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# EVALUATION OF CHIMERISM WITH DNA POLYMORPHISMS IN BONE MARROW TRANSPLANTATION*

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**Evaluation of chimeric status following allogenic BMT is an important tool for monitoring the replacement of host cells with donor cells and for determining the risk of relapse. Polymorphic DNA sequences can be used as powerful markers in identification of donor/recipient genotype differences, even between close relatives. Polymerase chain reaction (PCR) amplification of three variable number of tandem repeat (VNTR) loci and five single-locus polymorphisms (SLP) was used to identify chimerism in 40 recipient-donor pairs. Mixed chimerism was present in 11 patients, and complete chimerism in 29. This PCR method is a rapid and sensitive assay to detect engraftment and evaluate relapse potential, and thus is very useful in the clinical management of BMT patients.** *Key words: bone marrow transplantation, chimerism, polymerase chain reaction, polymorphism, VNTR, relapse.*

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Bone marrow transplantation (BMT) is the treatment of choice for some inborn errors of metabolism, and in many hematological and oncological diseases. BMT involves eradication of recipient stem cells by total body irradiation and/or high-dose chemotherapy followed by introduction of healthy donor bone marrow cells. This method creates a chimeric status in the patient's hematopoietic system. The patient may exhibit either "mixed chimerism" in which there is persistence of host hematopoietic cells along with donor cells, or "complete chimerism" in which there is complete conversion to the donor's cell type.

Several techniques have been used to evaluate chimerism following BMT, including analysis of protein polymorphisms, chromosomal studies and DNA typing[1-5]. These techniques have different sensitivities in clinical use. Recently, polymerase chain reaction (PCR) has been used for the rapid and sensitive analysis of human DNA variations that are inherited in a Mendelian fashion[6]. They include highly polymorphic, tandemly repetitive minisatellites and single-locus polymorphisms[7].

This study aimed to evaluate the clinical utility of PCR-based detection of human polymorphisms for the evaluation of chimerism in patients treated by allogenic BMT.

## Material and Methods

### *Patients*

A total of 40 recipient-donor pairs who underwent allogenic BMT for different diseases were evaluated for the development of chimerism. Informed consent was obtained from the recipient-donor pairs or parents who served as subjects prior to DNA analysis experiments. The diagnoses were as follows: aplastic anemia (AA) n = 3, thalassemia (T) n = 1, Fanconi anemia (FA) n = 1, chronic myeloid leukemia (CML) n = 14, acute lymphoblastic leukemia (ALL) n = 9, and acute non-lymphoblastic leukemia (ANLL) n = 12. Thirty-nine of the patients were from three different BMT units of İstanbul University. Of these, eight patients (Cases1-8) were from the BMT unit of the Department of Pediatrics, and 17 patients (Cases17-23, 29, 30, 33-35, 37, 39, 40) were from the Department of Internal Medicine at İstanbul Faculty of Medicine. Fourteen patients (Cases11-14, 16, 24, 28, 31, 32, 36, 38) were from the BMT unit of the Department of Internal Medicine at Cerrahpaşa Faculty of Medicine. The remaining patient (Case 9) was from the BMT unit of the Department of Internal Medicine at Marmara University Medical Faculty. All the patients were grafted from HLA-identical siblings. Patient data (disease type, age, sex, conditioning regimen, graft-versus-host disease, phase of BMT); DNA analysis results, such as time of PCR analysis, informative loci and chimeric status; and finally the present status are summarized in Table I.

## Table I: Clinical Characteristics of Patients and Results of Molecular Studies

| Case | Disease | Age | Sex (D/R) | Conditioning Regimen | GVHD | | Phase of BMT | Time of PCR Analysis | Informative Loci | Chimeric Status | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Acute | Chronic | | | | | |
| A. Pediatric Age-group | | | | | | | | | | | |
| 1. (KIT.5) | β-TM | 4 | M/M | TBI+CTX | – | – | – | +395 | HBGG, GYPA | MC | Alive |
| 2. (KIT12) | AA | 16 | M/F | TLI+CTX | – | – | – | +365 | GYPA | MC | Alive |
| 3. (KIT.7) | AA | 14 | F/M | TLI+CTX | – | – | – | +385 | LDLR, GYPA, GC | MC | Alive |
| 4. (KIT.8) | FA | 12 | M/F | TLI+CTX | I | – | – | +180 | D1S80, ApoB | MC | Died |
| 5. (KIT.6) | ALL | 8 | F/M | TBI+CTX | – | – | 2nd CR | +420 | D7S8 | MC | Alive |
| 6. (KIT.19) | ANLL | 5 | M/M | TBI+CTX | I | – | 1st CR | +180 | ApoB | CC | Relapse |
| 7. (KIT.9) | ANLL | 6 | M/M | TBI+CTX | – | – | 1st CR | +210 | PAH | MC | Alive |
| 8. (KIT.20) | CML | 10 | F/F | TBI+CTX | II | – | CP | +120 | PAH, ApoB | MC | Alive |
| 9. (KIT.21) | CML | 15 | M/F | TBI+CTX | I | – | 1st CR | +90 | ApoB | CC | Died |
| B. Adult Age-group | | | | | | | | | | | |
| 10. (KIT.13) | AA | 24 | M/M | ATG+Cy | – | – | – | +48 | HBGG, GC | MC | Alive |
| 11. (KIT.37) | ALL | 16 | F/F | TBI+Cy | – | – | 1st CR | +90 | LDLR,D7S8,GC,GYPA,Apo B | MC | Alive |
| 12. (KIT.14) | ALL | 20 | M/M | TBI+Cy | – | – | 1st CR | +325 | PAH | CC | Alive |
| 13. (KIT.25) | ALL | 23 | M/F | TBI+Cy | I | – | 1st CR | +90 | PAH | CC | Alive |
| 14. (KIT.28) | ALL | 26 | M/F | TBI+Cy | – | – | 1st CR | +240 | ApoB, PAH | CC | Alive |
| 15. (KIT.17) | ALL | 25 | M/F | TBI+Cy | I | – | 2nd CR | +75 | D7S8, GC | MC | Relapse |
| 16. (KIT.27) | ALL | 23 | M/M | TBI+Cy | – | – | 1st CR | +250 | D1S80 | CC | Alive |
| 17. (KIT.40) | ALL | 25 | M/M | TBI+Cy | I | – | 2nd CR | +720 | ApoB | CC | Alive |
| 18. (KIT.46) | ALL | 28 | M/M | TBI+Cy | – | – | 1st CR | +260 | D1S80 | CC | Died |
| 19. (KIT.16) | ANLL | 43 | F/M | Bu+Cy | I | – | 1st CR | +120 | GYPA, D7S8, GC | CC | Alive |
| 20. (KIT.29) | ANLL | 37 | M/M | Bu+Cy | – | – | 1st CR | +45 | D1S80 | CC | Alive |
| 21. (KIT.31) | ANLL | 18 | M/M | Bu+Cy | – | – | 1st CR | +110 | ApoB | CC | Alive |
| 22. (KIT.32) | ANLL | 29 | M/M | Bu+Cy+VP-16 | I | – | Relapse | +90 | D1S80 | CC | Alive |
| 23. (KIT.36) | ANLL | 29 | M/F | Bu+Cy+VP-16 | I | Lim. | 1st CR | +200 | D1S80 | CC | Alive |
| 24. (KIT.15) | ANLL | 18 | F/M | TBI+Cy | I | Lim. | 1st CR | +330 | GYPA, D7S8, GC | CC | Alive |
| 25. (KIT.24) | ANLL | 19 | F/M | TBI+Cy | I | Lim. | 1st CR | +425 | ApoB | CC | Alive |
| 26. (KIT.44) | ANLL | 23 | F/M | TBI+Cy | I | – | 1st CR | +210 | D1S80 | CC | Alive |
| 27. (KIT.49) | ANLL | 24 | F/F | Bu+Cy | II | – | 2nd CR | +120 | D1S80 | CC | Alive |
| 28. (KIT.50) | ANLL | 33 | M/F | Bu+Cy | – | – | 1st CR | +90 | D1S80 | CC | Alive |
| 29. (KIT.23) | CML | 30 | F/M | Bu+Cy | I | Ext. | AP | +119 | PAH | CC | Died |

Table I: Clinical Characteristics of Patients and Results of Molecular Studies

| Case | Disease | Age | Sex (D/R) | Conditioning Regimen | GVHD | | Phase of BMT | Time of PCR Analysis | Informative Loci | Chimeric Status | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Acute | Chronic | | | | | |
| 30. (KIT.26) | CML | 30 | F/F | TBI+Cy | I | Ext. | 2nd CP | +60 | ApoB | CC | Alive |
| 31. (KIT.11) | CML | 37 | F/M | TBI+Cy | – | – | 1st CR | +160 | PAH | MC | Died |
| 32. (KIT.30) | CML | 39 | F/F | TBI+Cy | – | – | 1st CR | +112 | ApoB | CC | Alive |
| 33. (KIT.22) | CML | 36 | M/F | Bu+Cy I | – | – | CP | +60 | ApoB | CC | Alive |
| 34. (KIT.34) | CML | 34 | M/M | TBI+Cy III | – | – | CP | +68 | PAH | CC | Died |
| 35. (KIT.39) | CML | 33 | F/F | Bu+Cy I | – | – | Relapse | +600 | PAH | CC | Alive |
| 36. (KIT.42) | CML | 35 | F/F | TBI+Cy | – | – | 1st CR | +120 | PAH | CC | Alive |
| 37. (KIT.43) | CML | 35 | F/M | TBI+CY | – | – | 1st CR | +180 | ApoB | CC | Alive |
| 38. (KIT.45) | CML | 29 | M/M | Bu+Cy I | – | – | CP | +90 | PAH | CC | Alive |
| 39. (KIT.38) | CML | 23 | M/M | Bu+Cy I | – | – | CP | +67 | D1S80 | CC | Alive |
| 40. (KIT.47) | CML | 25 | M/M | Bu+Cy I | – | – | CP | +90 | D1S80 | CC | Alive |

D : Donor
R : Recipient
Age of patients : Age in years at BMT
ANLL : Acute non-lymphoblastic leukemia
ALL : Acute lymphoblastic leukemia
CML : Chronic myeloblastic leukemia
AA : Aplastic anemia
FA : Fanconi anemia
β-TM : Beta thalassemia major
well : Disease free
Lim. : Limited
Ext : Extensive

MC : Mixed chimerism
CC : Complete chimerism
+ : Number of days after BMT (on which sample collected for analysis)
CR : Complete remission
CP : Chronic phase
AP : Accelerated phase
TBI : Total body irradiation
TLI : Total lymphoid irradiation
Bu : Busulphan
VP-16 : Etoposide
CTX and Cy : Cytoxane and Cyclophosphamide

### Conditioning Regimens

Twenty-six patients were treated with radiotherapy and chemotherapy, and the remaining 14 patients with chemotherapy alone (Table I).

### DNA Extraction

Genomic DNA was isolated from peripheral blood samples of donors and recipients (after BMT) by overnight proteinase K and sodium dodecyl sulfate digestion at 56 °C, ammonium acetate extraction and ethanol precipitation as described elsewhere[8]. The original genotype of the recipient was determined from DNA extracted from hair root cells. The hair roots were plucked, cut with scissors approximately 1 cm from the root, and rinsed in distilled $H_2O$ followed by absolute ethanol. After drying they were digested in 0.5 ml lysis buffer (NaCl 100 mM; disodium EDTA 25 mM) containing 50 µg/ml proteinase K and one percent SDS. DNA was extracted with 1.5 M NaCl and chloroform: isoamylalcohol (24:1), and precipitated with isopropanol as described elsewhere[9].

### PCR Analysis

As shown in Table I, eight genomic loci were analysed in this study. These include three VNTR and five SLP. The VNTR loci were phenylalanine hydroxylase (PAH) gene[10], apolipoprotein B (ApoB) gene[11], and D1S80 locus[12]. The remaining five loci were two or three allele polymorphisms: low-density lipoprotein receptor (LDLR)[13], glycophorin (GYPA)[14], hemoglobin G gammaglobulin (HBGG)[15], D7S8[16] and human group-specific component (GC)[17]. PCR reactions were performed in an automated thermal cycler (Perkin-Elmer, 480). Primers for PAH, D1S80 and ApoB loci were synthesized by a commercial company (Genomed Ltd.). AmpliType PM kits (Perkin-Elmer) were used for typing the LDLR, GYPA, HBGG, D7S8, and GC loci. For VNTR analysis, 0.5 µg of DNA was amplified in a final volume of 50 µL in the presence of 50 mmol/L KCl, 10 mmol/L tris-HCl pH 8, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 40 pmol of each primer and 0.5 U Taq DNA polymerase (Boehringer-Mannheim). The PCR conditions for the AmpliType PM kit were 60 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C for 32 cycles followed by a seven-minute extension at 72 °C; for PAH locus, 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C for 30 cycles, for D1S80 locus, 60 s at 94 °C, 60 s at 65 °C, and eight minutes at 72 °C for 30 cycles, and for ApoB locus, 60 s at 94 °C and six minutes at 60 °C for 32 cycles. The primers used are cited in the references.

After amplification of the PAH, D1S80 and ApoB loci, 20 µl of the sample volume was electrophoresed through a three percent agarose gel, stained with ethidium bromide and photographed under UV light. For the other five loci, amplified DNA samples were hybridized to strips onto which allele-specific oligonucleotide

probes were blotted. Specifically bound, amplified DNA was visualized by the enzymatic precipitation of biotin-labelled probes that give a color reaction. Determination of the genotypes was carried out as suggested by the manufacturer (Perkin Elmer Cetus, 1990). In order to determine the state of chimerism, the genotype of the recipient's post-BMT genotype was compared to the genotype of the hair root and the genotype of the donor.

## Results

In this study, polymorphic DNA markers were used to evaluate chimerism post-BMT in 40 donor-recipient pairs. Nine patients were in the pediatric age-group and 31 were adults. Genotype analyses were done at intervals during the 45 to 720 days post-BMT. Complete chimerism was documented in 29 patients and mixed chimerism in 11 (Table I). Figure 1 shows an example of complete chimerism detected by the PAH-VNTR polymorphism (Case 13).

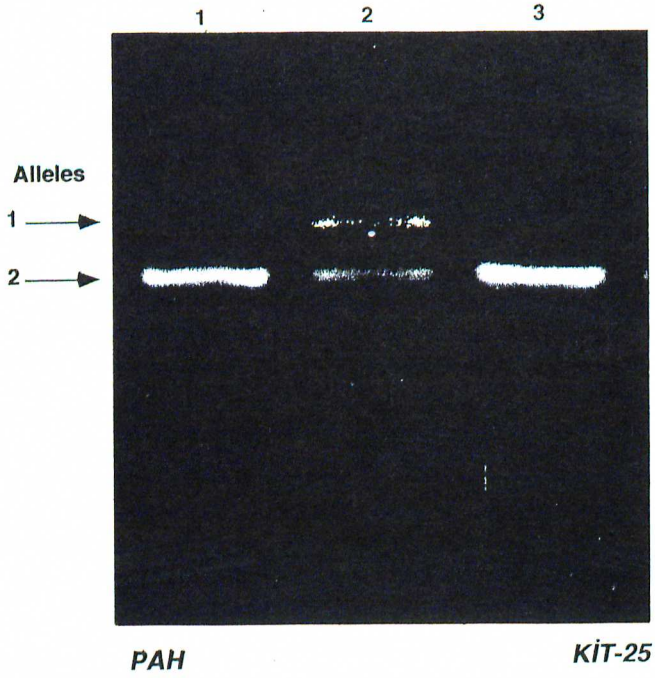


Fig. 1: An example of complete chimerism detected by PAH-VNTR polymorphism (Case 13).

Chimerism was evaluated initially using the VNTR polymorphisms. AmpliType PM analysis was used if the VNTR polymorphisms were not informative. VNTR polymorphism analysis alone was informative for 31 patients, while nine patients required analysis with the single-locus polymorphisms to determine chimerism.

Of the donor-recipient pairs, ApoB was fully informative in 13 pairs, D1S80 in 11 pairs, and PAH in 11 pairs. In nine cases requiring AmpliType PM analysis, GC and GYPA was informative in six pairs, D7S8 in five pairs, HBGG in two pairs, and LDLR in two pairs. In four patients (Cases 4, 8, 11, 14) single-locus typing was only partially informative. The evaluation of chimerism in these four patients required typing with two or more loci.

## Discussion

VNTR (ApoB, D1S80, PAH) and AmpliType PM (LDLR, GYPA, HBGG, D7S8, GC) analyses were successful in evaluating post-BMT chimerism in 40 recipient-donor pairs. This represents the largest series of bone-marrow-grafted patients analyzed with DNA markers in Turkey. Twenty-nine patients (72.5%) were mixed chimeric (MC). The CC/MC ratios in the adult and pediatric age-groups were different. Whereas 87 percent of the adult patients were CC, only 22 percent of the pediatric group were CC. Reports from different series of patients indicate the incidence of MC in the early phase of BMT to be approximately 50 percent when PCR is used. Disease type, conditioning regimen, and the time of PCR analysis are the factors that affect the chimeric status of the patients[18]. We believe these factors have been effective in our study group as well since MC/CC ratios differed between the adult and pediatric age-groups.

Several markers have been utilized to evaluate chimerism after BMT, including chromosomes, red blood cell antigens, immunoglobulin allotypes, cell enzymes, HLA typing, in-situ hybridization and DNA polymorphisms[1-5, 19]. PCR-based analysis of DNA polymorphisms is a very sensitive technique that is particularly useful in cases where the number of blood cells is low, for example during the early post-transplantation period and also during graft rejection. In addition, the use of highly polymorphic loci increases the power of discrimination in cases with HLA-identical, sex-matched donor/recipient pairs[1].

An important factor in the DNA-based evaluation of chimerism is the choice of polymorphic loci. Generally, VNTR polymorphisms are more informative and therefore more suitable for molecular analysis. In our study, the ApoB polymorphism was informative in 13 of 17 (76%) donor-recipient pairs, D1S80 was informative in 11 of 16 (68.7%) pairs, and PAH was informative in 11 of 24 (45%) pairs. Our standard protocol involves testing first with the ApoB and D1S80 loci followed by PAH typing if these are not informative. The AmpliType PM loci, although informative, are not routinely used because of their high cost.

Several groups have reported a correlation between mixed chimerism and leukemic relapse[20-22]. Generally the recipient's cells are responsible for the leukemic relapse[23]. Therefore, detection of these cells in the circulation may prove useful in monitoring patients susceptible to leukemic relapse[18]. At present

the significance of persistent low levels of recipient cells detected by PCR is not clear. The evaluation of the prognostic value and clinical significance of the DNA polymorphisms detected by PCR results will require further analysis of the BMT patients participating in this study for longer periods.

In conclusion, PCR-based detection of VNTR and AmpliType PM polymorphisms appears to be a sensitive and powerful technique for the evaluation of chimeric status in patients treated by BMT. Furthermore, polymorphic markers can be used instead of direct mutation-based tests to monitor relapse in hematopoietic malignancies, which represent a group of disorders that have a wide range of disease-specific genetic abberrations[23].

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