ALLELE FREQUENCIES OF THE HUMTH01 LOCUS IN THE ITALIAN POPULATION

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SUMMARY

Human identification through DNA analysis has been revolutionized in the last years. The technique of in vitro DNA amplification using the polymerase chain reaction (PCR) (Mullis and Faloona, 1989) has facilitated the study of genetic variations in terms of simplicity and sensitivity (Reynold et al., 1991).

Microsatellites, stably inherited short runs of tandemly repeated nucleotide sequences (STRs), are a rich source of higly polymorphic markers in Human population and easily detecte by PCR.

Very recently, a new dimension in DNA typing analysis was opened with the development of instruments for automated analysis of fluorescent amplification products.

STR systems were evaluated from Fourney R. and Fregeau G. J. (1993) for sensitivity and accurancy using the Gene Scanner and compared to other DNA typing methods currently in use. Their results strongly indicate that excellent precision and accurancy can be obtained using non-denaturing gel systems with the added advantage that the majority of STR fragments can be more rapidly resolved because of increased electrophoretic migration.

We report DNA typing of the tetrameric repeat (AATG) STRs HUMTH01 mapped to chromosomal locus 11.p. 15. 5 (Edwards A. et. al., 1991) with fluorescent STR primers. The objective of the project is to establish the TH01 allelic frequencies in the Italian population by comparing unrelated individuals selected from northern Italy (100 samples) to the whole Italian population (100 samples). We have found any significant allele variations in our comparision of northern Italians to rest of Italian population.

Introduction

A large proportion of the human genome (ca. 25%) consists of repetitive DNA sequences whose inherent polymorphic characteristic provides information for forensic identification.

In recent years, through the use of the PCR, a class of repetitive elements, microsatellites, has become the focus of attention for discrimination between individuals (2).These small stretches of DNA (less than 300 bp.) are composed of tandemly repeated monomers of 2 to 5 bp. in length and like minisatellites, are widespread throughout the genome.

Numerous STR systems have been documented to date, many of which have been used to study polymorphisms within several population databases. It is estimated that the human genome contains approximately 500.000 STRs (6000 to 10.000 trimeric and tetrameric repeats) (3) each characterized by a limited number of easily identified alleles but which collectively offer unlimited potential for a highly discriminatory test for human identification.

The automated analysis of fluorescently labeled DNA products using an instruments as Applied Biosystems Division (ABD, Foster City, CA) Model 373A with GeneScan software provides a welcome alternative to the manual processing of STRs. Automated real-time analysis allows electrophoretic information to be immediately stored. In addition, automatic allele sizing and quantitation are made possible by running an internal lane standard that is recognized by the computer to generate a calibration curve.

Thus, electrophoretic mobility variations (from lane to lane and gel to gel) that can lead to inconsistent allele sizing are automatically normalized. Furthermore, multiple primers, each "tagged"with a distinct fluorescent dye, can be used. This allows the simultaneous analysis of multiple VNTR profiles.

This study examines the potential of automated fluorescent detection of STR alleles as a sensitive and accurate approach for DNA typing.

We report DNA typing of the tetrameric repeat (AATG) STR HUMTH01 with fluorescent primer and STR products have been run and analyzed on an automated sequencer (4). Our study have established the TH01 allelic frequencies in the Italian population on DNA extracted from blood by comparing unrelated individuals selected from northern Italy (100 samples) to the whole Italian population.

Materials and Methods

Human genomic DNA was prepared from peripheral blood by standard methods involving cell lysis, proteinase K digestion and ethanol precipitation. Microsatellite locus STRs HUMTH01 (Edwards et al. 1991) was used in this study. All oligonucleotide primers used were synthesized using a Model 380 DNA synthesizer (Applied Biosystems, F.C., CA). For the automated detection a fluorescent dye (TAMRA) was attached to the 5'- end of the forward PCR primer used for amplifying of the locus using Aminolink 2 (Applied Biosystems, F.C., CA) and HPLC purified.

We used and internal lane size standards consisted of a Pst I digest of Lamda phage, labeled with a second red dye (ROX) by a ligation method. (1). Nonfluorescent primers were synthesized using the same sequences.

PCR were carried out for fluorescence primers for 25 cycles with 50-100 ng of DNA in 50 µl reaction volume. STRs HUMTH01 locus was labelled with TAMRA- dye and amplified with single PCR.

For gel analysis with fluorescent amplification products, 0.5 µl of the internal lane standards and 2 µl of PCR reaction were combined with 3 µl of deionized formamide. Were electrophoresed in a 6% acrylamide gel (BIORAD) with 8M urea in 1x TBE.

Gels were electrophoresed for 6 hr. at 30 W constant power on an Applied Biosystems, (F.C., CA) automated DNA sequencer model 373A. The size of PCR products is determined automatically and precisely by GeneScan 672 Software (ABD, F.C., CA) using the labelled size standard in each lane.

Results

We have not found any significant alleles variation in our comparison with northern italians (100 samples Lombards) and the rest of the Italian population (100 samples) (see figure). Six alleles were observed ranging in frequencies from 0.01 to 0.27. The observed overall heterozygosity was 76%.

Sometimes extra peaks were occasionally present, but they have not caused any difficulties in typing the HUMTH01 locus.

We believe that the adoption of the fluorescent technology should give us the appropriate choice of markers to obtain population data rapidly and accuratly.

Fig 1

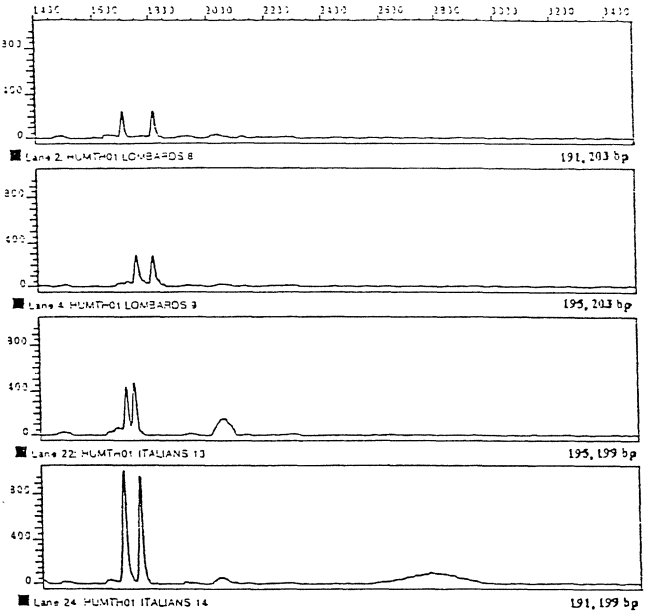
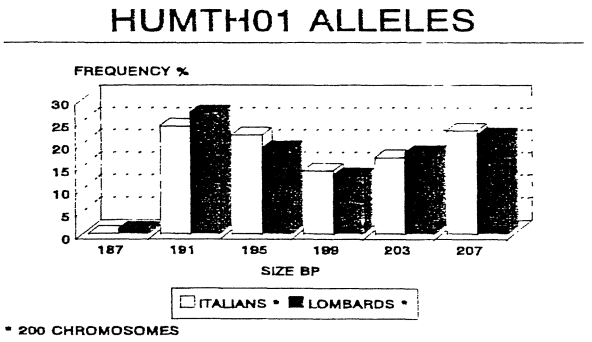


Fig 2



References

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