were compared between the groups using chi-squared and Student's *t*-tests, respectively. Student's *t*-test with the Bonferroni correction was used to compare volumetric data and postoperative laboratory data (ADAMTS13 activity and the vWF/ADAMTS13 ratio) between groups at each observation. Multiple linear regression was performed to evaluate the impact of donor age on liver regeneration. Recipient survival was compared using the log-rank test. Differences were considered significant at $p < 0.05$. Results of measured variables were expressed as mean values ± standard deviation.

Results

Right lobe LDLT

Donors

As shown in Table 1, there were no significant differences in the characteristics and surgical outcomes between the two groups, except for the male to female ratio. The incidences of total steatosis and mild steatosis were significantly higher in group O/R than in group Y/R: 47.1% versus 19.6% ($p = 0.03$), 41.2% versus 13.0% ($p = 0.014$), respectively.

Changes in donor remnant liver regeneration after LDLT are shown in Figure 1a. The RemLV/TLV ratio at 6 months was lower in group O/R than in group Y/R, although there were no statistical differences between the two groups by *t*-test with the Bonferroni correction (rough p value = 0.02 at 6 months, and rough p value >0.05 at 1, 3, and 12 months). As a result of multiple regression analysis including the three factors (donor age, steatosis, and donor gender) that were suggested to have statistical significance in univariate analysis, donor age (≥50 years old) was independently correlated with remnant liver regeneration at 6 months ($p = 0.04$) (Table 2a).

Recipients

Background characteristics of the recipients are summarized in Table 3. There were no significant differences in the characteristics between the two groups except for the male to female ratio. The ascites output on postoperative day 14 was significantly more increased in group O/R than in group Y/R: 2,309 ± 3,808 ml versus 664 ± 1,919 ml

Table 1 Characteristics and surgical outcomes of donors in right lobe living donor liver transplant (LDLT)

	Group Y/R ($n = 51$)	Group O/R ($n = 17$)	p Value
Age, years (median)	18–49 (33)	50–62 (55)	
Gender, male:female	25:26	3:14	0.046
BMI	21	23	N.S.
L/S	1.2	1	N.S.
Operative time, min	383 ± 74	357 ± 67	N.S.
Intraoperative blood loss, ml	754 ± 414	677 ± 341	N.S.
Actual graft weight, g	677 ± 124	640 ± 109	N.S.
Actual GRWR	1.08 ± 0.21	1.03 ± 0.15	N.S.
Donor wedge biopsy (steatosis)	9 /46 (19.6%)	8 /17 (47.1%)	0.03
Minimal (<10%)	3/46 (6.5%)	1/17 (5.9%)	N.S.
Mild (10–30%)	6/46 (13.0%)	7/17 (41.2%)	0.014
Minimum albumin, mg/dl	2.8 ± 0.3	2.7 ± 0.2	N.S.
Maximum total bilirubin, mg/dl	3.0 ± 1.8	2.8 ± 2.3	N.S.
Max PT-INR	1.47 ± 0.2	1.43 ± 0.2	N.S.
Postoperative complications	5 (9.8%)	2 (11.7%)	N.S.
Biliary leakage	3	0	N.S.
Fluid collection	0	1	N.S.
Duodenal ulcer	1	0	N.S.
Bleeding	0	1	N.S.
FUO	1	0	N.S.
Hospital stay, days	16 ± 5	15 ± 5	N.S.

N.S. not significant; *BMI* body mass index; *L/S* the liver to spleen computed tomography (CT) ratio; *GRWR* graft to recipient weight ratio; *PT-INR* prothrombin time international normalized ratio; *FUO* fever of unknown origin

($p = 0.03$). There were no significant differences in the rates of postoperative complications between the two groups.

The GLV/SLV ratios were lower in group O/R than in group Y/R during the first month after LDLT, although there were no statistical differences between the two groups by t-test with the Bonferroni correction (rough p value = 0.03 at 1 week, and rough p value >0.05 at 2 weeks, 1 month, and 3 months) (Fig. 1b). As a result of multiple regression analysis including the three factors (donor age, graft steatosis, and recipient gender) that were suggested to have statistical significance in univariate analysis, donor age (≥50 years) was independently correlated with graft liver regeneration at 1 week ($p = 0.02$) (Table 2b). There were no significant differences in patient survival between the two groups. The 1-, 3-, and 5-year cumulative survival rates were 80, 73, and 70% in group Y/R, and 77, 71, and 71% in group O/R, respectively. Because hepatitis C virus (HCV) infection and donor age are known to affect recipient survival, we compared the survival rates in HCV-positive recipients between group Y/R ($n = 22$) and group O/R ($n = 6$): the 1-, 3-, and 5-year cumulative survival rates were 75, 65, and 59% in group Y/R, and 67, 50, and 50% in group O/R, respectively, showing no significant differences between the two groups.

ADAMTS 13 activities were lower in group O/R than in group Y/R at all time points during the first month after LDLT, although there were no significant differences by t-test with the Bonferroni correction (rough p value = 0.049 on POD 1 and = 0.049 on POD 28 and rough p value >0.05 on POD 7 and 14) (Fig. 2a). The vWF/ADAMTS13 ratio on POD 14 was higher in group O/R than in group Y/R, although there were no significant differences by t-test with the Bonferroni correction (rough p value = 0.044 on POD 14 and rough p value >0.05 on POD 1, 7, and 28) (Fig. 2b). Postoperative platelet counts were not significantly different between the two groups (Fig. 3).

Left lobe LDLT

Donors

As shown in Table 4, there were no significant differences in the characteristics and surgical outcomes between the two groups except for the male to female ratio and the L/S ratio. We could not evaluate RemLV/TLV because CT scans were not routinely performed for the donors with left hepatectomy. Nor could we compare the incidence of steatosis because of a data shortage.

Recipients

Background characteristics of the recipients are summarized in Table 5. There were no significant differences in

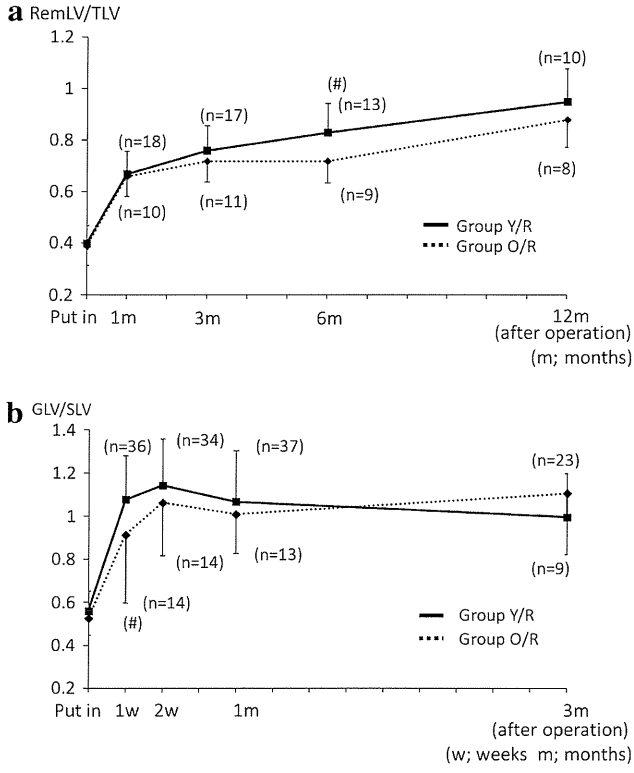

Fig. 1 The changes of donor's and recipient's liver regeneration after right lobe living donor liver transplant (LDLT). **a.** In donors, the remnant liver to volume/total liver volume (RemLV/TLV) ratio at 6 months was lower in older donor group (O/R) than in younger donor group (Y/R), although there were no statistical differences between the two groups by *t*-test with the Bonferroni correction (rough p value = 0.02 at 6 months (#), and rough p value >0.05 at 1, 3, and 12 months). **b.** In recipients, the graft liver volume to standard liver volume (GLV/SLV) ratios were lower in group O/R than in group Y/R during the first month after LDLT, although there were no statistical differences between the two groups by *t*-test with the Bonferroni correction (rough p value = 0.03 (#) at 1 week, and rough p value >0.05 at 2 weeks, 1 month, and 3 months)

the characteristics between the two groups except for Child-Pugh score. There was no significant difference in the rate of complications between the two groups.

The GLV/SLV ratios showed no significant differences between the two groups at all time points by *t*-test with the Bonferroni correction (rough p value >0.05 at all time points) (Fig. 4). As a result of multiple regression analysis including two factors (donor age and Child-Pugh score) that were suggested to have statistical significance in univariate analysis, donor age (≥50 years) was independently correlated with graft liver regeneration at 1 week ($p = 0.05$) (Table 2c). The survival rates were not significantly different between the two groups. The 1-, 3-, and 5-year cumulative survival rates were 85% in group Y/L and 67% in group O/L at all time points, respectively.

Graft regeneration in all recipients

In the present study, graft regeneration was examined according to graft type and donor age, and thus the number of patients in each group became too small to analyze statistically. Therefore, we compared the graft regeneration between the younger donor ($n = 77$: group Y) and older donor ($n = 24$: group O). As a result, the initial graft size (graft weight/SLV) did not differ between the two groups (0.53 ± 0.10 in group Y versus 0.50 ± 0.09 in group O), and the difference of GLV/SLV ratio at 1 week became nearly statistically significant: 1.02 ± 0.21 in group Y versus 0.87 ± 0.28 in group O (rough p value = 0.014).

Regeneration patterns in liver recipient graft and donor remnant

We compared the regeneration patterns between graft liver of the recipient and remnant liver of the donor by using the data on the whole left lobe LDLT recipients and on the whole right lobe donors. Comparing the regeneration ratios at the same time points between GLV/SLV (left lobe graft) and RemLV/TLV (remnant left lobe), they were significantly higher in the recipient graft livers than in the donor remnant livers: 0.99 ± 0.19 versus 0.66 ± 0.09 ($p < 0.001$) at

Table 2 Multiple regression analysis of factors influencing the liver regeneration

	Coefficient	95% Confidence interval	p Value
a. Factors influencing remnant liver regeneration at 6 months in right lobe LDLT donors			
Donor age ≥50	−0.12	−0.23 to −0.01	0.04
Steatosis	0.068	−0.05 to 0.19	0.3
Donor gender	−0.048	−0.15 to 0.06	0.3
b. Factors influencing graft liver regeneration at 1 week in right lobe LDLT recipients			
Donor age ≥50	−0.22	−0.42 to −0.03	0.02
Steatosis	0.067	−0.14 to 0.27	0.5
Recipient gender	−0.005	−0.20 to 0.19	0.7
c. Factors influencing graft liver regeneration at 1 week in left lobe LDLT recipients			
Donor age ≥50	−0.16	−0.33 to 0.00	0.05
Child-Pugh score	−0.015	−0.04 to 0.01	0.2

Table 3 Characteristics and surgical outcomes of recipients in right lobe LDLT

	Group Y/R ($n = 51$)	Group O/R ($n = 17$)	p Value
Age (median)	20–69 (53)	26–67 (54)	N.S.
Gender, male:female	33:18	17:0	0.004
MELD	18	16	N.S.
Child-Pugh score	9.7	8.8	N.S.
Etiology of disease			
Hepatitis C (HCC)	22 (17)	6 (3)	N.S.
Hepatitis B (HCC)	6 (5)	4 (3)	N.S.
Cholestatic	22	6	N.S.
Other	1	1	N.S.
Complications, total	19 (39.6%)	11 (64.7%)	N.S.
Biliary complication	6 (12.5%)	3 (17.6%)	N.S.
Postoperative bleeding	7 (14.5%)	4 (23.5%)	N.S.
Vascular complication	3 (6.3%)	2 (11.8%)	N.S.
Other	3 (6.3%)	2 (11.8%)	N.S.
Small-for-size syndrome	0 (0%)	2(11.8%)	N.S.
Total bilirubin on POD 14, mg/dl	5.53 ± 6.8	5.71 ± 6.2	N.S.
Ascites, ml	664 ± 1.919	2.309 ± 3.808	0.03

HCC hepatocellular carcinoma; *MELD* model for end-stage liver disease

1 month, and 0.90 ± 0.21 versus 0.75 ± 0.09 ($p = 0.002$) at 3 months.

Discussion

One of the most important factors in LDLT, in contrast to DDLT, is graft regeneration, and donor age may have an adverse effect on graft liver regeneration. Among the published articles on the subject, several indicate a negative effect of donor age [12, 29, 30]. In left lobe LDLT, Ikegami et al. [30] revealed that early graft regeneration was significantly impaired in their older donor group (age ≥50 years) than in their younger donor group (age <30 years) at 1 week after LDLT, but it was almost same at 1 month. Our results showed a similar pattern of graft regeneration, and the difference in the GLV/SLV ratio at 1 week in all recipients became nearly statistically significant. In addition, when we evaluated multivariate analysis in graft regeneration at 1 week, donor age (≥50 years) was an independent factor correlated with graft liver regeneration in both right and left lobe LDLT. Therefore, for recipients of older donor grafts, we must take extra care with the operative procedure and early postoperative management of the recipients, because older donor grafts are considered vulnerable to surgical insults.

Although the mechanisms by which donor age affects graft liver regeneration have not been fully clarified, several previous basic studies may offer a partial explanation. Experimental studies on liver regeneration after partial hepatectomy in rodent models revealed that the aging liver has a dramatically reduced ability to proliferate normally through the cell cycle [14, 15]. In a clinical study, Iwamoto et al. [31] compared the surgical outcomes and signaling enzyme in liver of LDLT recipients between their younger (age <50 years) and older (age ≥50 years) donors, and suggested that the lack of STAT3 overexpression after reperfusion is a potential cause of apoptosis and oxidative injury, resulting in an unfavorable prognosis for recipients of older donor grafts. Severe steatosis is recognized as another factor inducing impaired liver regeneration through aggravated Kupffer cell-mediated inflammatory responses [32]. In our study, the rate of steatosis was significantly higher in the livers of the older donor group, which also might have had an adverse effect on graft regeneration.

We considered ADAMTS13 activity a possible factor influencing graft regeneration. We recognized a tendency for ADAMTS13 activity to be lower and the vWF/ADAMTS13 ratio higher in the recipients in group O/R. In our previous study on serum ADAMTS13 levels in 81 patients after LDLT, 17 patients had both severe thrombocytopenia and hemolytic anemia with fragmented red cells and were diagnosed as having thrombotic microangiopathy-like syndrome (TMALS). In these TMALS patients, ADAMTS13 activity was significantly lower and vWF/ADAMTS13 ratios were significantly higher as compared to those without TMALS [23]. We speculated that the recipients from the older donor group might have an increased risk of developing thrombotic microangiopathy.

The use of older donor grafts might lead to unfavorable recipient outcomes in both DDLT [3–5, 7] and LDLT [10, 31]. However, in studies of right lobe LDLT from high-volume centers, there were no significant differences in the survival rates of recipient from older and younger donor

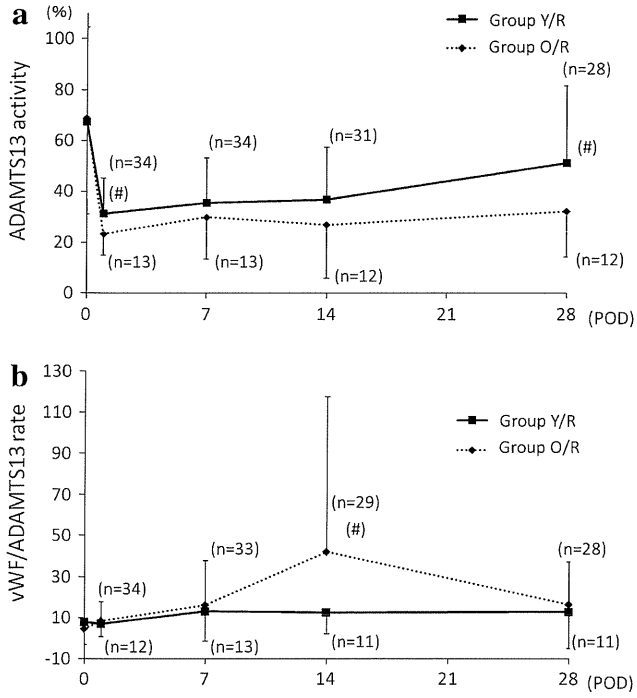

Fig. 2 The change in ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13) activity in recipients after right lobe LDLT. **a.** ADAMTS 13 activity was lower in group O/R than in group Y/R during the first month after LDLT, although there were no significant differences by *t*-test with the Bonferroni correction (rough p value <0.05 on postoperative day (POD) 1 and POD 28 (#) and rough p value >0.05 on POD 7 and 14). **b.** The change in von Willebrand factor (vWF)/ADAMTS13 ratios in recipients after right lobe LDLT. The vWF/ADAMTS13 ratio on POD 14 was higher in group O/R than in group Y/R, although there were no significant differences by *t*-test with the Bonferroni correction (rough p value <0.05 on POD 14 (#) and rough p value >0.05 on POD 1, 7, 14, and 28)

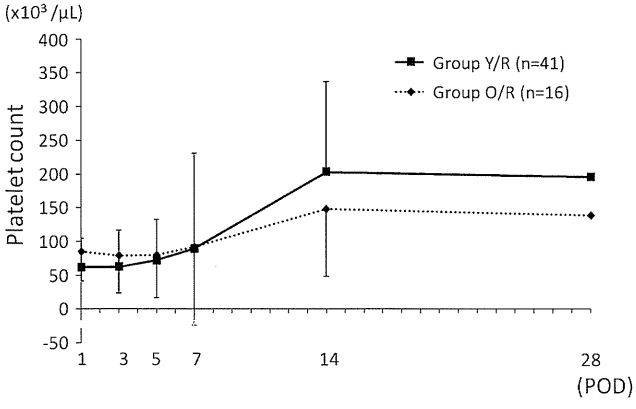

Fig. 3 Postoperative platelet count in recipients after right lobe LDLT. There were no significant differences in postoperative platelet count at all time points between the two groups

groups [11, 26, 33]. Similarly, in our study, overall survival of right lobe LDLT was almost same between the two groups, and that of left lobe LDLT was slightly lower in group O/L, although there were no significant differences. In left lobe LDLT, the donor aging effect is thought to be more influential on recipient outcome because of the smaller graft volume. Further study of left lobe LDLT, with larger numbers of cases, is required. In addition, it has been reported that steatosis had adverse effect on recipient outcomes even if its grade was less than 30% [34]. In our study, older donors have increased tendency toward steatosis. Therefore, when using a graft from an older donor, we should consider not only the effect of donor age itself, but also the indirect effect of steatosis.

As to the risk factors affecting recipient's survival, HCV infection has been well recognized, and it was recently revealed that increased donor age is associated with more severe HCV recurrence [4, 5, 10]. In our study, there were no significant differences in the survival rates of HCV-positive recipients between the older and younger donor groups, probably because of the small number of cases. In recipients with HCV infection, therefore, we should pay careful attention to donor age, recipient age, and HCV infection status.

Regarding complications of recipients, previous studies [11] have shown that the incidence of small-for-size syndrome in left lobe LDLT was significantly greater in older donor groups. In our study, the incidence of small-for-size syndrome in left lobe LDLT was not significantly different between the two groups. However, the recipients in group O/R had significantly greater amounts of ascites than group Y/R in right lobe LDLT. The frequency of complications was higher in the older than in the younger donor group for both left and right lobe LDLT, although the differences were not statistically significant.

Donor safety is the top priority in LDLT. We should pay great attention to the postoperative course, especially with older donors. A previous study showed that the hospital stay in right lobe LDLT was significantly longer among older donors (age ≥60) than younger donors [26]. In an analysis of the postoperative course of donors, total bilirubin levels were significantly higher among older donors (age ≥50), and the rate of major complications significantly increased in the older donor group who underwent right hepatectomy with middle hepatic vein [35]. In our study, the frequency of complications, postoperative liver damage and length of hospital stay in older donors were comparable to the same factors in younger donors for both left and right lobe LDLT. In older donors, however, the incidence of hepatic steatosis was significantly higher. In view of the increased risk of steatosis in older donors, it is important to ensure sufficient remnant liver volume for them. In addition, according to our previous study [36], we had been able to use a smaller graft by controlling portal pressure after reflow such as splenectomy. Therefore, we changed our institutional policy for the minimum RemLV from >30 to >35% in 2005.

Table 4 Characteristics and surgical outcomes of donors in left lobe LDLT

	Group Y/L ($n = 26$)	Group O/L ($n = 7$)	p Value
Age, years (median)	35 (19–48)	56 (50–64)	
Gender, male:female	25:1	2:5	0.0014
BMI	23	23	N.S.
L/S	1.17	1.32	0.006
Operative time, min	467 ± 98	466 ± 46	N.S.
Intraoperative blood loss, ml	1.424 ± 1.185	1.197 ± 538	N.S.
Actual graft weight, g	491 ± 82	470 ± 19	N.S.
Actual GRWR	0.91 ± 0.19	0.78 ± 0.05	N.S.
Minimum albumin, mg/dl	2.7 ± 0.4	2.5 ± 0.2	N.S.
Max total bilirubin, mg/dl	2.1 ± 1.1	2.3 ± 1.0	N.S.
Max PT-INR	1.3 ± 0.2	1.3 ± 0.1	N.S.
Postoperative complication	4 (15%)	1 (17%)	N.S.
Biliary leakage	3	0	N.S.
Ileus	1	1	N.S.
Hospital stay, days	20 ± 11	15 ± 4	N.S.

Table 5 Characteristics and surgical outcomes of recipients in left lobe LDLT

	Group Y/L ($n = 26$)	Group O/L ($n = 7$)	p Value
Age, years (median)	50 (17–70)	53.5 (23–62)	N.S.
Gender, male:female	9:17	4:3	N.S.
MELD	18	11	N.S.
Child-Pugh score	10.7	7.0	0.0057
Etiology of disease			
Hepatitis C (HCC)	9 (6)	0	N.S.
Hepatitis B (HCC)	5 (0)	3 (3)	N.S.
Alcoholic (HCC)	2 (1)	0	N.S.
Cholestatic	8	2	N.S.
Other	2	2	N.S.
Complications, total	8 (30.8%)	4 (57.1%)	N.S.
Biliary complication	1 (3.8%)	0 (0%)	N.S.
Postoperative bleeding	2 (7.7%)	1 (14.3%)	N.S.
Vascular complication	3 (11.5%)	0 (0%)	N.S.
Other	2 (7.7%)	3 (42.9)	N.S.
Small-for-size syndrome	4 (15.4%)	1 (14.3%)	N.S.
Total bilirubin on POD 14, mg/dl	7.54 ± 7.12	6.43 ± 8.48	N.S.
Ascites, ml	551 ± 917	508 ± 777	N.S.

It remains controversial whether older donor age affects remnant liver regeneration. There have been a few clinical studies on how age affects remnant liver regeneration after right hepatectomy in LDLT [37–39]. The previous CT volumetric study did not show any significant differences between younger and older donor groups [39]. However, this calculation method might not precisely reflect the regenerative ability of the liver remnant, because the regeneration volume % is affected by the initial volume of remnant liver. In another volumetric study, Kawasaki et al. [40] used the ratio of liver volume to SLV to evaluate graft regeneration for the first time, recognizing that SLV more precisely reflects the maximum volume that the remnant liver can grow to achieve. In our study, we used TLV instead of SLV as the maximum regeneration index, and observed that remnant liver regeneration was lower in group O/R than in group Y/R at 6 months after LDLT (not statistically significant). In view of these data, the regenerative ability of older donor liver might be impaired in the late period. Therefore, for older donors, it is important to attend to liver function more carefully and for a longer time than for younger donors.