Analysis of HumFABP$_2$ as a polymorphic human genetic marker in the Turkish population

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ABSTRACT

Objective: To examine the allele frequencies of HumFABP$_2$ locus in 155 individuals from different regions of Turkey.

Methods: The study was carried out in Cumhuriyet University Hospital, Sivas, Turkey, between March and June 2006. The allele and genotype frequencies for HumFABP$_2$ were determined by polymerase chain reaction (PCR) using the manufacturer's recommended protocol, and using the commercially available Macherey-Nagel DNA isolation kit. The PCR amplification was carried out in a Perkin-Elmer GeneAmp PCR System 9600 thermal cycler following the manufacturer's recommendations. The allele frequencies in the Turkish population was computed, and the heterozygote rate was calculated.

Results: In this population study of 155 samples, we found 75 (48.39%) heterozygote and 80 (51.61%) homozygote. The results showed heterozygotic cases as 150/250 bp, and homozygotic cases as 150 bp.

Conclusion: Allele frequency data of HumFABP$_2$ as a PCR-based genetic marker could be used in identity testing to estimate the frequency of a multiple PCR based profile in the Turkish population.

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Human identification through DNA analysis has faced tremendous changes in the last few decades. The advent of polymerase chain reaction (PCR) technology allows allelic profiles to be obtained with minute amounts of target DNA even in a degraded state.[1] The DNA analysis studies became a powerful tool in the field of forensic identification and paternity determination, as well as for research in human gene mapping.[2,3] The collective tombs, anthropological, and archeological remnants being revealed in the world have shed light on past history. Recently, DNA analysis is very useful in the identification determination of the corpses of war slaves, smuggled emigrants, and illegal refugees.[2-4] Genetic studies are important in the populations, as our country consists of different ethnic groups. Therefore, the forensic genetics science in forensic medicine has a great importance in the world. The use of polymorphic markers permits the formation of a unique profile for each individual. The studies of allele frequencies in the populations allow the scientist to estimate the probability of a particular allele combination.[2,3,5,6]

The HumFABP$_2$ can be analyzed by PCR. The characteristic of the HumFABP$_2$ locus makes it a very useful marker for population genetic research, genetic linkage studies, forensic identification of individuals, and for determination of biological relatedness of individuals. Also, the use of PCR has facilitated the typing of human identity testing genetic markers.[2,3,5,7,8] The DNA typing analysis offers significant advantages for the routine processing of a large numbers of DNA samples, greatly facilitates and expedites the generation of allelic profile databases, and enables investigators to perform the simultaneous survey of several different loci from individuals or forensic samples, or both.[1] Recently, forensic DNA analysis studies have been extensively investigated in many populations, however, studies on the Turkish population are limited. This study presents allele frequency data in the Turkish population for the HumFABP$_2$ locus, and we aim to determine the allele and genotype frequencies of HumFABP$_2$ as a PCR-based genetic marker in the Turkish population, and to support the connected forensic genetic studies.

Methods. This study was carried out in Cumhuriyet University Hospital, Sivas, Turkey, between March and June 2006. The subjects were randomly selected from unrelated healthy volunteer students from different regions of Turkey, which was our presupposition for a uniform study population. The study was approved by the ethical committee of the Cumhuriyet University Hospital. We selected 155 samples comprising of 90 male and 65 female, and an informed consents were given. Five ml blood samples were collected in ethylenediamine tetraacetic acid tubes, and were stored at -20°C until analyzed. ***DNA extraction.*** The DNA was extracted from 200 µl of blood by DNA isolation kit (Macherey-Nagel, Deutschland). The PCR amplification was performed in a 20 µl reaction containing 4.5 µl Water Nuclease free buffer (Fermantas, Lithuania), 12.5 µl 2 x PCR Master Mix (Fermantas, Lithuania), 0.5 µl of each primer set, and (0.5 µl forward, 0.5 µl reverse), 2 µl of the sample.

PCR amplification. The PCR conditions were denaturation at 93°C for one minute, primer annealing at 60°C for one minute, and primer extension at 73°C for 1.5 minutes, for a total of 28 cycles. A GeneAmp PCR System 9600 (Perkin-Elmer Inc, USA) thermal cycler was used for the PCR.

Agarose run. The marker HumFABP$_2$ was run in an agarose gel of 1% by using 1 x TAE. Four microliters of PCR's production were mixed with 2 µl of loading dye and were run.

Results. The marker HumFABP$_2$ (**Table 1**) was amplified in 155 samples, 90 (58.1%) males and 65 (41.9%) females, representing a 58/42 ratio of male/female. In this population study, we found 75 (48.4%) heterozygote (150/250 bp) and 80 (51.6%) homozygote (150 bp). The results showed heterozygotic cases as 150/250 bp, and homozygotic cases as 150 bp.

Discussion. The DNA analysis has become a standard method in forensic genetics as it currently applied by most laboratories for most of the forensic genetic types of expertise, especially in criminal forensic casework.[9-11] The DNA typing of human traces by PCR has become one of the most convincing instruments in forensic casework.[1,12-15] Since DNA analysis is a safe method used in human identification, it is very useful in forensic cases. Modern methods of DNA polymorphism typing are now widely used in criminology for identifying people whose biological traces are material testimony.[6,7,16]

Our survey focuses on the frequency analysis of polymorphic sites in Turkey. The historical settings of Turkey render it a perfect candidate for population studies. The Turkish population consists of Kurd, Laz, Armenian, Circassian, Georgian, and others. Hence, an induced gene flow is expected from these populations. Generally, there is a linguistic and religious boundary prohibiting the free gene flow among these populations. For forensic applications of DNA typing, the existence of subpopulation heterogeneity is an important factor in determining match probabilities. Databases for forensic statistical calculations generally contain 100 (although sometimes less) to several hundred individuals.[16] The sample sizes are adequate for forensic applications. Nei[17] demonstrated that population substructure analysis could be performed on databases containing less than 50 individuals, with loci containing only 2 - 6 alleles. Our study group comprised of 155 individuals, randomly selected university students from different regions of Turkey. We could not, therefore, say that it is a good percentage of the total population, however it might also give the result of the total population of Turkey of almost 73 million,[17] as the individual samples came from different sites of culture, religious, and ethnic backgrounds. However, due to the small sample size, further accurate population genetic surveys in Turkey are necessary.

The study of HumFABP$_2$ locus provided us with frequencies that compared to international data. In our results, we found HumFABP$_2$ in 75 (48.4%) people as heterozygote and 80 (51.6%) people as homozygote. These results will be used as support for other identification methods. Tsopanomichalou et al[16] demonstrated 56.6% of the cases were heterozygous, in the study of the allele and genotype frequencies for HumFABP$_2$ gene marker. The results are very close to each other. However, the heterozygous of Turkish population was 48.4%, and this data could be very important on the identification of the Turks. We found heterozygote as 150/250 bp in our cases by electrophoresis, and homozygote as 150 bp. We could not find 250 bp as homozygote. It has shown that the Anatolia genetic poll carry 150 bp as homozygote and 150/250 bp as heterozygote. The HumFABP$_2$ gene markers of the mother and father claimed are compared with children's HumFABP$_2$ gene markers for identification. However, some population movement has occurred from one region to other region of the world.

Table 1 - Sequences of marker HumFABP$_2$.

HumFABP$_2$	Sequences
Forward	5'- GTAGTATCAGTTTCATAGGGTCACC - 3'
Reverse	5'- CAGTTGGTTTCCATTGTGTGTTCCG - 3'

The HumFABP$_2$ allele frequencies could be evaluated to distinguish Turks from other populations, thus, will help to identify their origins. This study would help in determination of pioneer movement in the Anatolia, Turkish population. If a 150 bp and 250 bp sequences were determined for repeat alleles, this data would give more efficient information on forensic identification of a person's origin. This study would be of help in determination of identification and diseases (especially in the pharmacologic-genetics field studies), and in finding out the origin of pioneers.

In conclusion, the certain alleles of the HumFABP$_2$ locus could be used for discriminating the Turkish population, as a result of their unique presence. The data demonstrate that this locus can be useful for providing estimates of the frequencies of a DNA profile in forensic identity testing.

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