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Original Article

Single Nucleotide Polymorphism; A Molecular Marker for Genetic Variations

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ABSTRACT

Objective: Single nucleotide polymorphisms are the most common genetic variations in a human DNA. The aim of the study was to determine the genotype frequency of single nucleotide polymorphisms by a simple PCR technique in order to find the genetic variations in general population.

Design: Descriptive study.

Place and Duration: It was conducted at Genetic Resource Centre Lab Rawalpindi from Oct 2017- May 2018.

Methods: A total of hundred unrelated healthy individuals were selected and assessed for the genotype frequency of eighteen biallelic single nucleotide polymorphisms. Extraction of DNA was done from the whole blood and single nucleotide polymorphism were amplified by using conventional PCR assay. Electrophoresis was carried out and 6% polyacrylamide gels were used for the resultant amplified DNA products.

Results: A positive reaction was shown by a discrete band of DNA on the polyacrylamide gel. The most commonly found SNPs were S01, S03, S06, S07a, and S10a.

Conclusion: Our results demonstrate the successful screening and genotype frequency of each single nucleotide polymorphism by using a PCR amplification of polyacrylamide gel electrophoresis in general population. This research has provided a new and comprehensive methodology for carrying out further studies using SNPs as a marker of discrimination in the donor/recipient pairs having undergone haematopoietic stem cell transplantation in Pakistan. This PCR assay of SNPs appears to be a simple, rapid, reliable and technically feasible method for a use in a lab equipped with PCR.

Key words: Single Nucleotide Polymorphism; PCR; genotype frequency

Introduction

Regarding human genomic DNA the most common genetic variation is Single nucleotide polymorphisms (SNPs).[1] These variations may present at some particular position in the human genomic pattern.[2] Based on genetic pattern an individual may be different from the other individual by million of SNPs.[3] On account of their allelic variations these SNPs are found to be present in more than 1% of general population.[4]

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In clinical context theses SNPs may have been shown to be useful in the genetics of populations,[5] ancestry,[6] pharmacogenomics[7] and also the genes which are involved in certain diseases.[8] Moreover by using a large panel of SNP genotyping markers, the possibility of studying the genetic basis of complex diseases may be considered significantly increased. Nevertheless, Various researchers have also identified SNPs associated with the pathogenesis of certain cancers .[9,10,11,12]

Moreover in a sensitive molecular assay, SNPs, because of their stability and uniqueness, may be demonstrated easily and have shown to be a useful molecular marker involved in the studies of genetic disorders.[13] There is no study done locally which could present the usefulness of SNPs by a PCR method for determining the genotype frequency in general population..

Methodology

This study was done on a total of hundred healthy unrelated individuals. The individuals were selected by non-probability convenient sampling from Oct 2017- May 2018.The study was approved by the Ethical Review Committee of Islamic international Medical College (Appl. # Riphah /IRC/ 21/06). Every subject participating in the study has signed an informed consent.

Eighteen heterozygous SNPs were chosen as described by Alizadeh et al.[14] From each subject approx. 2-3 ml venous blood was taken in EDTA vial. DNA was extracted by the Chelex™ method [15]. The PCR conditions for allele specific primers of each SNP was developed, adjusted and set. A total of hundred healthy individuals were assessed and analyzed for the eighteen selected SNP regions for determining the frequency of these SNPs by a PCR method.

PCR Conditions: For the PCR of SNPs, DNA samples from hundred individuals was taken and assessed for the selected SNP regions. The sequence of primers for the selected SNPs were followed and selected as described by Alizadeh et al. [14] The primers were synthesized by Integrated DNA technologies, USA. The PCR was done in a reaction mixture accounting about 25 µl in volume. The reaction mixture consisted of 30 mM of each dNTPs, 5pM of each primer, 10 mM Tris HCl (pH 8.3), 50 mM KCl, , 0.5 units of Taq polymerase (Fermentas, Lithuania), , 100 mg/ml gelatin (Sigma, UK), 1.5 mM $MgCl_2$ and 0.2µg of genomic DNA. For thermocycling, 9700 (Perkin Elmer, USA) was used constituiting 28 cycles of: Denaturation 94^0C for 30 sec, Annealing 63^0C for 30 sec, and extension 72^0C for 1min. At the end of thermocycling the PCR was continued for the final extension of 3 minutes. Once the amplification of PCR products was done, these were loaded on polyacrylamide gels of 1 mm x 10 cm x 10 cm on Mini-protean electrophoresis apparatus (Bio-Rad, USA). Electrophoresis was done at 200V for 27 minutes. The gels were stained by silver nitrate and dried at 80^oC for fifteen minutes in a drier and results were then read.

Results

Genotype frequencies of SNP in the general population: The allele specific PCR and silver stained polyacrylamide gels were run for 30 minutes at 150 volts. The SNP marker was clearly able to distinguish between a positive and a negative result (Fig1 & 2). The number of amplification cycles in SNP-PCR was critical. Best results were obtained at 28 cycles of amplifications. Increasing the number of cycles resulted in false positive results.

The genotype frequencies in the 100 individuals are summarized in Table 1. The allele frequencies of the SNPs varied from 9% to 56%. The positive allele at most of the SNP loci had a frequency between 35% and 50%. The SNPs S01, S03, S06, S07a and S10a were the most polymorphic (>50% frequency of the positive alleles).

Table 1. Allele Frequencies of the 18 SNPs in 100 Unrelated Individuals

SNP Marker	Positive	%	Negative	%
S 01 (n=100)	51	51 (0.51)	49	49 (0.49)
S 02 (n=100)	26	26 (0.26)	74	74 (0.74)
S 03 (n=100)	52	52 (0.52)	48	48 (0.48)
S 04a (n=100)	48	48 (0.48)	52	52 (0.52)
S 04b (n=100)	36	36 (0.36)	64	64 (0.64)
S 05a (n=100)	44	44 (0.44)	56	56 (0.56)
S 05b (n=100)	28	28 (0.28)	72	72 (0.72)
S 06 (n=100)	54	54 (0.54)	46	46 (0.46)
S 07a (n=100)	56	56 (0.56)	44	44 (0.44)
S 07b (n=100)	39	39 (0.39)	61	61 (0.61)
S 08a (n=100)	44	44 (0.44)	56	56 (0.56)
S 08b (n=100)	32	32 (0.32)	68	68 (0.68)
S 09a (n=100)	43	43 (0.43)	57	57 (0.57)
S 09b (n=100)	09	09 (0.09)	91	91 (0.91)
S 10a (n=100)	51	51 (0.51)	49	49 (0.49)
S 10b (n=100)	36	36 (0.36)	64	64 (0.64)
S 11a (n=100)	48	48 (0.48)	52	52 (0.52)
S 11b (n=100)	28	28 (0.28)	72	72 (0.72)

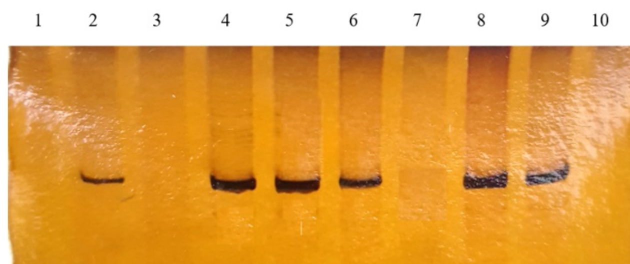

Fig 1 PCR amplified products of the SNP S-04a run on polyacrylamide gel and stained in silver nitrate. The + alleles are shown by a sharp band whereas the "-" alleles do not show any amplification. Lane 1, 3, 7, and 10 show absence of SNP allele whereas the lanes 2, 4, 5, 6, 8, and 9 show the positive (+) allele.

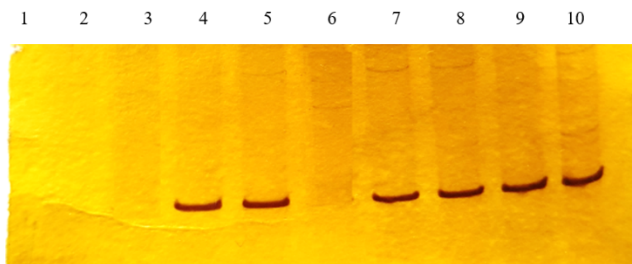

Fig 2. PCR amplified products of the SNP S07a run on polyacrylamide gel and stained in silver nitrate. The "+" alleles are shown by a sharp band whereas the "-" alleles do not show any amplification. Lane 1, 2, 3, and 6 show absence of the SNP allele whereas the lanes 4, 5, 7, 8, 9, and 10 show the "+" SNP allele.

Discussion

A human genome differs from a typical genome at 4 to 5 million sites, most of which consists of SNPs and short indels [16] In the last decade, a large number of SNPs have been identified that better explain the genetic pathology of complex diseases such as cancer, vascular complications, diabetes, mental illnessess, and a no of other disorders. [17] However the detection of these SNPs is being considered clinically important.[8] The availability of a number of molecular techniques for SNP genotyping on a large scale assays are fluorescence resonance energy transfer (FRET) probe or molecular beacon methods.[18] and Taqman probe technology,[18] For small scale assays, the SNP genotyping assays available and used are gel electrophoresis based, such as single strand conformation polymorphism (SSCP), conformation sensitive gel electrophoresis and denaturing gradient gel electrophoresis (DGGE), [19]

This article describes the allele frequency of 18 human biallelic SNPs in hundred unrelated individuals based on conventional PCR of SNPs. All the 18 SNPs have clearly shown to detect allele frequency in all the unrelated individuals. In this study the optimization of SNP panel has been done for the Pakistani population in a local setting. Moreover, different SNPs with high allele frequency may be tested and added for a use in other population groups. Regarding allele frequency, the frequently found SNP accounts 56% and the least commonly found SNP was 9% with four SNPs being highly informative. A Persian group of researchers have studied the frequency of ten SNPs in hundred people and found out three SNPs highly informative for Persian population.[20] A group of researchers conducted a study on 40 SNPs in 923 chile people and investigated that SNPs may be used to estimate individual genetic ancestry. [21] Few researchers have studied a panel of 22 SNPs on 641 Shanghai people and found three SNPs being highly informative. [22] This concept of SNPs detection in population may also be used in the depiction of the informativeness in potential donor/recipient pairs before haematopoietic stem cell transplantation by a PCR assay. Various researchers have analyzed and investigated the informativeness of SNP marker by PCR and found an informativity of 90% in recipient and donor pairs studied. [23,24]

Conclusion

Our results demonstrate the genotype frequency of SNPs in general population by a simple and fast method of PCR that uses a general PCR apparatus, PCR mixture and SNP primers in a single PCR tube. It is a useful technique for detecting variations in human genome. Based on the findings observed in our study, SNP being a molecular marker for detecting genomic variations, this SNP-PCR based method may further help the clinical researchers to the detect an informative region in potential donor and recipient pairs having undergone bone marrow transplantation. Since the set of SNP panel used in our study is optimally set for a use in Pakistani population, it may need further optimization and modification for a other populations groups.

Conflict of Interest: None.
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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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