ORIGINAL ARTICLE

Chimerism monitoring following allogeneic hematopoietic stem cell transplantation

S Talwar[1], F Khan[1,2], S Nityanand[3] and S Agrawal[1]

[1]Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical School, Lucknow, Uttar Pradesh, India; [2]Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada and [3]Department of Hematology, Sanjay Gandhi Post Graduate Institute of Medical School, Lucknow, Uttar Pradesh, India

Information regarding the chimeric status of hematopoietic stem cell transplantation (HSCT) recipients is of great significance when comparing different conditioning and prophylactic therapies. In recent years, short tandem repeats/variable number tandem repeats (STRs/VNTRs) have emerged as the best tool for chimerism monitoring. However, the polymorphisms of STR/VNTR markers vary within and between ethnic groups. The issue is further complicated in a heterogeneous population such as occurs in the Indian subcontinent. In the present study, we attempted to devise a robust scheme to identify a set of polymorphic STRs/VNTRs most suitable for chimerism evaluation in north Indian HCST recipients. At first, we did genotyping of 11 STR and one VNTR in 1000 randomly chosen north Indian individuals to quantify different diversity parameters. Resulting data indicated that ApoB3$'$HVR, FES, VWA, D3S1358 and D16S310 were most polymorphic loci with the average heterozygosity being 0.756 ± 0.17. Furthermore, all markers were genotyped in 77 HLA-matched donor–recipient pairs to evaluate the informativeness in differentiating donor's and recipient's cells. A panel of seven markers (ApoB3HVR-D3S1358-HUM-THO1-VWF-1-D16S310-FES-VWA) differentiated 98.70% of donor–recipient pairs. This set of markers also successfully monitored the graft status in 14 HSCT cases during multiple time points following HSCT. The results were compared to the commercially available AmpF/STR SGM Plus multiplex PCR kit (Applied Biosystems, Foster City, CA, USA). Our findings established that the panel of seven markers we identified was more cost-effective and informative.
Bone Marrow Transplantation (2007) **39**, 529–535. doi:10.1038/sj.bmt.1705626; published online 5 March 2007
Keywords: chimerism; hematopoietic stem cell transplantation; STR; VNTR; heterozygosity; polymorphism information content

Correspondence: Dr S Agrawal, Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow, Uttar Pradesh, 226014, India.
E-mail: suraksha@sgpgi.ac.in

Introduction

Quantification and assessment of the pattern and distribution of genetic variation among different human populations is an essential step targeted in the Human Genome diversity program.[1] It was established that humans are considerably more similar to each other than other species, as any two randomly chosen humans differ at ~ 1 in 1000 nucleotide pairs.[2] However, there are ~ 3 billion nucleotides on the human haploid genome and therefore any two randomly chosen humans differ at ~ 3 million nucleotides that constitutes ~ 0.1–0.2% of the total genome.[3] This tiny genomic fraction leads to the disparity in human susceptibility to diseases and differential human response to pharmacological agents and produces varied phenotypes. The same fraction of genetic variation is also used for a range of applications including 'chimerism'-based graft monitoring following hematopoietic stem cell transplantation (HSCT).[4]

Allogeneic HSCT is considered to be the best treatment modality for various malignant and non-malignant hematological disorders. In human HSCT, complete donor-derived hematopoiesis is considered essential for sustained engraftment and for preventing relapse of the underlying disease.[5] The critical task following HSCT is the qualitative and quantitative examination of the donor-specific cells in the recipient's body. Such analysis is based on chimerism monitoring that varies from exclusively autologous cells to mixed (both autologous and donor cells) and complete chimerism (only donor cells). Chimerism monitoring has therefore become essential for identifying/predicting the success or failure of HSCT.[6]

The underlying principle of chimerism monitoring is differentiation of donor and recipient cells based on their genetic constitution. The genotypic profile of polymorphic genetic markers or those of their products in recipient and donor act as a specific tag to identify and quantify the presence of specific cell types in the post HSCT recipient. However, the key task would be in identifying the markers that have maximum chances of being informative (i.e. having a different genotype in donor and recipient). In the recent years, various methods including erythrocyte phenotyping,[7] RFLP[8] and fluorescence *in situ* hybridization[9] and quantitative real-time PCR[10] have been used to establish chimerism status. Fluorescent primer-based

530

PCR genotyping of highly polymorphic STRs/VNTRs is widely regarded as the most successful and robust genotyping technique,[11] largely because of the uniform repeat lengths and high degree of polymorphism of STR/VNTR markers with high sensitivity and quantification ability.

In the present study, we set out to develop a cost-effective and robust scheme for chimerism analysis for north Indian HCT patients based on the identification of a set of highly informative markers. Currently, more than 15 transplant centers carry out stem cell transplants in India and have used this potentially curative mode of therapy in upwards of 800 patients. Therefore, more dedicated efforts are required to identify a panel of markers that possess maximum probability of genetically differentiating HLA-matched donor/recipient pairs. The study requires more consideration in the Indian population, due in part to the high level of socio-cultural and geographical diversity often creating varied gene pools. The characteristic of genetic heterogeneity led to the screening of a significant number of markers culminating in an informative panel for chimerism evaluation.

We have designed our search directive towards an effective set of markers based on three criteria: the level of genetic diversity within randomly selected individuals, the informativeness of the markers in donor–recipient pairs and the success rate in graft monitoring. To meet each of these criteria, we analyzed a set of 11 markers (10 STR and one VNTR) in three groups: (i) 1000 randomly selected samples of varied socio-religious groups of north India by determining the allelic variability for each marker; (ii) 77 pairs of HLA-matched HSCT donor/recipient pairs by assessing the informativeness of each marker and (iii) we analyzed the identified set of most informative markers in 14 HSCT patients at different time periods following HSCT by assessing their success in monitoring different engraftment status. The findings were then compared with that of a commercially available kit to evaluate the cost-effectiveness of the identified set of markers.

Materials and methods

Subjects
To meet our objective, this study included 1077 subjects (1000 randomly selected north Indian individuals and 77 HSCT donor–recipient pairs, matched at all five HLA class I and II loci including those 14 patients that were transplanted at SGPGIMS, Lucknow since July 2000). Whole blood specimens were obtained from all the study subjects by peripheral venipuncture and approximately 5 ml of blood was collected in EDTA vacutainer tubes after obtaining informed consent from each subject. The study was performed following approval by the Institutional Ethical Reviewing Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow.

Random north Indian samples. A total of 1000 randomly selected individuals belonging to different socio-cultural strata were collected from different regions of the northern state (Uttar Pradesh) of India. All individuals were adult with a mean age of 38.8 ± 3.4 years and resident in Uttar Pradesh for the last three generations. Before collecting the blood specimens, regional addresses and detailed computerized list of the subjects were prepared. Random numbers were generated by computer and adults were questioned regarding their ethnicity and the birthplace of their parents. Unrelated subjects were considered eligible for participation in the study. The demographic profile and other ethnical and familial information were recorded in a detailed Performa. Three-generation pedigrees were prepared to assure non-related status of subjects.

HLA-matched HSCT donor–recipient pairs. A total of 167 families were registered with the Department of Hematology, SGPGIMS for HSCT from 1996 to 2006. Families of each patient were referred to the Department of Medical Genetics for HLA class I and II matching. Donors were identified for 77 patients based on complete matching at five HLA loci–class I (A, B and C) and class II (DRB1 and DQB1).

Patient treated with HSCT. A total of 14 patients transplanted with non-myeloablative regimens since July 2000 were analyzed for chimerism-based graft monitoring at different time intervals following HSCT. The graft monitoring was performed on the 14th day following transplantation, then by weekly intervals until 1 month and then every month for next 6 months, if complete chimerism was achieved. Six months post transplant, we began following the patients at 3-month intervals, provided complete chimerism persisted. In case of recipients with mixed chimerism, weekly chimerism evaluation was preferred.

DNA extraction
High molecular weight genomic DNA was extracted through a salting out method using phenol–chloroform and purified by ethanol precipitation.[12]

STR/VNTR genotyping
Genotyping methodology. A panel of 10 STR markers (Tho1, D3S1358, D16S310, F13A, TPO, FES, VWA, D4S243, DHFRP2 and VWF-1) and one VNTR (ApoB-3′HVR) were genotyped as described previously[13] using PCR-based locus-specific amplification. One primer for each marker was labeled with a fluorochrome–VIC (D4S243, VWA, D16S310), Ned (TPO, FES, DHFRP2) and 6-FAM (D3S1358, Tho1, F13A1, VWF-1 and ApoB). Six STR markers were co-amplified by two multiplex PCR. The remaining four STR and ApoB3HVR were amplified using flanking primers in a single PCR reaction. Size fractionation of the fluorochrome labeled amplicons was carried out by capillary electrophoresis in an ABI-310 PRISM fragment analyzer (Applied Biosystems, Foster City, CA, USA). Size calling of the alleles at individual locus was performed with GENESCAN v3.1.2 software using 500-ROX and 1000-ROX (ABI, USA) size standard. Once the size calling was completed, GENOTYPER v2.5.2

software was employed for assigning the allelic profile of an individual for each marker.

Statistical analysis

Allele frequencies were obtained through a direct counting method. Hardy–Weinberg equilibrium at genotypic frequencies for all markers was estimated using Fischer's exact test based on 1000 Markov-Chain algorithm steps with Arlequin v2 software. Bonferroni correction to the P-value was applied. Genetic diversity parameters like gene diversity, observed heterozygosity, polymorphism information content (PIC), power of exclusion (PE) and mean numbers of pair wise differences were estimated using the following software: Pop Gene v16, Cervus v2 and Arlequin v2.

Results

Analysis of 11 repeat loci in randomly selected north Indian individuals

Analysis of a panel of 11 autosomal repeat loci in 1000 randomly selected north Indian individuals has revealed high allelic variability and level of genetic diversity. A total of 111 alleles were observed that ranged from seven to nine at seven STR loci (Tho1, TPO, FES, VWA, D4S243, DHFRP2 and VWF-1) whereas $\geqslant 11$ alleles[11–15] were observed at the remaining three STR loci (D3S1358, D16S310 and F13A). In contrast, 16 alleles were observed only at ApoB'3HVR (Table 1). The average number of observed alleles was 10.09 and none of the 11 repeat loci exhibited significant departures form HWE values.

In order to categorize the individual marker or a set of markers for maximum individual identification potential, different parameters like average gene diversity, average observed heterozygosity, mean number of pair wise

differences, PIC and PE were calculated (Table 1). The mean average gene diversity was found to be as high as 0.781 ± 0.188. Individually, maximum average gene diversity was observed at ApoB-3′HVR (85%), followed by D3S1358 (84%), D16S310 (83%) and VWA (81%). The average observed heterozygosity (H_o) was found to be 0.756 ± 0.17. Locus wise average observed heterozygosity ranged between 0.835 at ApoB-3′HVR to 0.675 at F13A. A high value of mean number pair wise differences (9.46 ± 3.67) was also observed. PIC was calculated to determine the extent of polymorphism at each locus. It was observed that PIC varied from 0.646 at TPO to 0.840 at ApoB-3′HVR. The mean PIC value of the 11 repeat loci was found to be 0.758 suggesting high prospective of the analyzed set of markers in chimerism analysis. The PE was also calculated as an index of individualization potential of a set of markers. A high value of total PE (0.99915) was observed where ApoB-3′HVR (0.545) and D3S1358 (0.522) showed the maximum PE whereas TPO (0.283) showed the least.

Analysis of 11 repeat loci in 77 HLA-matched HSCT donor–recipient pairs

The 77 donor–recipient pairs matched at all five HLA class I and class II loci were genotyped for the same panel of 11 markers to identify the most informative marker (that has a different genotype in donor and recipient). The informativeness analysis revealed that individually, FES was most informative (56%) closely followed by ApoB3′HVR (54%) and VWF1 (52%) as shown in Table 2. Average informativeness over all 11 STR loci was 46.27%; however, total informativeness of all 11 STR loci, if analyzed together was 100%.

Furthermore, we calculated the percentage of informativeness for different combinations of STR/VNTR markers (Table 2). A group of markers was considered informative

Table 1 Locus wise values of genetic diversity indices evaluated among north Indian populations based on 11 STR/VNTR loci

Locus	Genetic diversity indices			
	Expected heterozygosity	Observed heterozygosity	PIC	Power of exclusion
THO1	0.785	0.765	0.765	0.417
D3S1358	0.840	0.807	0.824	0.522
D16S310	0.830	0.774	0.807	0.487
F13A	0.735	0.675	0.748	0.397
TPO	0.710	0.715	0.646	0.283
FES	0.788	0.775	0.772	0.438
VWA	0.810	0.795	0.783	0.447
D4S243	0.711	0.731	0.666	0.306
DHFRP2	0.746	0.699	0.742	0.390
VWF-1	0.782	0.752	0.725	0.406
ApoB3′HVR	0.850	0.835	0.840	0.545
Genetic diversity indices				
Total number of alleles			111	
Average gene diversity			0.782 ± 0.188	
Average observed heterozygosity			0.756 ± 0.170	
Mean number of pair wise differences			9.460 ± 3.670	
Mean polymorphism information content			0.758	
Total power of exclusion			0.999915	

Abbreviations: PIC = polymorphism information content; STR/VNTR = short tandem repeats/variable number tandem repeats.

Table 2 Informativity values of individual and group of STR/VNTR markers among 77 HSCT donor–recipient pairs

					Average % informativity					
Single locus[a]										
Apo	FES	VWF1	TPO	VWA	Tho1	D4S	D3S	F13	D16	DHF
54.0	56.0	52.0	43.0	50.0	51.0	32.0	48.0	49.0	51.0	36.0

Three locus			
APO-FES-VWF1	D4S-F13-TPO	Tho1-TPO-F13-TPO	D3S, D16, DHFRP2
92.2	83.1	81.1	79.2

Five locus[a]		
APO-FES-D3S-VWA-D16	APO-FES-D4S-Tho1-F13	APO-TPO-D4S-FES-DHF
96.1	93.5	92.2

Seven locus[a]		
APO-D3S-vWF1-FES-VWA-Tho1-D16	APO-D4S-TPO-VWA-FES-F13-VWF1	APO-Tho1-TPO-F13-D16-DHF-D4S
98.7	96.5	95.4

Abbreviations: HSCT = hematopoietic stem cell transplantation; STR/VNTR = short tandem repeats/variable number tandem repeats.
[a]Apo = ApoB3′HVR; D4S = D4S243; D3S = D3S1358; F13 = F13A; D16 = D16S310; DHF = DHFRP2.

if any marker of that group was found to be informative in a donor–recipient pair. Significantly, average informativeness revealed average number of donor–recipient pairs for which a particular group of markers was informative. The 3 STR group comprising ApoB3′HVR, FES and VWF1 exhibited maximum informativeness (92.2%). In comparison, the group of D3S1358, D16S310 and DHFRP2 was least informative (79.2%). The five STR group consisting of ApoB3′HVR, FES, D3S1358, VWA and D16S310 was informative for 96.10% of donor–recipient pairs. The group of seven markers carrying ApoB3′HVR, D3S1358, VWF1, FES, VWA, Tho1 and D16S310 enhanced the informativeness index to 98.70%. This panel of seven markers (six STR and one VNTR) was considered to be the most effective set for chimerism monitoring. No further elevation in the informativeness index was observed when an additional pair of markers was included. In addition, the combination of four and six loci did not reveal significant changes in informativeness when compared to the set of three or five loci, respectively.

*Analysis of the panel of seven STR in 14 HSCT cases*
The selected panel of the most informative seven markers (ApoB3′HVR, D3S1358, VWF1, FES, VWA, Tho1 and D16S310) was then used for graft monitoring in 14 recipients following HSCT. The graft monitoring was performed at varying intervals beginning at the 14th day after transplant. The identified panel of seven markers was 100% successful in differentiating donor's cells from those of the recipient during all the stages after engraftment.

Among those 14 HSCT cases transplanted at SGPGIMS, we identified three different chimeric stages: complete chimerism, mixed chimerism and only recipient cells in the span of 3 years. We also observed that four markers each (in the set of seven markers) were informative in eight donor–recipient pairs, whereas five and six markers, respectively, were informative in three pairs each.

Discussion

The search for a HLA compatible donor to facilitate a successful HSCT has been the major area of concern for the transplantation geneticists in the last few decades. However, in the recent years efforts have been made to identify an ideal chimerism study tool, which could not only evaluate the exact status of engraftment but also assist in deciding the treatment modalities. In the present study, we analyzed the use of 11 polymorphic STR/VNTR markers in randomly selected north Indian individuals and HLA-matched donor–recipient pairs to identify a highly informative and successful STR/VNTR panel for chimerism studies. We identified a set of seven markers (ApoB3HVR, D3S1358, VWF1, FES, VWA, Tho1 and D16S310), which differentiated 98.70% of the 77 HLA matched donor–recipient pairs and successfully monitored the status of engraftment in 14 cases at different time points following HSCT. These results have major implications considering the significant rise in the HSCT performed within India during the last decade. The importance increases as clinicians consider non-myeloablative transplantation[14] and T-cell depletion HSCT or peripheral blood stem cell transplantation (PBSCT) instead of the traditional HSCT approach (myeloablative HSCT). In such cases, chimerism monitoring is a prerequisite for manipulating engraftment by altering patient immunosuppression and donor's lymphocyte infusion.[10,14]

Both random population and donor–recipient pair analysis was considered necessary to identify an informative set of marker before assessing its success in chimerism monitoring. This tripartite analysis aided in developing a robust scheme and a reliable method of chimerism evaluation, which could be effective for a large set of populations irrespective of the genetic or socio-cultural heterogeneity existing between sects and sub-groups. In the first phase of analysis, we genotyped 11 markers in 1000 randomly selected north Indian subjects and identified a high range of allelic variability. A total of 111 observed alleles with as many as 16 alleles were observed at
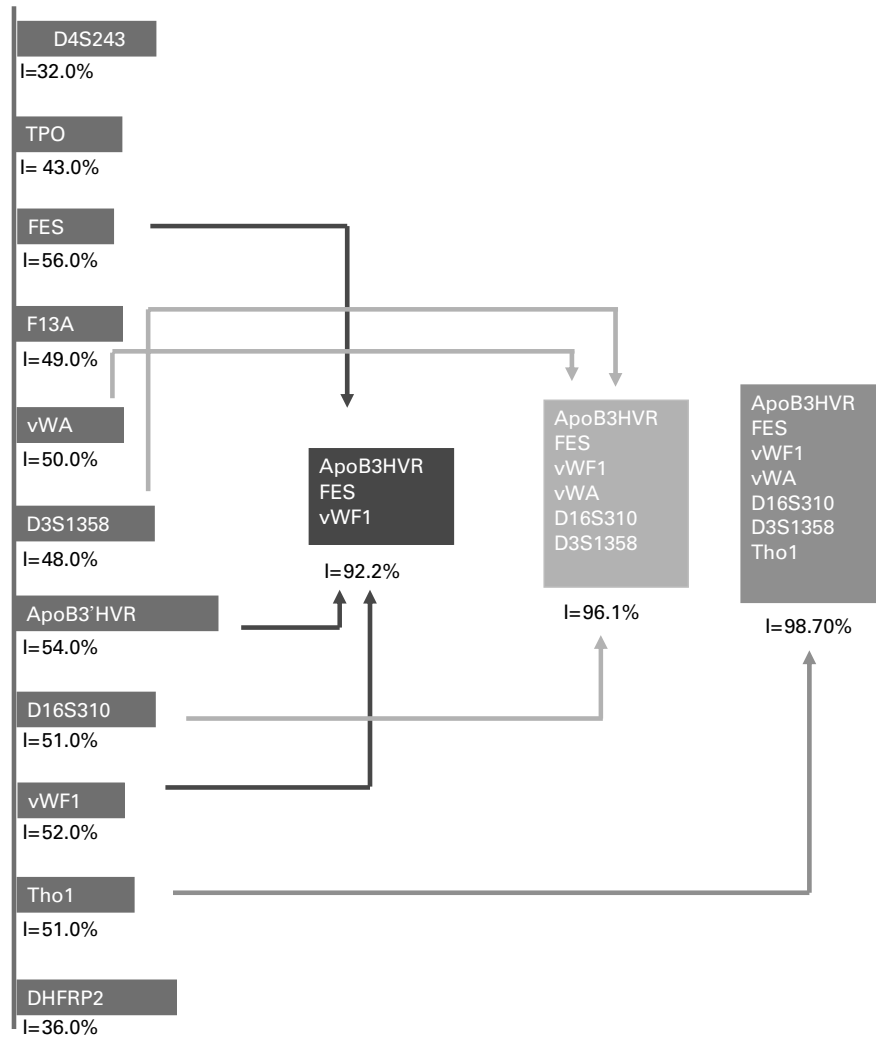
Figure 1 Algorithm of seven STR/VNTR for chimerism evaluation.

ApoB3′HVR, which is in accordance with other studies.[15] STR and VNTR markers are the most accepted regions of the human genome for assessing the levels of genetic diversity owing to their high mutation rates and high degree of polymorphism.[16] We also calculated different diversity parameters in order to quantify the range of polymorphism and genetic diversity. The analysis helped in categorizing an individual marker or set of markers that would have maximum individual identification potential. The majority of STR loci are known to possess a heterozygosity level of more than 60%,[16] possibly accounting for their success in chimerism monitoring. The average observed heterozygosity was found to be 0.756 ± 0.17 confirming the findings of other studies based on different Indian populations.[17–18] Other parameters (PIC and PE) also indicated a relatively equal distribution of large number of alleles and an optimistic potential for success in chimerism monitoring. The random population analysis determined that individually ApoB3′HVR, D3S1358, FES, VWA and D16S310 possess maximum variability and chimerism evaluation potential.

Markers with high informativeness for chimerism studies were selected from the second step of analysis carried out in 77 HLA-matched donor–recipient pairs. Three markers, FES, ApoB3′HVR and D3S1358 were found to be most informative. The findings corroborated the population study results; however, the individual locus differentiated only up to 56% of donors and recipients pairs. Interestingly, two more loci VWF1 and Tho1 also exhibited high informativeness despite depicting less diversity in the random population analysis. Sellathamby et al.,[11] in their work on 310 recipient-donor pair, has also shown that Tho1 and VWF1 exhibited an informativeness of ~54%. The informativeness percentage of different marker groups was calculated based on the informativeness of each locus. A set of seven markers (ApoB3HVR, D3S1358, VWF1, FES, VWA, Tho1 and D16S310) was found most informative with total informativeness being 98.70%. Recently, Thiede et al.[19] have calculated the informativeness of 27 STR markers in 203 matched related donor–recipient pairs and found Penta E and SE33 (ACTBP2) to be highly informative. Similarly, Hassan et al.[20] studied eight STR/VNTR markers in a patient/donor cohort ($n = 195$) and found that at least one informative marker was detected in 177 pairs (91%). Sellathamby et al.[11] attempted to develop an algorithm

534

based on the analysis of five markers. They reported the combined informativeness of 95% in a cohort of 310 patient–donor pair. Our attempt to develop such an algorithm (Figure 1) was based on the cumulative results obtained from the three-level analysis of both random north Indian population and HLA-matched donor–recipient pairs. This approach magnified the probability of the identified STR/VNTR panel to be more successful for larger set of populations. The population study identified ApoB3′HVR, D3S1358, FES, VWA and D16S310 as the most valuable markers based on different diversity parameters whereas patient–donor pair analysis identified Tho1 and VWF1 as other two compelling markers based on their high informativeness index. Finally, the panel of seven STR was 100% successful in differentiating donors cells from that of recipient at all the stages after engraftment. Three chimerism stages: complete chimerism (only donor's cells), mixed chimerism (both donor's and recipient's cells) and no chimerism or relapse of the disease (only recipient's cell) were identified with 100% accuracy.

In order to evaluate the cost-effectiveness of the panel of seven markers in graft monitoring, the results were compared from the 14 HSCT cases using SGM plus multiplex PCR kit. Our findings indicated that SGM plus PCR kit was informative in 11 HSCT cases (78.5%) in comparison with the 100% informativeness of our panel. The fragment size calling was >99% accurate and precise with both the SGM kit and our panel of markers. Additionally, the cost of analysis of seven markers as multiplex system (two PCR and one electrophoresis run using 1000 ROX as size standard) was less than $5 per sample using ABI 310 genetic analyzer. Moreover, we also successfully genotyped all seven markers by separating the amplicons of each locus individually using 10% PAGE followed by the silver staining. This would provide an option of manual allele scoring in labs where an automated fragment size analyzer is not available.

In conclusion, the identification of the panel of seven markers based on the chimerism evaluation algorithm developed in the present study can be a useful, practical and reliable tool for chimerism monitoring following allogeneic HSCT.

Acknowledgements

References

1 Cavalli-Sforza LL, Feldman MW. The application of molecular genetic approaches to the study of human evolution. *Nat Genet* 2003; **33** (Suppl): 266–275.

2 Fischer A, Wiebe V, Paabo S, Przeworski M. Evidence for a complex demographic history of chimpanzees. *Mol Biol Evol* 2004; **21**: 799–808.

3 Jorde LB, Wooding SP. Genetic variation, classification and 'race'. *Nat Genet* 2004; **36**: S28–S33.

4 Khan F, Phadke S, Nityanand S, Talwar S, Agrawal S. Use of ApoB′ hyper variable region in studying mixed chimerism and maternal contamination in North Indian populations. *J Clin Forensic Med* 2004; **11**: 183–188.

5 Slavin S, Nagler A, Kapelushnik Y, Aker M, Cividalli G, Varadi G et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.

6 Khan F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. *Bone Marrow Transplant* 2004; **34**: 1–12.

7 Schaap N, Schattenberg A, Bar B, Mensink E, de Man A, Geurts van Kessel A et al. Red blood cell phenotyping is a sensitive technique for monitoring chronic myeloid leukaemia patients after T-cell-depleted bone marrow transplantation and after donor leucocyte infusion. *Br J Haematol* 2000; **108**: 116–125.

8 Roy DC, Tantravahi R, Murray C, Dear K, Gorgone B, Anderson KC et al. Natural history of mixed chimerism after bone marrow transplantation with CD6-depleted allogeneic marrow: a stable equilibrium. *Blood* 1990; **75**: 296–304.

9 Seong CM, Giralt S, Kantarjian H, Xu J, Swantkowski J, Hayes K et al. Early detection of relapse by hypermetaphase fluorescence *in situ* hybridization after allogeneic bone marrow transplantation for chronic myeloid leukemia. *J Clin Oncol* 2000; **18**: 1831–1836.

10 Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 2002; **99**: 4618–4625.

11 Sellathamby S, Balasubramanian P, Sivalingam S, Shaji RV, Mathews V, George B et al. Developing an algorithm of informative markers for evaluation of chimerism after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2006; **37**: 751–755.

12 Coomey CT, Koons BW, Presley KW, Smerick JB, Sobieralski CA, Stanley DM. DNA extraction strategies for amplified fragment length polymorphism analysis. *J Forensic Sci* 1994; **39**: 1254–1269.

13 Perez-lezaun A, Calafell F, Mateu E, Comas D, Bosch E, Bertranpetit J. Allele frequency for 20 microsatellites in a worldwide population survey. *Hum Hered* 1997; **47**: 189–196.

14 Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood* 1999; **94**: 3234–3241.

15 Frossard PM, Lestringant GG. Analysis of an apolipoprotein B gene 3′ hypervariable region among Abhu Dhabi emirates. *Ann Saudi Med* 2001; **19**: 490–494.

16 Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA et al. Genetic structure of human populations. *Science* 2002; **298**: 2381–2385.

17 Agrawal S, Muller B, Bharadwaj U, Bhatnagar S, Sharma A, Khan F et al. Microsatellite variation at 24 STR loci in three endogamous groups of Uttar Pradesh, India. *Hum Biol* 2003; **75**: 97–104.

18 Das B, Chauhan PS, Sheshadri MS. Genetic polymorphism study at four minisatellite loci (D1S80, D17S5, D19S20 and

APOB) among five Indian population groups. *Hum Biol* 2002; **74**: 345–361.

19 Thiede C, Bornhauser M, Ehninger G. Evaluation of STR informativity for chimerism testing–comparative analysis of 27 STR systems in 203 matched related donor–recipient pairs. *Leukemia* 2004; **18**: 248–254.

20 Hassan R, Bonamino MH, Braggio E, Lobo AM, Seuanez HN, Tabak DG *et al.* A systematic approach to molecular quantitative determination of mixed chimerism following allogeneic bone marrow transplantation: an analysis of its applicability in a group of patients with severe aplastic anaemia. *Eur J Haematol* 2004; **73**: 156–161.