

## Research Article

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# Study of whole blood and T cell chimerism in monitoring HLA identical allogeneic hematopoietic transplantation

## Abstract

Chimerism analysis is an important method for monitoring outcome of allogenic hematopoietic stem cell transplantation. Variable number tandem (VNTR) analysis is considered an informative technique to follow up chimerism state. The aim of the work is to study certain number of VNTRs to identify their potential value in the detection of chimerism in transplanted patients in conventional ablative transplants. Also to demonstrate the value of T cells subset analysis in detecting transplantation outcomes. This study included 17 pairs undergoing HSCT. Informative loci pre-transplantation using five VNTR loci and two gene loci were identified. After transplantation the informative loci were used to detect chimerism status. After DNA extraction from blood samples, amplification of VNTR loci was performed using a conventional PCR protocol. Extra sample post transplantation was collected and pre-treated with resetting technique in order to separate T cells, its product was subjected to DNA extraction then amplification of informative loci was done. Amplified product of DNA samples was run on 2% agarose gel stained using ethidium bromide. Fourteen recipients showed full done chimerism in both whole blood and T cells separated cells, one recipient died after HSCT, one recipient showed split chimerism and one pair failed to detect informative locus. VNTR analysis using a panel of five loci is suitable to detect state of chimerism after HSCT.

**Keywords:** VNTR, T cell, hematopoietic, chimerism, resetting

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative procedure for a variety of haematological malignant and non-malignant diseases.<sup>1,2</sup> Successful outcome after allogeneic bone marrow transplantation has been correlated with a state of stable hematopoietic chimerism (full donor chimerism).<sup>3</sup> In this context, chimerism analysis is an important method in monitoring post-hematopoietic stem cell transplantation outcomes.<sup>4-6</sup> Many techniques have been used to detect chimerism statuses such as single-nucleotide polymorphism (SNP), short tandem repeats (STR), restriction fragment length polymorphism (RFLP) and variable number tandem repeats (VNTR).<sup>7,8</sup> VNTR loci are groups of DNA sequences that represent a source of highly polymorphic markers for individual identification.<sup>8</sup> They are repetitive DNA motifs with a core repeat length ranging from 10–100bp.<sup>9</sup>

Many advantages are known for VNTR analysis: the need of lower amount of DNA, easy and rapid processing protocol, no need for the use of restriction enzymes or the use of radioisotopes and more importantly, the overall cost is much lower than other techniques.<sup>8,10</sup> The most frequently used material for chimerism analysis is peripheral blood and bone marrow. If chimerism is analysed within certain cellular fractions, e.g. T cells, B cells, or myeloid cells, the term 'subset chimerism' is commonly used. A 'complete donor chimerism' denotes the condition when all cells within a given compartment are derived from the donor, while mixed chimerism denotes a mixture of donor and recipient cells. If one compartment, e.g. the T cells, is completely of recipient origin, whereas the others are donor-derived, this is a situation of 'split chimerism'.<sup>9,11</sup> The aim of the work is to study certain number of VNTR to identify their potential value in the detection of chimerism in conventional ablative transplanted patients. Also to demonstrate the value of T-cells subset analysis added to

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whole blood chimerism in detecting transplantation outcomes.

## Patients and methods

The study was conducted at Alexandria University (MOASSAT Hospital) –Bone marrow transplantation unit during the period from June 2016 to February 2018. Seventeen patients and their donors were included. Patients were transplanted for malignant and non-malignant haematological diseases from HLA identical siblings. Twelve patients were transplanted for acute myeloid leukaemia, one for acute lymphoblastic leukaemia, two patients for aplastic anaemia, one for thalassemia and one for paroxysmal nocturnal haemoglobinuria. Six out of the seventeen patients were children and the rest were adults. The median duration of follow-up was six months. The patients were followed up according to the centre of International Blood and Bone Marrow Transplant Research (CIBMTR) guidelines. Three out of the seventeen patients died during the follow-up period, either due to relapse or severe infections. One patient developed acute graft versus host disease and five patients developed chronic graft versus host disease.

Peripheral blood samples were collected from both recipients and their HLA identical sibling prior to transplantation. A second peripheral blood sample was collected from the recipients on 28<sup>th</sup> day after transplantation. The three samples were subjected to DNA extraction using ABIO pure<sup>TM</sup> extraction, Cat No: M501DP100 USA. Another sample was collected from recipients on 28<sup>th</sup> day after transplantation for its processing using Rosette Sep<sup>TM</sup> Human T Cell Enrichment Cocktail from Stem cell technologies, Cat No: 15021 USA then processed for DNA extraction. The concentration of DNA was estimated by UV spectrophotometry.

VNTR analysis was performed at Clinical Pathology Department, Faculty of Medicine, and Alexandria University. A panel of 5 VNTR

loci (D1S80, D17S30, YNZ-22Apo B and 33.6) and 2 gene loci (SRY and ZP3) was used. Amplification of these loci was performed as described in Tables 1 & 2. All reactions were performed in a volume of 25µL containing 13µL Thermo Scientific Dream Taq Green PCR

Master Mix (catalogue number: K1081), 1µL forward primer, 1µL reverse primer, 5µL RNase-free water and 5µL template DNA followed by visualization in 2% ethidium bromide-stained gel.

**Table 1** Representing VNTR sequences

Primer	Sequence	Reference
D1S80	Forward primer 5'-GAAACTGGCCTCCAAACACTGCCGCCG	26
	Reverse primer 5'-GTCTTGTGGAGATGCACGTGCCCTTGC	
D17S30	Forward primer 5'-CACAGTCTTATTCTT-CAGCG-3'	8
	Reverse primer 5'-CGAAGAGTGAAGTGCACAGG-3'	
YNZ22	Forward primer 5' AAA CTG CAG AGA GAA AGG TCG AAG AGT GAA GTG	27
	Reverse primer 5' AAA GGA TCC CCC ACATCC GCT CCC CAA GTT	
Apo B	Forward primer 5'CCTTCTCACTTGGCAAATAC	26
	Reverse primer 5'ATGGAAACGGAGAAATTATG	
33.6	Forward primer 5'TGTGAGTAGAGGGAGACCTCAC	26
	Reverse primer 5'AAAGACCACAGAGTGAGGAGC	
SRY gene	Forward primer 5'CATGAACGCATTCATCGTGTGGTC	8
	Reverse primer 3'TTCTTAACGTAAACGAAGGGCGTC	
ZP3 gene	Forward primer 5'AGCCATCCTGAGACGTCCGTACA	8
	Reverse primer 3'TACCTGTGTCTTACACCAGTCC	

**Table 2** Representing VNTR amplification protocols

VNTR Locus	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final extension	Reference
D1S80	1min at 94°C	1min at 94°C	1min at 65°C	5min at 70°C	28 cycle	7min at 72°C	28
D17S30	1min at 95°C	1min at 94°C	1min at 55°C	2min at 72°C	28 cycle	7min at 72°C	8
YNZ 22	1min at 95°C	1min at 95°C	45 sec at 60°C	1min at 72°C	30 cycle	10min at 72°C	28
Apo B	5min at 94°C	1min at 94°C	1min at 58°C	2min at 66°C	30 cycle	10min at 72°C	29
33.6	2min at 95°C	1min at 95°C	1min at 55°C	1.5min at 72°C	35 cycle	5min at 72°C	30
ZP3 gene	5min at 95°C	1min at 95°C	1min at 60°C	2min at 72°C	35 cycle	10min at 72°C	31
SRY/ ZP3 genes	1min at 94°C	1min at 94°C	1min at 65°C	2min at 72°C	30 cycle	10min at 72°C	8
SRY gene	2min at 94°C	30sec at 94°C	30sec at 57°C	1min at 72°C	35 cycle	10 min at 72°C	32
SRY gene	11min at 95°C	1min at 94°C	45sec at 60°C	1min at 72°C	28 cycle	60 min at 72°C	33
SRY gene	2min at 94°C	15sec at 94°C	20sec at 65°C	20sec at 72°C	35 cycle	10 min at 72°C	34

## Results

In order to identify the informative VNTR for each donor and recipient pair, we prescreened a pre-transplantation recipient sample and a donor sample for our 5 VNTR loci. The VNTR is considered informative if there is an allelic difference between donor and recipient alleles.

In this study, out of 17 cases, D1S80 VNTR was found to be informative in 59% of cases (10 cases), D17S30 and YNZ 22 VNTR were found to be informative in 47% of cases (8 cases), Apo B VNTR was found to be informative in 41% of cases (7 cases) and 33.6 VNTR was found to be informative in 18% of cases (3 cases).

### Analysis of chimerism in post-transplantation samples showed

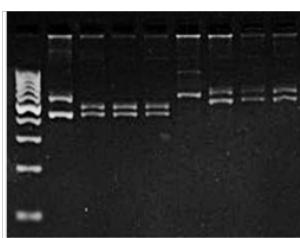
Full donor chimerism could be detected in 14 cases (Figure 1). One case showed the persistence of recipient DNA chimerism which

denoted the failure of implantation. This case was an acute myeloid leukaemia case that relapsed after transplantation and eventually died.

One case showed split chimerism in which whole blood chimerism post-transplantation showed full donor DNA chimerism while T cell separated cells showed recipient DNA chimerism. This case was a case B-Acute Lymphoblastic Leukaemia who showed positivity for minimal residual disease before transplantation (Figure 2).

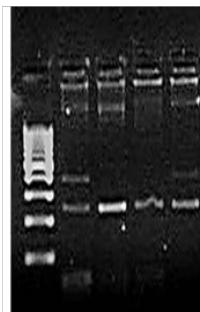
In one case none of the analysed VNTR was found to be informative. We did not investigate further other VNTRs although we recommended the use a wider spectrum of VNTRs in this case or shifting to another method like STRs.

We were able to collect a follow-up sample for 4 patients after 4 months of transplantation. These cases showed earlier full donor chimerism and showed the persistence of full donor chimerism in the second follow up sample at 4 months.



Lane 1: molecular marker (100 bp)  
Lane 2: pre transplant pattern of case 17 for D1S80 VNTR  
Lane 3: his donor pattern  
Lane 4: represent the patient at day +28 (whole blood sample) showing only donor pattern indicating full donor chimerism  
Lane 5: represent the patient at day +28 (T cell separated sample) showing only donor pattern indicating full donor chimerism  
Lane 6: pre transplant pattern of case 16 for D1S80 VNTR  
Lane 7: his donor pattern  
Lane 8: represent the patient at day +28 (whole blood sample) showing only donor pattern indicating full donor chimerism  
Lane 9: represent the patient at day +28 (T cell separated sample) showing only donor pattern indicating full donor chimerism

**Figure 1** Full donor chimerism after transplantation.



Lane 1: molecular marker (100 bp)  
Lane 2: pre transplant pattern of case 15 for D17S30 VNTR  
Lane 3: his donor pattern  
Lane 4: represent the patient at day +28 (whole blood sample) showing only donor pattern  
Lane 5: represent the patient at day +28 (T cell separated sample) showing recipient pattern indicating split chimerism

**Figure 2** Full donor chimerism before transplantation.

## Discussion

Selecting the most informative VNTR locus is the main key in chimerism analysis.<sup>3,12</sup> In this study, we enrolled seventeen patients with their HLA identical siblings. We studied the potential value of various VNTR in the detection of chimerism status post-transplantation. We found that D1S80 VNTR was the most informative allele. D17S30 VNTR and YNZ22 VNTR showed similar discriminative power which is expected as D17S30 VNTR and YNZ 22VNTR are both located on chromosome 17p13. Scientists now are considering YNZ 22 VNTR as part of D17S30 VNTR.<sup>13,14</sup> Apo B VNTR and 33.6 VNTR were informative to some extent.

Muniz et al.<sup>15</sup> reported a frequency of 43% and 37% for D17S30 and D1S80 respectively in their recipient–donor pairs while in our study it was 47% and 59% respectively; whereas Stuppia et al.<sup>16</sup> reported frequencies of 44% for APO-B and 25% for D1S80 compared to 41% for Apo B and 59 % for D1S80 in our work. Considering Sreenan et

al.<sup>3</sup> they reported a frequency of 69% for D1S80, 35% for D17S30, 11.5% for APO-B whereas in our study it was 59% for D1S80, 47% for D17S30 and 41% for APO-B. This difference could be related to the difference in race or ethnic groups of the studied subjects. It may be also explained by a difference in VNTR sequence used or even amplification protocols applied.<sup>3</sup>

Unfortunately, both gene loci (ZP3/SRY) were not informative in our study. Although trying different amplification protocol none of them seemed to be successful. This is consistent with the previous observation of Wang et al.<sup>8</sup> that such failure could be explained by the difference in GC amount in the amplified VNTR sequence which led to a difference in melting temperature optimal for each reaction. In our work, all patients included received myeloablative regimen. Fourteen patient demonstrated full donor chimerism for samples collected on day 28 following transplantation, which is consistent with Devine et al.<sup>17</sup> findings that conventional myeloablative regimens typically promote full donor hematopoietic chimerism.<sup>17</sup>

Graft failure with autologous recovery was detected in one patient with acute myeloid leukaemia who died shortly after 45 days of transplantation due to relapse which was consistent with our finding of persistent recipient chimerism. Another case with acute lymphoblastic leukaemia demonstrated split chimerism, which could be explained by incomplete myeloablation pre-treatment (transplantation was done with a positive minimal residual disease result). This is explained by Hans et al.<sup>18</sup> who stated that donor chimerism in T cell subset occurs later than other cell subsets, especially when transplanted with residual malignant cells which is the case here (having a positive MRD at the time of transplant).<sup>18</sup>

Several methods have been used for detecting chimerism status after allogeneic bone marrow transplantation, we chose the VNTR method as Uguzzoli et al.<sup>19</sup> demonstrated that VNTR has many advantages over RFLP which include that VNTR required less DNA (approximately 250ng); there was no need for DNA digestion, and VNTR was more rapid and sensitive. VNTR analysis has many advantages as we have listed before and is a good predictor of chimerism status in post-transplantation follow up but sometimes we face difficulties in differentiation between donor and recipient allelic distribution which denote the importance of the use of wider scope of genetic markers. Nowadays, short tandem repeats are considered the standard test for quantitative chimerism analysis,<sup>20</sup> genotyping in parentage testing<sup>21</sup> and forensic human identity testing.<sup>22</sup> Many studies compared the precision and efficacy of VNTR in comparison with STR. Mossallam et al.<sup>12</sup> concluded that there is concordance in chimerism results when they compared both techniques results together. Their sensitivity was almost the same<sup>23</sup> but STR showed better results in discrimination capacity. VNTR discriminatory power could be potentiated with the use of smaller size VNTR. Unlike STR, VNTR is used as a qualitative or even semi-quantitative test if we constructed a standard curve using serial dilutions of the recipient and donor DNA. We may also use band intensity as an indicator for the degree of donor-recipient DNA concentrations. In spite of the stated advantages of STRs, the cost of the analysis is very high including the instrumentation and the kits which are not applicable in under-resourced countries. We, therefore, recommend the VNTR as a preliminary cost-effective tool that can be informative in most of the cases. The number of VNTRs could be further extended to solve cases where there are no informative markers, hence saving more expensive procedures as a second line for selected cases that showed no success with VNTRs.

Some studies have confirmed that chimerism analysis in T cells

is more informative for the evaluation of graft-versus-host disease risk than that of whole blood peripheral blood. We could not assess any additional value for T cell chimerism as only one of our cases showed a disparity between whole blood and T cell subset chimerism results.<sup>24,25</sup> This study was initiated with the start of HSCT practice in our hospital and would be further extended on a larger scale.

## Conclusion

VNTRs analysis is a cost-effective method in chimerism detection in most of the cases. D1S80 is the most informative VNTR in our studied population. It is not preferred to use gene loci ZP3 and SRY. The value of subset analysis is yet to be studied on a larger number of patients.

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## Conflict of interest

Authors declare that there is no conflict of interest.

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