D1S111 and 33.4 Polymorphism as a Tool of Genetic Identity among Egyptians

Thesis

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List of Abbreviations

Abb.	Full term
A	Absorbance
AFLP.	Amplified fragment length polymorphism
ALL	Acute lymphoblastic leukemia
ALLO-HSCT...........	Allogenic haematopoietic stem cell transplantation
APO B	Apolipoprotein B
ATP	Adenosine triphosphate
BMT	Bone marrow transplantation
CC	Complete chimerism
CCD	Charge-coupled device
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
EDTA	Ethylenediamine tetraacetic acid
ERV	Endogenous retrovirus
EST	Expressed sequence tag
FISH	Fluorescent in situ hybridization
HSCT	Haematopoietic stem cell transplantation
LINEs	Long interspersed nuclear elements
LTR	Long terminal repeat
MC	Mixed chimerism
Mg	Magnesium
Mt DNA	Mitochondrial deoxyribonucleic acid

List of Abbreviations (Cont…)

Abb.	Full term
NK	Natural killer
NRY	Non-recombining region of Y chromosome
OD	Optical density
PARs	Pseudoautosomal regions
PCR	Polymerase chain reaction
Pmoles	Picomoles
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RQ-PCR	Real-time quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SINEs	Short interspersed nuclear elements
SNP	Single-nucleotide polymorphism
SPSS 20	Statistical package for social science
SSM	Slipped strand mispairing
STR	Short tandem repeats
SYBR	Sybricons
TEs	Transposable elements
tRNA	Transfer ribonucleic acid
uL	Microliter
UTRs	Untranslated regions
VNTR	Variable number tandem repeats
WBC	White blood cells

ABSTRACT

Background: The variable number tandem repeats (VNTR) are highly polymorphic markers. Due to their high polymorphic content, VNTR constitute useful tools in population genetic studies in understanding population and ethnic migrations throughout history. They also constitute preferred systems for DNA "fingerprinting", criminal and forensic examinations and paternity testing, evaluating hematopoietic chimerism and in determining the origin of leukemic cells in patients with recurrent disease after BMT.

Aim of the Work: To standardize PCR protocols of 2 VNTR loci (D1S111/33.6 and 33.4) in order to detect allelic polymorphism among Egyptians as a tool in following up the chimeric status after allogenic stem cell transplantation.

Patients and Methods: The current study was conducted at Clinical Pathology Department of Ain Shams University Hospital between January 2018 and December 2019, a total of 130 subjects [65 pairs (allogenic stem cell transplantation donors and their recipients)] were included. D1S111/33.6 and 33.4 VNTR loci were evaluated in every subject by PCR technique to study their relative distribution and number of informative alleles detected in order to be used in chimerism follow up and population studies.

Results: Five alleles were found at D1S111/33.6 in 130 subjects with molecular weight ranging from 364 to 993 bp, frequency of homozygous alleles were 30.8% and it was 69.2% for heterozygous, 90 out of the total 130 subjects were heterozygous for 33.6, that means that the heterozygosity index for D1S111/33.6 is 69.2%, from the 65 pairs tested 31 pair were informative or discriminative with discriminative power 47.69%. For 33.4 we tried many PCR programs but no bands were detected at all.

Conclusion: D1S111/33.6 VNTR polymorphism is a dependable cost effective method in following up of allogenic stem cell transplantation recipients.

Keywords: *Chimerism, VNTR, Allogenic stem cell transplantation, Repetitive DNA*

INTRODUCTION

Tandemly repeated DNA is organized as multiple copies of a homologous DNA sequence of a certain size (repeat unit), that are arranged in a head to tail pattern to form tandem arrays. They are not evenly dispersed throughout the genome but they tend to cluster toward the telomeric end of chromosome. Thus, they represent a distinct type of sequence organization shared by all sequenced genomes ***(Gelfand et al., 2007).***

Variable number of tandem repeats (VNTR) are highly polymorphic markers. Although each VNTR locus is usually associated with a large number of different alleles in any population, each individual carry two alleles only—one on each of the two homologous chromosomes. Due to their high polymorphic content, VNTR constitute useful tools in population genetic studies, in understanding population and ethnic migrations throughout history. They also constitute preferred systems for DNA “fingerprinting,” or determination of unique sets of genetic markers for individual identification. The direct practical applications of which include criminal and forensic examinations, solving of immigration cases, paternity testing, evaluating hematopoietic chimerism and in determining the origin of leukemic cells in patients with recurrent disease after BMT ***(Babushkina and Kucher, 2011).***

Since VNTR loci are abundant in the human genome, and the probability of having the same pattern between two persons is unlikely. VNTR loci contain variations of multiple alleles in various human population. Thus, with the use of molecular techniques, these polymorphic genetic markers can be employed for monitoring chimerism status and individual polymorphism ***(Richard et al., 2008).***

Since the 1980s, a variety of techniques that employ polymorphic markers have been established, such as the following: fluorescent in situ hybridization (FISH) with XY chromosome specific probes or polymerase chain reaction(PCR), single-nucleotide polymorphism (SNP) analysis, short tandem repeats (STR), restriction fragment length polymorphism (RFLP) and variable number tandem repeats (VNTR). However, several limitations have been reported associated with these techniques namely low sensitivity, time-consuming, limited to sex-mismatched transplantations, high DNA requirement and limited degree of polymorphism. For all these clinical applications, the optimal methodological approach needs to be informative, sensitive, accurate, reproducible and cost effective ***(kletzel et al., 2013).***

Currently, PCR-based amplification of a highly polymorphic VNTR system is considered to be the most sensitive technique for discriminating individuals. By using PCR primers that flank the VNTR loci, the whole allele is amplified, and therefore, the size of the PCR product is determined by the length and number of tandem repeats ***(Choong et al., 2011).***

The discriminative power of VNTR loci depends on the number of alleles detected and their distribution in the population ***(Talkhan et al., 2016).***

In case of patients undergoing haematopoietic stem cell transplantation, informativity determination always precedes the monitoring of cell chimerism. Recipient and donor DNAs are tested by a panel of highly polymorphic STRs, VNTRs and/or SNPs. A comparison of the donor's and the recipient's DNA profiles allows selection of the specific informative markers suitable for the monitoring of cell chimerism during the post transplantation course. So variable number tandem repeats VNTR loci is a perfect source of highly polymorphic markers that can be employed for the evaluation of the state of chimerism. Also, offer substantial cost savings, faster turnaround time, easier preparation of the DNA and smaller DNA requirements ***(Antonio Blanco et al., 2017).***

AIM OF THE WORK

The aim of this work is to standardize the PCR protocols of 2 VNTR loci (33.4 and D1S111) in order to detect allelic polymorphism among Egyptians to be used in following up the chimeric status after allogenic Haematopoietic stem cell transplantation.

Every cell in human body contains deoxyribonucleic acid (DNA). On average, about 99.9 percent of the DNA between two humans is the same. The remaining percentage is what makes us unique. Although this might sound like a small amount, it means that there are around three million base pairs that are different between two people, these differences can be compared to distinguish people ***(Nakamura, 2009).***

I- DNA Structure:

Biologists in the 1940s had difficulty in accepting DNA as the genetic material because of the apparent simplicity of its chemistry. DNA was known to be a long polymer composed of only four types of subunits, which resemble one another chemically. Early in the 1950s, DNA was first examined by x-ray diffraction analysis, a technique for determining the three-dimensional atomic structure of a molecule. The early x-ray diffraction results indicated that DNA was composed of two strands of the polymer wound into a helix. The observation that DNA was double-stranded was of crucial significance and provided one of the major clues that led to the Watson-Crick structure of DNA. Only when this model was proposed, did DNA's potential for replication and information encoding become apparent ***(Sinden and Richard, 2012).***

A DNA molecule of two long polynucleotide chains composed of four types of nucleotide subunits, each of these chains is known as a DNA chain or a DNA strand. Hydrogen bonds between the base portions of the nucleotides hold the two chains together. Nucleotides are composed of a five-carbon sugar to which are attached one or more phosphate groups and a nitrogen-containing base. In the case of the nucleotides in DNA, the sugar is deoxyribose attached to a single phosphate group (hence the name deoxyribonucleic acid), and the base may be either adenine (A), cytosine (C), guanine (G), or thymine (T) **(Figure 1)**. The nucleotides are covalently linked together in a chain through the sugars and phosphates, which thus form a "backbone" of alternating sugar-phosphate/sugar-phosphate ***(Alberts et al., 2002)***.

The way in which the nucleotide subunits are lined together gives a DNA strand a chemical polarity. This polarity in a DNA chain is indicated by referring to one end as the hydroxyl 3′ end and the other as the 5′ phosphate end ***(Griffith et al., 1992).***

In each case, a two-ring base (a purine) is paired with a single-ring base (a pyrimidine); A always pairs with T, and G with C. This complementary base-pairing enables the base pairs to be packed in the energetically most favorable arrangement in the interior of the double helix. In this arrangement, each base pair is of similar width, thus holding the sugar-phosphate backbones at equal distance apart along the DNA molecule. To maximize the efficiency of base-pair packing, the two sugar-phosphate backbones wind around each other to form a double helix, with one complete turn every ten base pairs ***(Lodish et al., 2000).***