Blackwell Publishing

*Asian J Androl 2006; 8 (4): 477–482*
DOI: 10.1111/j.1745-7262.2006.00147.x

AJA
http://www.asiaandro.com

·*Clinical Experience*·

# Presence of donor-and-recipient-derived DNA microchimerism in the cell-free blood samples of renal transplantation recipients associates with the acceptance of transplanted kidneys

Yao-Wen Fu[1], Wei-Gang Wang[1], Hong-Lan Zhou[1], Lu Cai[2,3]

[1]*Renal Transplantation Center of Jilin Province, and Department of Urology, the China-Japan Union Hospital, Changchun 130033, China*
[2]*Department of Hematology and Oncology, the First Teaching Hospital, Jilin University, Changchun 130021, China*
[3]*Departments of Medicine, Radiation Oncology, and Pharmacology and Toxicology, the University of Louisville, Louisville, Kentucky 40202, USA*

**Abstract**

**Aim:** To examine whether the existence of the donor-and recipient-derived DNA chimerism in recipient's plasma can be a predictive marker for the status of transplanted organ. **Methods:** One hundred and twenty-six female patients who had been transplanted with male kidneys were enrolled in the present study. In these female recipients, the $SRY_1$, $DYZ_1{}^{1st}$ and $DYZ_1{}^{2nd}$ genes on the Y chromosome from the plasma were prospectively examined using reverse transcription polymerase chain reaction (RT-PCR). **Results**: *SRY*, $DYZ_1{}^{1st}$ and $DYZ_1{}^{2nd}$ sequences were detected in the cell-free blood (plasma) of 97 (77%) of 126 female patients with male kidney. The average time that the transplanted kidneys functioned was 8.7 years and 5.4 years among microchimerism-positive and microchimerism-negative recipients, respectively. The frequency of the patients who had acute rejection after renal transplantation was approximately 10% and 28% in microchimerism-positive and microchimerism-negative recipients, respectively. Serum creatinine levels in microchimerism-positive patients were significantly lower than those in microchimerism-negative patients. **Conclusion:** These results suggest that plasma DNA microchimerism present in certain patients following renal transplantation and measurement of plasma DNA microchimerism using quantitative RT-PCR might be a useful predictor for the acceptance of transplanted kidneys. *(Asian J Androl 2006 Jul; 8: 477–482)*

**Keywords**: renal transplantation; donor-and-recipient-derived DNA microchimerism; immunotolerance; renal rejection

## 1 Introduction

Renal transplantation is the best alternative for the treatment of chronic or acute renal diseases in the terminal phase [1]. The development of microchimerism, a phenomenon of the persistence of donor cells in the peripheral blood of renal transplant recipients, has been considered to be positively associated with the acceptance of transplanted organs [1–3]. However, studies have shown that donor microchimerism is not always detectable in the patients surviving organ grafts [4–6].

Correspondence to: Dr Yao-Wen Fu, Renal Transplantation Center, the China–Japan Union Hospital, Changchun 130031, China.
Tel: +86-431-499-5771, Fax: +86-431-464-1026
E-mail: wangweigang@medmail.com.cn
Received 2005-11-17 Accepted 2006-02-01

The discrepancy might be mainly a result of the small number of cases and the short post-transplant duration in these previous studies.

Detection of microchimerism following transplantation has been achieved predominantly by polymerase chain reaction (PCR)-based techniques [2, 4–7]. Male-specific Y-chromosomal sequences have been used for this purpose [5, 7, 8]. Recent studies also revealed the presence of donor-derived DNA in cell-free plasma of the liver and heart transplantation recipients, a phenomenon known as plasma DNA microchimerism.

In this report, therefore, we used the reverse transcription (RT)-PCR technique to detect donor-derived DNA in cell-free plasma samples of female recipients who received male kidneys. The existence of microchimerism was analyzed against the survival time and function of the transplanted kidneys.

## 2 Patients and methods

### *2.1 Patients*

The present study enrolled 126 female transplant recipients receiving male kidneys, aged from 24–47 (average: 35.4) years old from the Renal Transplantation Center, the China–Japan Union Hospital, Jilin University School of Medicine, between June 1986 and July 2000. Approval documents have been obtained from the Clinical Research Ethics Committee of the Jilin University. Among the 126 patients who received healthy male kidneys, 118 patients were married and 109 patients had been pregnant with male infants. All 126 recipients received grafts from male donors with the same blood types, and 56 patients had human leukocyte antigen (HLA) matching and 3 patients received re-renal transplant. The male donors were aged from 18 to 30 years old. Times of warm ischemia and cold ischemia were less than 7 min and 10 h, respectively, for all renal grafts. All patients received routine immunosuppression therapy for their lifetime after renal transplantation; however, the drugs used for the immunosuppression therapy varied among the patients during the wide time-period. They could be divided into four regimens: regimen I (38 patients) were given azathioprine (Aza) + Prednisone (PDS); regimen II (20 patients) were given Aza + PDS + cyclosporine (CsA); regimen III (36 patients) were given CsA + PDS + mycophenolate mofeti (MMF); and regimen IV (32 patients) were given MMF + PDS + tacrolimus (FK506). Among these patients, 71 cases survived for 1–5 years, which is within the range of survival time reported by the published literature [9], 27 cases for 5–10 years and 28 cases for more than 10 years.

### *2.2 Plasma DNA extraction*

Three milliliters of whole blood was collected from each recipient at each examination. The plasma was transferred carefully into another tube without disturbing the pellet at the bottom of the bottle and then centrifuged at 2 500 × $g$ for 5 min to make sure that cells and cellular debris were completely removed from the plasma. The samples were frozen at –20ºC until further use. Plasma DNA was extracted using a phenol/chloroform method. Blood samples were collected from each recipient twice a week within 1 month, once a week at 1–3 months and once a month after 3 months following renal transplantation. The results of the present study were mainly obtained from the examination at and after 3 months following renal transplantation.

### 2.3 *Reverse transcription polymerase chain reaction (RT-PCR)*

Three pairs of primers, *SRY-1F* and *SRY-2R, Y16F* and *Y14R,* and *DYZA-1* and *DYZB-1*, for $SRY_1$, $DYZ_1^{1st}$ and $DYZ_1^{2nd}$ genes were used with the sequences and conditions summarized in Table 1. Extracted cell-free plasma DNA (5 μL) was used as the template for PCR reaction. Each sample was analyzed in duplicate. PCR was carried out in a Perkin-Elmer Applied Biosystems 7700 Sequence Detector (Perkin-Elmer Corporation, Foster City, CA, USA). The compositions and conditions of the PCR assays for each of these three genes is described in Table 1.

Specificity, reproducibility and sensitivity of PCR products were analyzed using plasma samples from 10 healthy male and 10 healthy female volunteers for the present study. DNA was extracted and used to make PCR products with the aforementioned three primers and PCR reactive conditions. The specificity of the PCR products was determined between male and female blood samples. Reproducibility was evaluated by three separate examinations for each person's samples. For sensitivity of the PCR products, the DNA concentration to be used for each PCR product was gradually diluted from 1:1 to 1:1 × $10^7$ to define the sensitivity of the PCR amplification at the fixed condition described in Table 1. Microchimerism positive was defined as an individual whose blood sample was positive at least once among several examinations.


http://www.asiaandro.com; aja@sibs.ac.cn

Table 1. PCR primer sequences and reactive conditions. PCR, polymerase chain reaction.

| Genes/Locus | Primer sequences | PCR conditions |
|---|---|---|
| $SRY_1$ | 5'-CAGTGTGAAACGGG-AGAAAACAGT-3' | Denature: 94ºC, 5 min |
| | 5'-CTTCCGACGAGGTC-GATACTTATA-3' | Cycling profile: (94ºC, 30 s; 60ºC, 30 s) × 30 cycles |
| | | Extend: 72ºC, 1 min |
| $DYZ_1^{1st}$ | 5'-AATTTGAGCATTCG-TGTCCATTCT-3' | Denature: 94ºC, 2 min |
| | 5'-AATGCCCTTGAATT-AAATGGACT-3' | Cycling profile: (94ºC, 30 s; 60ºC, 30 s) × 20 cycles |
| | | Extend: 72ºC, 30 s |
| $DYZ_1^{2nd}$ | 5'-CGAGTCCATTCCAT-TACCGT-3' | Denature: 94ºC, 2 min |
| | 5'-CGGAATGGAATGCA-ACGCAA-3' | Cycling profile: (94ºC 30 s, 60ºC 30 s) × 25 cycles |
| | | Extend: 72ºC, 1 min |

*2.4 Statistical analysis*

Data were presented as mean ± SD for each group. SPSS 8.0 statistical analysis software was used for the statistical analysis using the unpaired *t*-test for quantitative data and the $\chi^2$-test for quality data. A significant difference is accepted when $P < 0.05$.

## 3 Results and discussion

*3.1 Assay precision*

To analyze the specificity of the PCR products, all samples from the 10 men showed three positive bands at 270 bp, 1 024 bp and 674 bp for $SRY_1$, $DYZ_1^{1st}$ and $DYZ_1^{2nd}$, respectively; whereas all samples from the 10 females were negative for these three genes (Figure 1A). The reproducibility was confirmed by the consistent positive and negative results for three separate examinations of each sample for the three genes (data not shown). Serial dilutions of DNA extracted from a man indicated that for $SRY_1$ PCR amplification the positive products were obtained until 1:1 × $10^5$ DNA dilutions (Figure 1B), for $DYZ_1^{1st}$ until 1: 1 × $10^5$ dilutions (Figure 1C) and for $DYZ_1^{2nd}$ until 1:1 × $10^6$ dilutions (Figure 1D). Based on these results of normal healthy volunteers, the detection of $DYZ_1^{2nd}$ using PCR seems the most sensitive marker. This is similar to the results reported by Tajik *et al*. [6] and by McDiabiel *et al*. [8] using PCR to detecting Y-chromosome markers.

*3.2 Existence of microchimerism in renal-transplanted recipients*

None of the samples collected from the 126 patients before they received male renal grafts showed the occurrence of positive microchimerism for any of the three probes; however, 96 of them (77%) had detectable three Y-chromosome genes in their blood samples at different times after renal transplantations. Based on the present study, we do not have direct evidence to indicate that the occurrence of microchimerism directly attributed to male grafts; however, the occurrence of positive microchimerism is unlikely attributed to the recipient's husband or to having-carried male babies becuase all healthy women as controls showed negative results for the detection of the Y-chromosome-gene-related microchimerism, even though they were married and some had delivered male babies, as mentioned above.

Most of the microchimerism-positive recipients showed positive results for all three PCR products (Figure 2A), except for a small number of recipients who showed either one or two of these three probes as positive. Sampling times varied among the patients depending on how long the patients survived after renal transplantations. We have randomly analyzed 42 microchimerism-positive patients of a total of 60 male-kidney recipients within a certain year for the first time of occurrence of positive microchimerism (Figure 2B). In general, two peaks of the positive rate could be found in these patients: one around 2 weeks and another one approximately 3–4 months after renal transplantation; and then a relative rate was maintained. The two peaks of the positive rate for the occurrence of microchimerism have been documented in a previous study [8]. In that study, they found that for patients without rejection ($n = 7$) within 1 year after receiving male kidneys, there was a peak of donor-

*Tel: +86-21-5492-2824; Fax: +86-21-5492-2825; Shanghai, China*

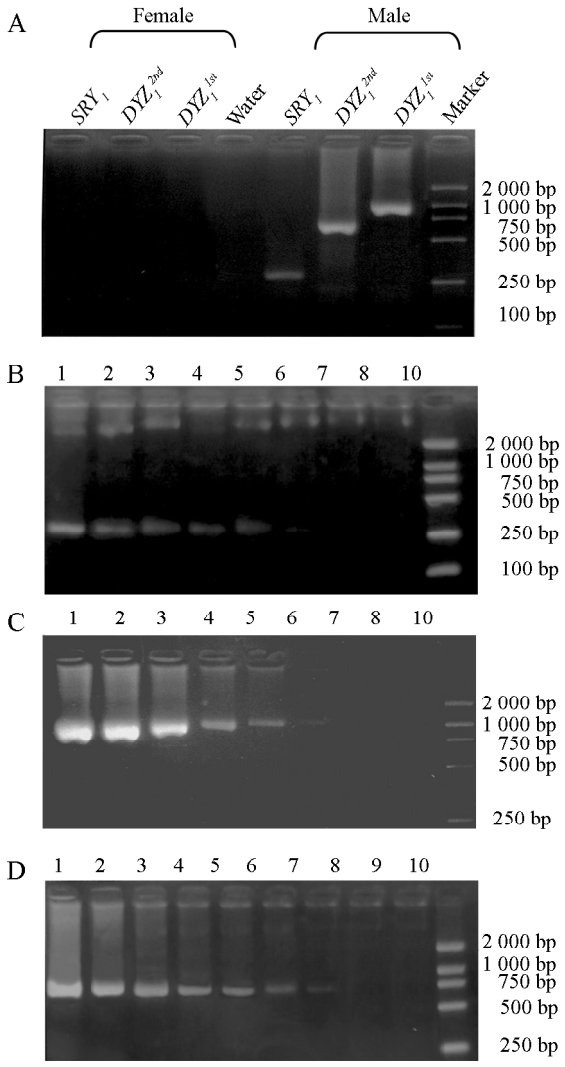


Figure 1. Detection of microchimerism by Y-chromosome markers using polymerase chain reaction (PCR). (A): Y-chromosome markers, $DYZ1^{1st}$ (1024 bp), $DYZ1^{2nd}$ (674 bp) and *SRY* (270 bp) were all detected in men and not in women. (B)–(D): The sensitivity to detecting three PCR products (B: $SRY1$, C: $DYZ1^{1st}$ and D: $DYZ1^{2nd}$); 1, $1:1 \times 10^0$; 2, $1:1 \times 10^1$; 3, $1:1 \times 10^2$; 4, $1:1 \times 10^3$; 5, $1:1 \times 10^4$; 6, $1:1 \times 10^5$; 7, $1:1 \times 10^6$; 8, $1:1 \times 10^7$.

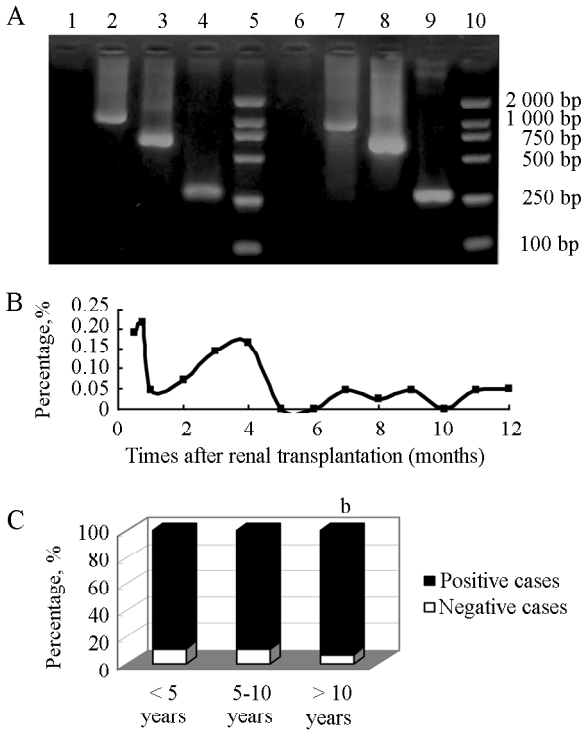


Figure 2. Female recipients with male kidney-transplants showed the existence of donor-DNA microchimerism as Y-chromosome markers. (A): The gel profiles of 2 female recipients with male kidney-transplants. (B): The first occurring times of the positive microchimerism among 42 randomly selected microchimerism-positive recipients of a total of 60 women receiving male kidneys within a certain year. Y axis represents the percentage of patients who were found for the first time to be positive for any of the three probes to the total 42 microchimerism-positive patients within 1 year. (C) presents the quantitative analysis for the association of the donor-DNA microchimerism positive rate with recipient survival times. $^bP < 0.05$ compared with other two groups. 1, $1:1 \times 10^0$; 2, $1:1 \times 10^1$; 3, $1:1 \times 10^2$; 4, $1:1 \times 10^3$; 5, $1:1 \times 10^4$; 6, $1:1 \times 10^5$; 7, $1:1 \times 10^6$; 8, $1:1 \times 10^7$.

DNA at 1–3 weeks post-transplantation followed by a second peak between 3 weeks and 4 months.

In addition, among the 126 recipients, 8 recipients who had not experienced acute rejection and also experienced better general health after the renal transplantation only showed positive PCR product of $DYZ1^{2nd}$ gene. This supports the concept that PCR product of $DYZ1^{2nd}$ gene might be the most sensitive marker.

Compared to previous studies, the present study shows a high rate of microchimerism-positive renal-transplant recipients (77%). For instance, Tajik *et al.* [6] examined 20 male-to-female renal allograft recipients for up to 30 months (2.5 years). Microchimerism was detected in 13 (65%) of the 20 recipients. Among the 60 kidney transplant recipients, examined for up to 1.5 years, Pujal *et al.* [10] found 43.7% with microchimerism. Microchimerism-positive rates in both studies were significantly lower than those in the present study (Table 2). This discrepancy might be a result of the different follow-up times because longer follow-up periods after renal transplantation might provide more opportunities for

Table 2. Comparison between microchimerism-positive and microchimerism-negative groups for clinical outcomes of transplanted kidneys (total cases: 126). $^{b}P < 0.05$ compared with corresponding negative groups.

| | Microchimerism | |
|---|---|---|
| | Positive | Negative |
| Case number (%) | 97 (77%) | 29 (23%) |
| Average survival time of transplanted kidneys (year) | 8.7 ± 3.5[b] | 5.4 ± 3.3 |
| Cases with renal rejection | 10 (10.3%)[b] | 8 (27.6) |
| Blood creatinine (mmol/L) | 74.3 ± 32.5[b] | 113.6 ± 37.8 |
| Annual times of infections for each person (year) | 1.3 | 1.5 |

the positive detection of the microchimerism in the recipients. As documented in previous studies [4–6], and as indicated in the present study (Figure 2B), microchimerism is a dynamic process, and the possibility of detecting microchimerism is significantly increased with a longer follow-up period.

### *3.3 Clinical outcomes of transplanted kidneys in microchimerism-positive recipients*

As summarized in Table 2, the survival time of transplanted kidneys was significantly higher in the microchimerism-positive recipients (8.7 years) than that in the microchimerism-negative recipients (5.4 years). The rate of patients with transplant rejection was significantly lower in the microchimerism-positive recipients than that in the microchimerism-negative recipients. There was no difference for the infection frequency per year between the two groups of recipients. However, the serum creatinine levels, measured at 1 year after transplantation, were significantly lower in the microchimerism-positive recipients than those in the microchimerism-negative recipients (Table 2). Regarding survival times of transplanted kidneys relative to microchimerism-positive incidence, approximately 89.0% of the patients who survived for 10 years or less after having transplanted kidneys were microchimerism-positive, whereas 93.0% of the patients who survived for more than 10 years were microchimerism-positive (Figure 2C). In the patients with the longest survival time (more than 10 years) the microchimerism-positive rate is significantly higher than that in the group with a survival time being either 5–10 years or less than 5 years ($P < 0.05$, Figure 2C). Furthermore, the patients who survived for a relatively longer time after renal translation and who were microchimerism-positive were randomly distributed in the four immunosuppression-therapeutic regimens; that is, the longer survival or positive microchimerism is not related to the difference of immunosuppressing regiments.

The present study used a larger number of renal recipients and longer observation post-transplantation period than previous studies. There are several case reports indicating that a microchimerism-positive finding in the recipients of renal transplantation is an index of acceptance of transplanted kidney, as shown by relative longer survival time of transplanted kidneys in the recipients [6, 8, 10]. Tajik *et al*. [6] documented that among the 20 recipients in their study only 3 recipients had an episode of acute rejection during the first week after transplantation, and all were in the non-microchimerism group. Pujal *et al*. [10] analyzed 51 renal transplant recipients for rejection within 18 months (1.5 years) and found that 11.5% of microchimerism-positive recipients showed rejection, whereas 28% of microchimerism-negative recipients showed rejection. The preliminary findings from these two small case studies were further confirmed by the present, large-case study (Table 2).

In addition, the positive correlation of microchimerism with the acceptance of the transplanted organs was also evident in recipients with other organ transplants. Pujal *et al*. [10] analyzed 17 heart recipients and found a microchimerism-positive rate of 47% (8 of 17) in these patients. The rejection rate was 25% in the microchimerism-positive recipients and 57% in the microchimerism-negative patients ($P < 0.05$). Araujo *et al*. [11] recently reported that 71.9% of 32 patients with liver transplant presented positive microchimerism. Among the 23 microchimerism-positive recipients, 16 did not experience rejection and only 7 recipients showed rejection. In contrast, among the 9 (28.1%) microchi merism-negative patients, 7 experienced rejection and 2 did not. Therefore, a negative correlation of microchipmerism-positivity with transplanted organ-rejection was significant based on these studies.

In summary, the detection of donor-DNA from the

*Tel: +86-21-5492-2824; Fax: +86-21-5492-2825; Shanghai, China*

blood of recipients with renal transplantation was positively associated with the immunotolerance of transplanted organs in the recipients. Although the exact mechanisms by which microchimerisms were formed remain largely unknown [10–15], the microchimerism were proposed to be derived from kidney cells, organ-contained leukocytes or blood stem cells [15]. From a clinical view, the microchimerism might be one of several immunological mechanisms that lead to long-term graft survival. Combined with recent advances in inducing transplantation tolerance using donor-bone marrow infusion with organ transplantation [12–14], microchimerism leading to transplantation tolerance will be of utmost importance for future clinical application and remains to be further explored [15].

## References


1 Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. Lancet 1992; 339: 1579–82.

2 Starzl TE, Demetris AJ, Trucco M, Ricordi C, Ildstad S, Terasaki PI, *et al*. Chimerism after liver transplantation for type IV glycogen storage disease and type 1 Gaucher's disease. N Engl J Med 1993; 328: 745–9.

3 Starzl TE, Demetris AJ, Trucco M, Zeevi A, Ramos H, Terasaki P, *et al*. Chimerism and donor-specific nonreactivity 27 to 29 years after kidney allotransplantation. Transplantation 1993; 55:1272–7.

4 Schlitt HJ, Hundrieser J, Hisanaga M, Uthoff K, Karck M, Wahlers T, *et al*. Patterns of donor-type microchimerism after heart transplantation. Lancet 1994; 343: 1469–71.

5 Elwood ET, Larsen CP, Maurer DH, Routenberg KL, Neylan JF, Whelchel JD, *et al*. Microchimerism and rejection in clinical transplantation. Lancet 1997; 349: 1358–60.

6 Tajik N, Singal D, Pourmand G, Ebrahimi-Rad M, Radjabzadeh M, Tavasoli P, *et al*. Prospective study of microchimerism in renal allograft recipients: Association between HLA-DR matching, microchimerism and acute rejection. Clin Transplant 2001; 15: 192–8.

7 Sahota A, Yang M, McDaniel HB, Sidner RA, Book B, Barr R, *et al*. Evaluation of seven PCR-based assays for the analysis of microchimerism. Clin Biochem 1998; 31: 641–5.

8 McDaniel HB, Yang M, Sidner RA, Jindal RM, Sahota A. Prospective study of microchimerism in transplant recipients. Clin Transplant 1999; 13: 187–92.

9 Xu LG, Shi SF, Qi XP, Huang XF, Xu HM, Song QZ, *et al*. Morphological characteristics of spermatozoa before and after renal transplantation. Asian J Androl 2005; 7: 81–5.

10 Pujal JM, Grinyo JM, Manito N, Gil-Vernet S, Hueso M, Caldes A, *et al*. Influence of hematopoietic microchimerism in organ tolerance after kidney or heart transplantation. Transplant Proc 2003; 35: 1775–7.

11 Araujo MB, Leonardi LS, Boin IF, Leonardi MI, Magna LA, Donadi EA, *et al*. Development of donor-specific microchimerism in liver transplant recipient with HLA-DRB1 and -DQB1 mismatch related to rejection episodes. Transplant Proc 2004; 36: 953–5.

12 Strober S, Lowsky RJ, Shizuru JA, Scandling JD, Millan MT. Approaches to transplantation tolerance in humans. Transplantation 2004; 77: 932–6.

13 Cosimi AB, Sachs DH. Mixed chimerism and transplantation tolerance. Transplantation 2004; 77: 943–6.

14 Claas F. Chimerism as a tool to induce clinical transplantation tolerance. Curr Opin Immunol 2004; 16: 578–83.

15 Starzl TE. Chimerism and tolerance in transplantation. Proc Natl Acad Sci U S A 2004; 101 (Suppl 2): 14607–14.