Title: Effect of recipient-derived cells on the progression of familial amyloidotic polyneuropathy after liver transplantation: a retrospective study

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A short title: Microchimerism on FAP patients after LT

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DECLARATIONS

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Hirofumi Jono, Masaaki Naka mura, Shogo Misu mi and Yuki Ohya were involved in protocol development, gaining ethical approv al, patient recruitment and data ana lysis. Yuki Ohya wrote the first draft of the m anuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Abstract

Background: Some familial amyloidotic polyneuropathy (FAP) patients show the post-transplant progression of the clinical symptoms. Although the presence of recipient-derived cells in transplanted livers has been reported, no studies investigating the functional significance of this post-transplant chimerism in tra nsplanted FAP patients was perform ed. The aims of this study were to evaluate am yloidogenic transthyretin (ATTR) production of recipient-derived cells and the relationship between the protein from recipient-derived cells and the progression of FAP symptoms after liver transplantation (LT).

Methods: Seven FAP ATTR Val30Met patients who underwent LT were included in this

study. In one male patient with sex-mismatched donor, fluorescence in situ hybridization (FISH) method was performed on a liver biopsy sample using DNA probes for visualizing X and Y chromosomes to detect the recipient-derived cells. In three patients including the FISH-analyzed patient, ATTR mRNA expression in transplanted livers was evaluated by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method and real-time quantitative reverse transcription-PCR. In five of the seven patients, ATTR in serum protein expression was measured by mass spectrometry.

Results: One FAP patient has 3.1% recipient-derived cells in the transplanted liver. The ATTR mRNA was not expressed in any of the three transplanted livers. The ATTR was not detected in any sera of measured patients.

Conclusion: Although the FAP patient had recipient-derived cells in the transplanted liver, the recipient-derived cells did not contribute to the production of ATTR in our specific case. Effect of recipient-derived cells on the post-transplant progression of FAP symptoms may be negligible.

Introduction

Familial amyloidotic polyneuropathy (FAP) is a disease, inherited in an autosomal dominant fashion, that is caused by mutant transthyretin (TTR).[1,2] The disease is characterized by sensory-dominant peripheral neuropathy, as well as symptoms in the gastrointestinal tract, heart, eyes, kidneys, and autonomic nervous system.[3-6] More than 90 amyloidogenic transthyretin (ATTR) mutations leading to FAP have been reported.[7] Of those, Val30Met is the most common type mutation. TTR is produced by the liver, retinal pigment epithelium, choroid plexus, and alpha cells of the pancreas. Because the liver synthesizes more than 90% of serum TTR,[8,9] liver transplantation (LT) has been performed to treat FAP since 1990. LT is now considered as a promising therapy to halt the progression of clinical symptoms in FAP.[9,10] However, it was reported that some FAP patients showed progression of FAP symptoms even after LT,[11-14] while the mechanism remain to be elucidated.

There is a possibility that the recipient-derived cells in transplanted liver have impact on the progression of FAP symptoms after LT. The presence of microchimerism was reported in transplanted livers and in other transplanted organs.[15-17] Chimerism is

defined as cell migration from the host to the transplanted organ. The mechanism of microchimerism was reported to be transdifferentiation and/or cell fusion of bone marrow cells.[18-20] Many reports have been published on the presence of microchimerism in transplanted liver.[21,22] It was reported that transplanted livers have recipient-derived cells and these cells play a role in liver regeneration.[21-23] In FAP cases, if transplanted liver has recipient-derived cells in FAP patients, these cells have a possibility to produce ATTR which may cause the progression of FAP symptoms. However, there have been no studies investigating recipient derived cells in FAP patients.

The aims of this study were to evaluate the protein production of recipient-derived cells and the impact of recipient–derived cells on the progression of FAP symptoms after LT. We evaluated recipient-derived cells in transplanted FAP patients by fluorescent in situ hybridization (FISH). We also evaluated the serum ATTR level and ATTR mRNA expression in transplanted liver in FAP patients.

Patients and methods

We performed a retrospective study of 7 Japanese FAP ATTR Val30Met patients in

Kumamoto University Hospital who underwent LT from 1994 to 2008 (Table 1). All FAP patients in this study had a definite diagnosis of FAP on the basis of genetic investigations and clinical manifestations of FAP. All patients had standard immunosuppression with tacrolimus or cyclosporine and corticosteroids with or without mycophenolate mofetil (Table 1). We evaluated preoperative factors, graft types, and the changes of clinical manifestations after LT by retrospective analysis of medical charts (Table 1). The patient who has sex-mismatched donor and shows the typical progression, would be an ideal and a suitable case to evaluate the effect of recipient-derived cells. Especially, Patient 4 was the ideal and suitable case. Therefore, we focused on Patient 4. Two frozen autopsy samples of Patient 1 and Patient 2 and one frozen biopsy sample of Patient 4 were used to evaluate ATTR mRNA expression in the transplanted livers. In three patients including the FISH-analyzed one, expression of ATTR Val30Met mRNA in transplanted livers was evaluated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) method. In five of the seven patients, ATTR in sera was measured by using matrix-assisted laser desorption

ionization/time-of-flight mass spectrometry (MALDI/TOF-MS).

The ethical committee at Kumamoto University School of Medicine approved this study and informed consent was obtained from patients after explanation about this study.

FISH

The biopsied liver at 7 years after LT of Patient 4 was analyzed. FISH was performed in Nihon Gene Research Laboratories. Formalin-fixed paraffin-embedded sections were sectioned at 6 μm. The sections were prepared for FISH by the NaSCN incubation, denaturation, and proteinase K digestion steps, one after the other. After the last formamide denaturation, the slides were dehydrated in graded ethyl alcohols and air dried. FISH was performed on pretreated slides using spectrum green labeled DXZ1 probe (Vysis, Downers Grove, IL) and spectrum red labeled DYZ1 probe (Vysis) for labeling X and Y chromosomes. Probe-applied, covered, and sealed slides were denatured on hot plate and hybridized over night at 37 °C using a hybrid incubation chamber (Vysis). DAPI was performed for nuclear counter-staining.

Determination of TTR using MALDI/TOF-MS

We examined the presence of ATTR Val30Met in sera of five post-transplanted FAP patients. Fifty microliter of test serum was mixed with 10 µl of 2.7 mM dithiothreitol and 20 µl of an anti-TTR polyclonal antibody (DAKO, Glostrup, Denmark). TTR in the serum was isolated as described by Ando *et al*.[24,25] All experiments were performed using a Bruker Reflex mass spectrometer (Bruker Franzen Analytik GmbH, Bremen, Germany) operated at a wavelength of 337 nm. The isolated TTR was assayed as described previously.[26]

Isolation of RNA and reverse transcription

Total RNA was extracted from each frozen samples using the RNeasy Mini Kit Isolation System (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was quantified by spectrophotometric analysis using NanoDrop ND-1000 spectophotometer (NanoDrop Technologies, DE). The RNA was reverse-transcribed into complementary DNA (cDNA) by one cycle at 37 °C 15 min followed by one cycle 85 °C for 5 s using

PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan).

PCR-RFLP

The generated cDNA was used as the template in real-time PCR reactions. PCR primers were designed to amplify the Val30Met mutation in exon 2 of the TTR gene. Primers were: forward 5'-CA TTCTTGGCAGGATGGCTTC-3' and reverse 5'-CT CCCAGGTGTC ATCAGCAG-3'. The PCR conditions were 5 min at 98 °C; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; followed by 7 min 72 °C. The PCR product of 199 bp was digested with 5 U *Nsi*I restriction enzyme (New England Biolabs, UK) that recognized mutation site at 37 °C for 2 h. PCR products were then separated on 3.5% NuSieve GTG agarose gel (BMA products, Rockland, ME) by electropheresis in 1× TBE buffer, approximately 2 h at 50 V. The expected PCR products are visualized using SYBR SafeTM DNA Gel Stain (Invitrogen, Carlsbad, CA). PCR products were evaluated using microchip electrophoresis system (Cosmo-I SV1210; Hitachi Electronics Engineering, Tokyo, Japan), too. Measurement was according to manufacture's manual.

Real-time quantitative RT-PCR

The generated cDNA was used as the template in real-time PCR reactions. Real-time PCR primers were designed to amplify the Val30Met mutation of the TTR gene. Primers were: forward 5'-CTCTGA TGGTCAAAGTTCTAGATGCT-3' and reverse 5'-GTGTCATCAGCAGCCTTTCTG-3'. We used FAM-labeled TaqMan probe and VIC-labeled TaqMan probe to detect the Val30Met TTR gene and wild-type TTR gene, respectively. Val30Met TTR specific FAM-labeled TaqMan probe was 5'-FAM-AATGTGGCCATGCAT-3'. Wild-type TTR specific VIC-labeled TaqMan probe was 5'-VIC-CAATGTGGCCGTGCAT-3'. The real time PCR conditions were 2 min at 50 °C, 10 min at 95 °C; 40 cycles of 95 °C for 15 s, 65 °C for 1 min. Real time RT-PCR was performed with TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA), according to the manufactures' protocol. Real-time PCR for wild-type and Val30Met TTR mRNA was validated on the Applied Biosystems 7500 Real-Time PCR system according to the manufacture's instructions. 50:50, 90:10, 98:2, 99.6:0.4, 99.92:0.08, and 100:0 of wild-type TTR genes and ATTR Val30Met genes served as

standards for analyses of TTR mRNA expression ratio in transplanted liver.

Results

Clinical outcome

Table 1 presents preoperative factors, graft types, and the changes of clinical manifestations after LT. Patient 1 and Patient 2 received whole liver grafts from deceased donors. Patient 1 showed the progression of cardiac symptoms even after LT. He underwent pacemaker implantation 8 years after LT due to complete atrioventricular block. In addition, although the ocular manifestation was not observed before LT, he developed mild degree of vitreous opacity in his right eye 12 years after LT. He did not show the progression of neuropathy after LT and died of hypokalemia-induced pulseless ventricular tachycardia caused by alcoholism 13.8 years after LT. Patient 2 showed the progression of cardiac symptom too. She underwent pacemaker implantation 5 years after LT due to heart failure. Because the progression of vitreous opacity and glaucoma was observed, she underwent trabeculectomys 7 and 8 years after LT and vitrectomy 9 years after LT. She did not show the progression of neuropathy after LT and died of

severe congestive heart failure with cardiac liver cirrhosis 9.2 years after LT. Patient 3, 5, 6 and 7 did not show the progression of FAP symptoms. Patient 4 received the right lobe graft from the living donor and underwent liver biopsy 7 years after LT. The donor was his daughter without pregnancy history. He showed the granular sparkling echo and circumferential ventricular wall thickening with echocardiography 4 years after LT and developed symptoms of heart failure. He underwent pacemaker implantation 5 years after LT due to trifascicular block. He underwent liver biopsy to check his liver, because he had ascites and pleural effusion 7 years after LT. The transplanted liver was almost normal according to the results of biopsy. These results showed that the cardiac amyloidosis caused the ascites and pleural effusion and cardiac symptoms progressed after LT. He showed the progression of neuropathy and deterioration in nerve conduction velocity gradually after LT. In addition, because he also exhibited the progression of vitreous opacity, he underwent anterior vitrectomy of his right eye 3 years after LT and vitrectomy of his left eye 4 years after LT.

Recipient-derived cells in liver allograft

FISH analysis was perform ed in Patient 4 with sex-mismatched donor to evaluate the ratio of recipient-derived cells in tr ansplanted liver. There were 31 cells that had XY signals out of 1000 cells in his transplanted liver, indicating that his transplanted liver had 3.1% recipient-derived cells 7 years after LT (Figure 1).

Serum ATTR in FAP patients after LT

TTR in sera of five transplanted F AP patients was m easured by MAL DI/TOF-MS to evaluate the serum ATTR after LT. The results of a FAP patient before LT as a positive control and Patient 4 we re shown in Figure 2. While the peak of approxim ately 13,761 kDa of wild-type TTR was detected, the peak of ATTR Val30Met was not detected in Patient 4. Other transplanted FAP patients did not exhibit the peak of ATTR Val30Met.

ATTR Val30Met mRNA expression in transplanted livers

ATTR mRNA levels in transplanted livers were evaluated by using PCR-RFLP and real time quantitative R T-PCR to elu cidate the ATTR expression of recipient-derived hepatocytes. In three tra nsplanted livers, PCR-RFLP analysis revealed wild-type TTR

mRNA expression, but not ATTR Val30Met mRNA expression in transplanted livers.

ATTR mRNA expression in transplanted livers was evaluated by using real time quantitative RT-PCR to elucidate the ATTR production of recipient-derived hepatocytes more sensitively. Delta threshold cycle (Ct) value was calculated by taking Ct of wild-type TTR from that of Val30Met ATTR in real time quantitative RT-PCR. The chart of standard curve that has delta Ct scale and logarithmic scale of mixing ratio was made. The approximation of standard curve was expressed as follows: $Y = -15.35\ln(X) + 33.218$. Using purified wild-type and ATTR Val30Met DNAs, we verified approximately 0.1% ATTR Val30Met mRNA expression at the lowest level. However, in three transplanted livers, ratio of ATTR mRNA expression was calculated by the approximation (Table 2). Ratio of ATTR expression in three transplanted livers was below the detectable level. In this analysis, ATTR mRNA was not expressed in transplanted livers.

Discussion

Results of this study indicate that the transplanted liver had recipient-derived cells

which did not produce ATTR. Although it has been reported the progression of FAP symptoms after LT,[11-14] effect of recipient-derived cells on the progression of FAP have not been reported until now. If recipient-derived cells have some effects on the progression, we need to consider the way of inhibition for the recipient-derived cells. Therefore, we enrolled FAP patients with or without progression of symptoms in our study.

In this study, the presence of recipient-derived cells in transplanted liver was first confirmed in the FAP patient by using FISH analysis, indicating that microchimerism occurred in the FAP patient after LT. Although FISH analysis was performed in only one FAP patient, the presence of recipient-derived cells were also confirmed with almost the equal frequency of previous reports in other diseases.[21,22] However, despite the presence of recipient-derived cells, our results showed that ATTR mRNA was not expressed in the transplanted liver and serum ATTR could not be detected. Previous studies reported that hepatocyte transdifferentiation from recipient bone marrow stem cells was rare,[27-30] suggesting that the recipient-derived cells in the transplanted liver may be too immature to synthesize TTR. In addition, previous studies have also shown

that the recipient-derived cells transdifferentiated into cholangiocytes or vein endothelium cells in transplanted livers, [21,30-33] implying that the recipient-derived cells may transdifferentiate into non-hepatocytes. Because the sample was limited in this study, further investigation will be needed to identify the type of recipient-derived cells by multiple methods such as immunohistochemical staining for hepatocyte marker. Taken together, in the view of microchimerism, values of LT for FAP patients were revalidated by showing that recipient-derived cells did not produce ATTR in our cases. However, because transplanted liver actually had recipient-derived cells in FAP patients, it still remains possible that these recipient-derived cells may have impact on the progression of FAP symptom in some FAP patients. Furthermore, the transplanted FAP patient who showed the progression and had sex-mismatched donor was very rare. We need to analyze in more number of FAP patients hereafter.

Although it has been reported that some FAP patients showed progression of FAP symptoms even after LT, the mechanism remains to be elucidated. In this study, our data did not confirm the effect of recipient-derived cells on the progression of FAP. It is well documented that TTR was produced by different organs, such as retinal pigment

epithelium, choroid plexus, and alpha cells of the pancreas. Even though ATTR levels were not detectable in our study, the trace amount of ATTR from extrahepatic organs may contribute to the progression of FAP symptoms after LT. In addition, because the part of amyloid deposits in some organs have been proven to be derived from the wild-type TTR, the TTR may have some impact on the progression of FAP symptoms after LT. Further investigations will be needed to clarify the detail mechanism of the progression of FAP symptoms after LT.

Another interesting finding in this study is that LT in FAP has possibility to be a unique and natural human model to evaluate the recipient-derived transdifferentiated hepatocytes by detecting ATTR gene or protein functionally, because only recipient-derived hepatocytes can express ATTR gene. Previously, the presence of recipient-derived hepatocytes was usually shown by sex-mismatch analysis.[21,22] FISH and immunohistochemical staining methods on transplanted tissues in sex-mismatched individuals cannot fully evaluate the function of recipient-derived cells. It is well documented that not only albumin but also TTR is produced by differentiated

hepatocytes. If recipient-derived cells transdifferentiate to hepatocytes in heterozygous FAP patients, these recipient-derived he patocytes should produce both wild-type TTR and ATTR. We could not detect the expression of A TTR Val30Met in this study. This result may imply that the recipient-derived cells did not transdifferentiate to hepatocytes in our cases. Although we need to study in m ore transplanted FAP patients, we m ight provide a new tool to evaluate functional ch imerism in tran splanted livers. However, because our data do not exclude the possibility that ATTR mRNA expression and serum ATTR protein levels were too low to be dete cted, we need to evaluate ATTR expression by more sensitive method such as radioimmunoassay procedure.[34]

In conclusion, although it wa s confirmed that the F AP patient had recipient-derived cells in the transplanted liver in our series, the recipient-derived cells did not contribute to the production of ATTR. Although the case volume was small, our study suggests that microchimerism do not have effect on the progression of FAP symptoms after LT in our cases. Our results provide one milestone into LT in FAP patients.

REFERENCES

1. Saraiva MJ, Costa PP , Birken S, G oodman DS. Presence of an abdom inal transthyretin (prealbumin) in Portuguese patients with fa milial amyloidotic polyneuropathy. *Trans Assoc Am Physicians* 1983;**96**:261-270

2. Ando Y, Nakamura M, Araki S. T ransthyretin-related familial amyloidotic polyneuropathy. *Arch Neurol* 2005;**62**:1057-1062

3. Ando Y, Suhr OB. Autonomic dysfunction in familial amyloidotic polyneuropathy (FAP). *Amyloid* 1998;**5**:288-300

4. Ando Y, Araki S, Ando M. Transthyretin and familial amyloidotic polyneuropathy. Intern Med 1993;**32**:920-922

5. Ando E, Ando Y, Okamura R, Uchino M, Ando M, Negi A. Ocular manifestations of familial amyloidotic polyneuropathy type I: long term follow up. *Br J Ophthalmol* 1997;**81**:295-298

6. Araki S. Type I familial amyloidotic polyneuropathy (Japanese type). *Brain Dev* 1984;**6**:128-133

7. Connors LH, Richardson AM, Théber ge R, Costello CE. T abulation of

transthyretin (TTR) variants as of 1/1/2000. *Amyloid* 2000;**7**:54-69

8. Makover A, Moriwaki H, Ramakrishnan R, Saraiva MJM, Blaner WS, Goodman

9. DS. Plasma transthyretin. Tissue sites of degradation and turnover in the rat. *J Biol Chem* 1988;**263**:8598-8603

10. Ando Y, Tanaka Y, Nakazato M, *et al.* Change in variant transthyretin levels in patients with familial amyloidotic polyneuropathy type I following liver transplantation. *Biochem Biophys Res Commun* 1995;**211**:354-358

11. Suhr OB, Holmgren G, Steen L, *et al.* Liver transplantation in familial amyloidotic polyneuropathy. Follow-up of the first 20 Swedish patients. *Transplantation* 1995;**60**:933-938

12. Parrila P, Ramirez P, Andereu LF, *et al.* Long-term results of liver transplantation in familial amyloidotic polyneuropathy type I. *Transplantation* 1997;**64**:646-649

13. Yamamoto S, Wilczek HE, Nowak G, *et al.* Liver transplantation for familial amyloidotic polyneuropathy (FAP): A single-center experience over 16 years. *Am J Transplant* 2007;**7**:2597-2604

14. Stangou AJ, Hawkins PN, Heaton ND, *et al.* Progressive cardiac amyloidosis

following liver transplantation for familial amyloid polyneuropathy: Implications for amyloid fibrillogenesis. *Transplantation* 1998;**66**:229-233

15. Hörnsten R, Wiklund U, Olofsson BO, Jensen SM, Suhr OB. Liver transplantation does not prevent the development of life-threatening arrhythmia in familial amyloidotic polyneuropathy, Portuguese-type (ATTR Val30Met) patients. *Transplantation* 2004;**78**:112-116

16. Quaini F, Urbanek K, Beltrami AP, *et al.* Chimerism of the transplanted heart. *N Engl J Med* 2002;**346**:5-15

17. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. *Lancet* 1992;**339**:1579 -1582

18. Lagaaij EL, Cramer-Knijnenburg GF, van Kemenade FJ, van Es LA, Bruijn JA, van Krieken JH. Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* 2001;**357**:33-37

19. Theise ND, Badve S, Saxena R, *et al.* Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000;**31**:235-240

20. Wang X, Willenbring H, Akkari Y, *et al*. Cell fusion is the principal source of

bone-marrow-derived hepatocytes. *Nature* 2003;**422**:897-901

21. Petersen BE, Bowen WC, Patrene KD, *et al*. Bone marrow as a potential source of hepatic oval cells. *Science* 1999;**284**:1168-1170

22. Theise ND, Nimmakayalu M, Gardner R, *et al.* Liver from bone marrow in humans. *Hepatology* 2000;**32**:11-16

23. Idilman R, Erden E, Ersoz S, *et al*. Recipient-derived hepatocytes in sex-mismatched liver allografts after liver tra nsplantatioon: early versus late transplant biosies. *Transplantation* 2004;**78**:1647-1652

24. Ng IO, Chan KL, Shek WH, *et al.* Neil. High frequency of chim erism in transplanted livers. *Hepatology* 2003;**38**:989-998

25. Ando Y, Ohlsson PI, Suhr O, *et al*. A new simple and rapid screening m ethod for variant transthyretin-related amyloidosis. *Biochem Biophys Res Commun* 1996;**228**:480-483

26. Ando Y, Suhr O, Y amashita T, *et al.* Detection of dif ferent forms of variant transthyretin (Met30) in cerebrospinal fluid. *Neurosci Lett* 1997;**238**:123-126

27. Misumi S, Kudo A, Azum a R, T omonaga M, Furuishi K , Shoji S. The p2 gag

peptide, AEAMSQVTNTATIM, processed from HIV-1 Pr55 gag was found to be a suicide inhibitor of HIV -1 protease. *Biochem Biophys Res Commun* 1997;**241**: 275-280

28. Wu T, Cieply K, Nalesnik MA, *et al.* Minimal evidence of transdifferentiation from recipient bone m arrow to parenchym al cells in regenerating and long-surviving human allografts. *Am J Transplant* 2003;**3**:1173-1181

29. Fogt F, Beyser KH, Porem ba C, Zi mmerman RL, Khettry U, Ruschof f J. Recipient-derived hepatocytes in liver transplants: a rare event in sex-mismatched transplants. *Hepatology* 2002;**36**:173-176

30. Tomiyama K, Miyazaki M, Nukui M, *et al.* Limited Contribution of Cells of Intact Extrahepatic Tissue Origin to Hepatocyte Regeneration in Transplanted Rat Liver. *Transplantation* 2007;**83**:624-630

31. Hove WR, van Hoek B, Bajem a IM, Ri ngers J, van Krieken JH, L agaaij EL. Extensive chimerism in liver transplants: vascular endot helium, bile duct epithelium, and hepatocytes. *Liver Transpl* 2003;**9**:552-556

32. Gao Z, McAlister VC, W illiams GM. Re population of liver endo thelium by

bone-marrow-derived cells. *Lancet* 2001;**357**:932-933

33. Baccarani U, Donini A, Risaliti A, Bres adola F. Replacement of liver venous endothelium. *Lancet* 2001;**357**:2137

34. Körbling M, Katz RL, Khanna A, *et al.* Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002;**346**:738-746

35. Ando Y, Tanaka Y, Nakazato M, *et al.* Change in va riant transthyretin levels in patients with f amilial amyloidotic polyneuropathy type I following liver transplantation. *Biochem Biophys Res Commun* 1995;**211**:354-358

Figure legends

Figure 1. Presence of recipient-derived cells is confirmed in the liver biopsy sample of male FAP recipient with sex-mismatched donor. Nuclear red spots were interpreted as Y chromosomal signal, green spots as X chromosomal signals. Recipient-derived cells (arrow) were detected in the transplanted liver.

Figure 2. Analysis of TTR forms by MALDI/TOF-MS in the serum of FAP patients. (A) A FAP patient before LT as a positive control. The peaks of 13,761 kDa and 13,792 were free forms of wild-type TTR and ATTR Val30Met, respectively. (B) Patient 4 after LT. The peak of 13,761 kDa was free form of wild-type TTR. The peak of ATTR was not detected.