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Assessment of 13 single nucleotide polymorphisms loci for their application in identification in forensic sciences for Turkish population

Abstract. Single nucleotide polymorphisms (SNPs) are used for genetic identification in forensic science. In this study, 13 loci (rs876724, rs1357617, rs2046361, rs717302, rs917118, rs735155, rs901398, rs729172, rs740910, rs1493232, rs719366, rs1031825, rs722098) selected among 52 SNP plex's whose importance in forensic sciences were determined before, were studied for the Turkish population. In addition to the discrimination power of the Turkish population as selected loci in this study, it was aimed to calculate the separation of powers showed in their Turkey geographic regions. For this purpose, blood samples taken from 50 volunteers' DNA samples were duplicated by polymerase chain reaction (PCR) and typed in a capillary electrophoresis device and genotype information was determined for 13 SNP loci. The data obtained showed parallelism with values published by the SNPforID Consortium. Obtained results proved the separation forces of the studied loci suitable for the Turkish population. The existence of Hardy-Weinberg Equation was tested for all studied loci and Hardy-Weinberg Equation was found in all loci except one loci (rs876724). Since the number of samples is insufficient for geographical studies, no statistically significant difference was observed in other loci in the regional context except for the significant results obtained for rs729172 and rs740910 loci.

Key words: single nucleotide polymorpism, population, electrophoresis, forensic genetics.

Introduction

DNA analyses in the field of forensic sciences are conducted for the purpose of identification, paternity testing, and identification of the relationship between the victim and the perpetrator with the help of biological evidence found at the crime scene. It has been proven by various studies that SNP (single nucleotide polymorphism) method is more successful within the identification of biological evidences in cases where biological evidences located in a crime scene are highly degraded or quantitatively inadequate than other identification methods [1-3]. PCR products of STR (short tandem repeats) loci commonly used in genetic identification range between 200 to 450 base pairs while PCR products of SNP loci range between 100 to 150 base pairs. SNP loci being shorter than STR loci causes higher discrimination power, especially in degraded samples. Mini-STR kits developed for cases where PCR product sizes are short are not preferred in analyses due to not having high discrimination power as STR kits as an outcome of the number of sites they contain. Moreover, SNPs having a low mutation rate ($2x10^{-8}$ mutations/generation) is significant in terms of it being preferred for genetic identification purposes in forensic sciences [4; 5]. This situation enables SNPs to be used for paternity and complicated kinship tests as well. Besides, the fact that SNP analysis methods are easier and cheaper than STRs, it is also advantageous in terms of time and cost as well [6; 7].

In this study, the ultimate aim is to have studies regarding 52 SNP loci completed to a large extent when the SNP method used routinely in many laboratories in the world is also wished to be applied in forensic laboratories in Turkey. Optimization of 29 out of 52 SNP loci significant for forensic genetic identification was completed in 2009 as part of a project [8]. This study aims the evaluation of the discrimination power for the Turkish population of 13 SNP loci which have not been studied before in our country and contribution to obtaining the necessary data for routine studies.

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Materials and methods

In this study, venous blood was used belonging to 50 persons (29 women, 21 men) taken after receiving informed consent from such persons following the detailed clarification regarding the study, who have no kinship with each other. Participants in the study were set proportionally to Turkey Geographical Region population distribution as to be 7 people from Mediterranean Region, 4 people from Eastern Anatolia Region, 8 people from the Aegean Region, 5 people from Southeastern Anatolia Region, 9 people from Central Anatolia Region, 6 people from the Black Sea Region and 11 people from Marmara Region. The research was approved by the Ethics Committee of Istanbul University Cerrahpaşa Medical Faculty (No. 88446, Date: 07.03.2016).

DNA Isolation and Quantitation. Within all of the samples in the study, QIAAmp® DNA Mini Kit (Germany) whichis suitable for studying in blood materials was used. DNA quantification was assayed asfluorometric by using Qubit® dsDNA HS Assay Kit (USA) and Qubit® Fluorometer (Invitrogen, USA). After the DNA quantities of the isolates were measured, their quantity was adjusted to be 0.1-10 ng DNA in each tube for PCR reactions.

PCR Analysis. 13 loci out of 52 SNP loci which are identified as significant by SNPforID Consortium were studied within the study as to be rs876724, rs1357617, rs2046361, rs717302, rs917118, rs735155, rs901398, rs729172, rs740910, rs1493232, rs719366, rs1031825, and rs722098 [9] and the primer sequences published by the consortium were used (Table 1). In the study, two separate PCR stages were applied for genotyping tests of 13 SNP loci.

Table 1- Primer information used in this study

SNP Name	NBCI rsCode	Forwardprimer 5'3'	Reverseprimer 5'3'	Bp
A02	rs876724	GCAGGCTCCATTTTTATACCACT	GAATATCTATGAGCAGGCAGTTAGC	83
A04	rs2046361	CCTATTTGTATGTATCTATTGTCTATGAACG	GTCATTGTTGACACTTCACCTTCTA	79
A05	rs717302	CTTTAGAAAGGCATATCGTATTAACTGTG	AACACAGAAAGAGGTTCATATGTTGG	86
A07	rs917118	GCCCTTTAGGGTCGGTTC	GTAAGAGATGACTGAGGTCAACGAG	87
A10	rs735155	GGAGAAAACCGGAGAGCTG	GAGTGTCACCGAATTCAACG	100
A11	rs901398	CTGGGTGCAAACTAGCTGAATATC	CTGGAATGTACTAGGCAAGAAACTAA	70
A16	rs729172	CATTAATATGACCAAGGCTCCTCT	ACATTTCCCTCTTGCGGTTAC	60
A17	rs740910	GTATAACAGTTTGCTAAGTAAGGTGAGTG	AGATAGGTTCGAGTTTTGGCTTTA	87
A18	rs1493232	CTATTCTCTCTTTTGGGTGCTAGG	CAAACTGTTTATTGTGAGGCCTGT	59
A19	rs719366	CCACAGCATCTTTTAACTCTTTTATTATCC	GTAAGGACTTATAGTGAGTAAAGGACAGG	105
A20	rs1031825	CTTATCTTTCCCACATTATGGTCCT	AAGATATAATCACTGCTTTCAAGTATGC	98
A21	rs722098	GGAAGTACACATCTGTTGACAGTAATGA	GGGTAAAGAAATATTCAGCACATCC	80

In the study, Qiagen Multiplex PCR Kit (Germany) was used and the procedure of the kit was followed. The 10µl volume mix required for PCR was prepared to be 4 µl Qiagen Master Mix, 3 µl Primer Mix, 2 µl DNA, and 1 µl of distilled water. PCR cycle program was set as to be; 5 minutes and 30 seconds at 94ºC for initial denaturation and denaturation at 94°C in 35 cycles, 30 seconds at 60ºC for primer annealing, 30 seconds at 65ºC for an extension, 7 minutes at 65ºC for a final extension. Escherichia coli Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (SAP) were used for the removal of unwanted primer residues and dNTPs left from PCR reaction. 0.5 µl Exo I and 1 µl SAP were added on 5 µl PCR products during downstream application. The resulting mix was centrifuged and incubated for 70 minutes at 37ºC. At the end of the incubation, the mix was left for 20 minutes at 72ºC to ensure the inactivation of enzymes.

SNaPshot Reaction. SNaPshot Multiplex Kit (USA) was used for the SNaPshot reaction. For each SNaPshot reaction, 2.5 µL SNaPshot reaction mix, 1.5 µl SNaPshot primer mixture, and 1.5 µl DNA/Control DNA mix were prepared. Positive and negative control samples were used in each study to determine possible contaminations. The SNaPshot reaction was performed using the Gene Amp 9700 (Applied Biosystem, the USA) device. SNaPshot

cycle program was set as to be; is 5minutes at 94ºC for initial denaturation and denaturation for 30 seconds at 95°C in 35 cycles, 30 seconds at 60ºC for primary annealing, 30 seconds at 65ºC for an extension, 7 minutes at 65ºC for a final extension. After the SNaPshot reaction, 1 µl SAP (1U/µl) was added to each 5 µl mix to remove ddNTPs left after the reaction. The resulting mix is vortexed and centrifuged for 30 seconds. Subsequently, it was incubated for 70 minutes at 37ºC and 15 minutes at 85ºC respectively. In the study, control reactions were performed to determine whether SNaPshot Multiplex Kit (Applied Biosystem) was working properly or not.

Analysis of Data. Typing of the samples obtained has been conducted in ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA) capillary electrophoresis device by using the Data Collection Software 3.0 (Applied Biosystems, USA) software. In the ABI Prism 310 Genetic Analyzer, GeneScan-120 LIZ Size Standard (Applied Biosystem) was used to control the analysis conditions while all samples were analyzed. Data analysis was performed by using Gene Scan Analysis Software 3.1.2 (Applied Biosystems) software. The NCSS 2007 (Number Cruncher Statistical System) (Kaysville, Utah, USA) software for statistical evaluation of data and Pearson Chi-Square Test and Fisher's Exact Test for comparing qualitative data were used. Distributions of genes according to their genotypes were evaluated by Hardy-Weinberg equilibrium.

Results and discussion

Sample electropherograms showing 13 SNP nucleotides belonging to people living in four different geographical regions were given in Figure 1, Figure2, Figure3, and Figure4.

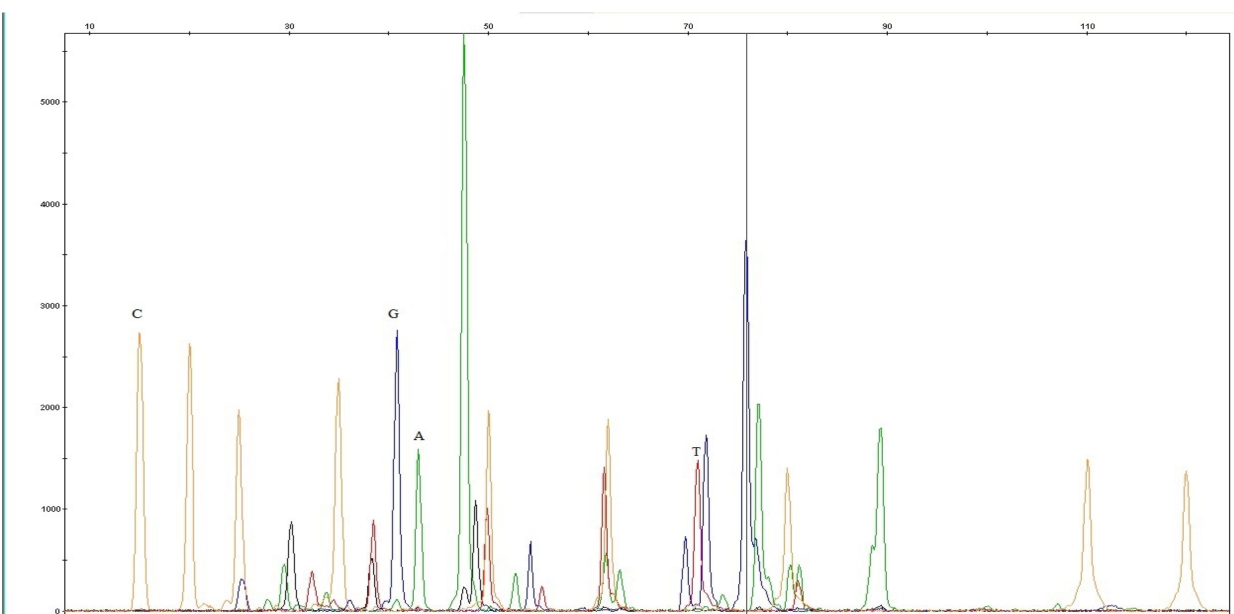

Figure 1 – Electrophoregram of a sample of a volunteer from the Marmara region

On the figures, the bases represented by the color of their peaks are indicated (Green: Adenine, Red: Thymine, Blue: Guanine, Yellow: Cytosine). When the distribution of blood samples taken from volunteers by region are analyzed, it is indicated that the samples were taken as to be; 28.6% from Mediterranean and Aegean, 18.4% from Eastern and Southeastern Anatolia, 14.3% from Central Anatolia, and the Black Sea and 40% from Marmara region. Pearson Chi-Square Test and Fisher's Exact Test were used to compare the qualitative data. The distribution of genes according to their genotypes was evaluated by the Hardy-Weinberg equation where genotype frequencies from allele frequencies enabled to be calculated in ideal conditions [10]. For each genotype, the values in Table 2 were obtained. Hardy-Weinberg equation was found to exist in all loci except for rs876724 loci.

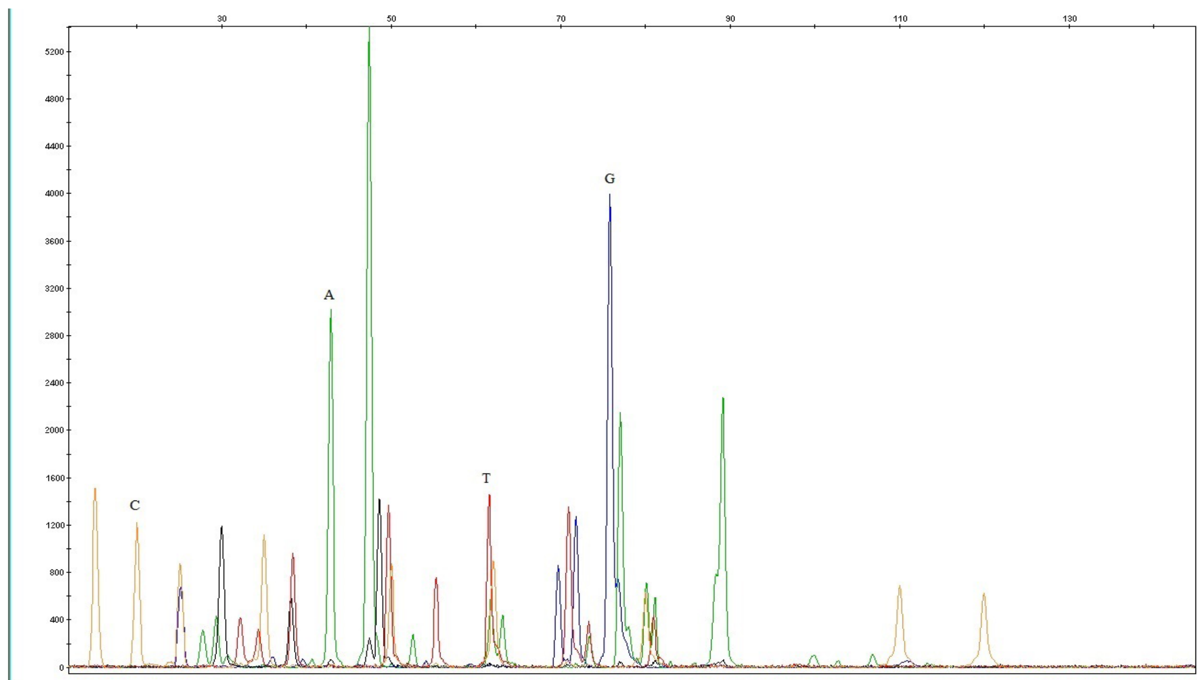

Figure 2 – Electrophoregram of a sample of a volunteer from the Central Anatolia region

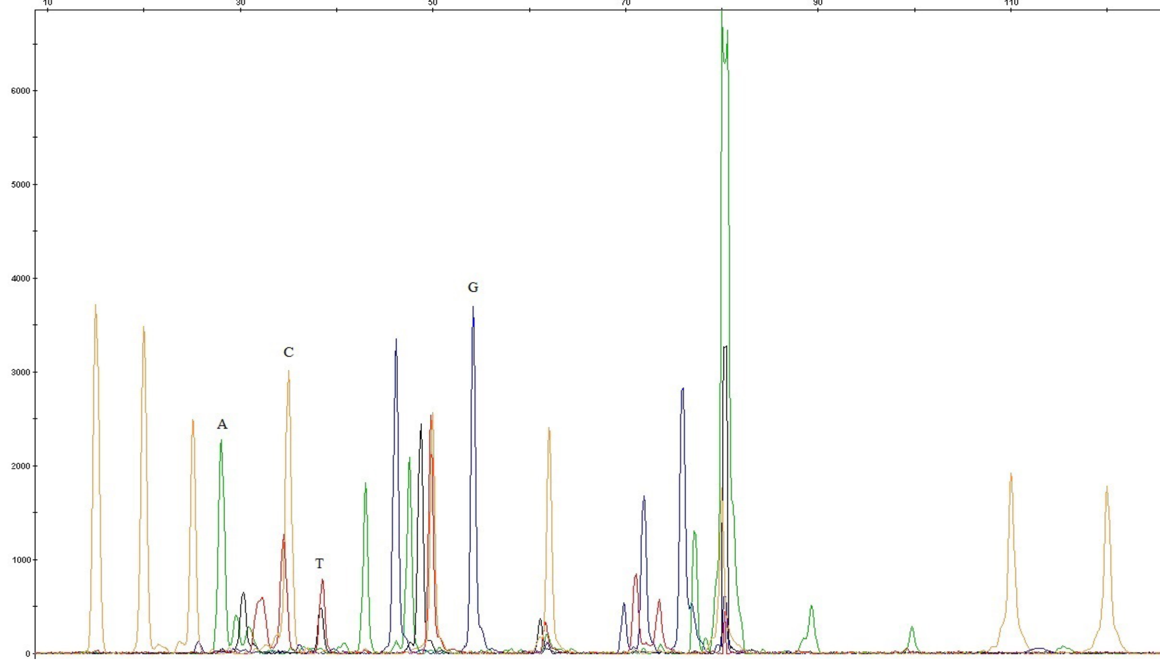

Figure 3 – Electrophoregram of a sample of a volunteer from the Black Sea region

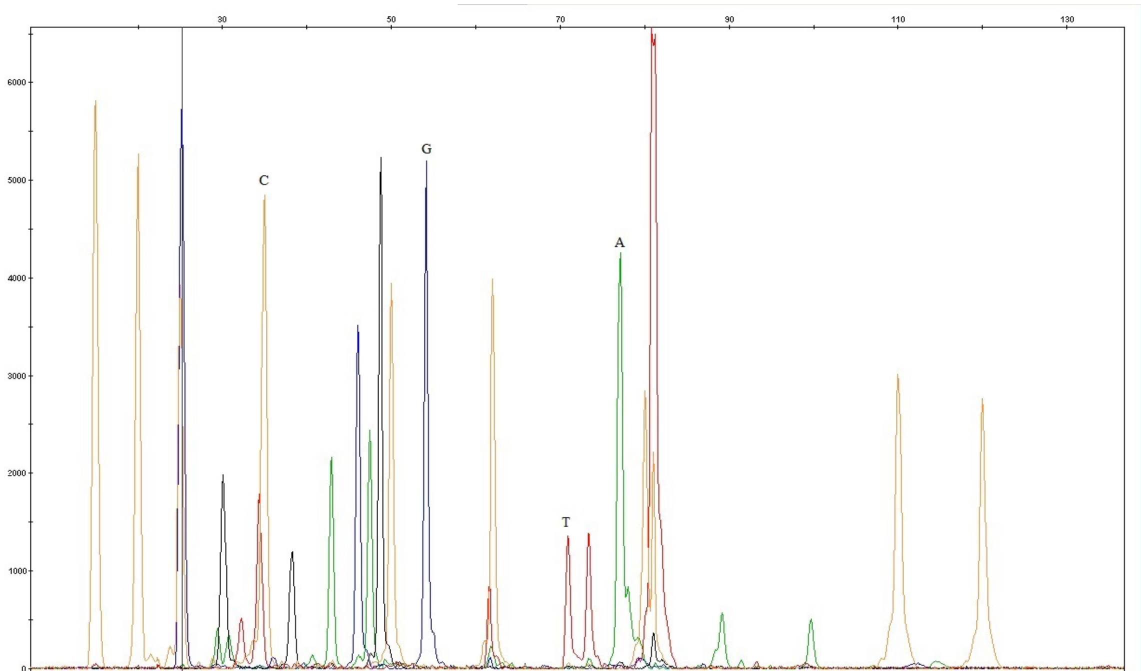

Figure 4 – Electrophoregram of a sample of a volunteer from the Southeastern Anatolia region

Table 2 – Values of Hardy-Weinberg Equilibrium

	Expected value	**Observed value**	**p**
rs876724	0.354	33.333	<0.001*
rs1357617	0.250	3.129	0.077
rs2046361	0.635	0.736	0.391
rs717302	0.479	0.089	0.765
rs917118	0.458	0.212	0.645
rs735155	0.510	3.000	0.083
rs901398	0.375	1.929	0.165
rs729172	0.510	0.350	0.554
rs740910	0.396	2.365	0.124
rs1493232	0.469	1.272	0.259
rs719366	0.438	0.434	0.510
rs1031825	0.458	3.515	0.061
rs722098	0.365	2.324	0.127

Note: * *Do not conform for Hardy-Weinberg Equilibrium*

Table 3 – Allele disturibitions by regions

		A/E	**D/G**	**İ/K**	**M**
rs876724	C/T	0.44 / 0.56	0.46 / 0.54	0.44 / 0.56	0.48 / 0.52
rs1357617	A/T	0.25 / 0.75	0.28 / 0.72	0.14 / 0.86	0.16 / 0.84
rs2046361	A/T	0.54 / 0.46	0.67 / 0.33	0.64 / 0.36	0.50 / 0.50
rs717302	A/G	0.68 / 0.32	0.78 / 0.22	0.64 / 0.36	0.68 / 0.32

Continuation of table 1

		A/E	D/G	İ/K	M
rs917118	A/G	0.46 / 0.54	0.22 / 0.78	0.43 / 0.57	0.26 / 0.74
rs735155	C/T	0.43 / 0.57	0.44 / 0.56	0.50 / 0.50	0.45 / 0.55
rs901398	C/T	0.29 / 0.71	0.44 / 0.56	0.29 / 0.71	0.24 / 0.76
rs729172	G/T	0.75 / 0.25	0.44 / 0.56	0.57 / 0.43	0.55 / 0.45
rs740910	A/G	0.89 / 0.11	0.72 / 0.28	0.93 / 0.07	0.61 / 0.39
rs1493232	G/T	0.36 / 0.64	0.22 / 0.78	0.50 / 0.50	0.24 / 0.76
rs719366	C/T	0.36 / 0.64	0.22 / 0.78	0.36 / 0.64	0.32 / 0.68
rs1031825	G/T	0.64 / 0.36	0.61 / 0.39	0.57 / 0.43	0.68 / 0.32
rs722098	A/G	0.79 / 0.21	0.67 / 0.33	0.64 / 0.36	0.79 / 0.21

Note: *A: Mediterranean E: Aegean D: Eastern Anatolia G: Southeast Anatolia İ: Central Anatolia K: Black Sea M: Marmara*

Distributions of allele percentages by region are presented in Table 3. Accordingly, no statistically significant difference was identified in terms of allele distributions of the loci; rs876724, rs1357617, rs2046361, rs717302, rs917118, rs735155, rs901398, rs1493232, rs719366, rs1031825 and rs722098 among regions. The percentage of G allele was identified to be higher in comparison with Eastern Anatolia/Southeastern region (p:0.036) when the distribution percentage of rs729172 locus is examined. There is no significant difference between allele distributions of other regions. Moreover, when analyzing the distribution percentages of rs740910 locus, the percentage of A allele is identified to be lower in the Marmara region in comparison with Mediterranean/Aegean and Central Anatolia/Black Sea regions (p:0.010, p:0.025, respectively). There is no significant difference between allele distributions of other regions ($p>0.05$).

As SNP analyses are used in pharmacogenomic studies in phenotyping and paternity analysis studies in forensic genetics, detection of migration routes, origin determination studies, they are also used within genetics polymorphisms and their effects over addictions as well [11-13]. Forensic identification studies constitute the largest and most basic areas of use of SNPs in forensic sciences.

Although SNPs have many advantages, they also have disadvantages as well. SNPs have limited discrimination power due to being biallelic [14;15]. An STR locus has about 4 times more discrimination power compared to an SNP locus. This disadvantage can be eliminated by working with more SNP loci while increasing the discrimination power. The low discrimination power of SNPs is considered to be as another disadvantage within mixed samples with DNA remains belonging to more than one person [16]. The STR method gives more successful results in identifying such examples. However, in degraded samples in mitochondrial DNA analyses, kinship, lineage and phenotypic properties of individual analyses as well as in forensic cases where sample quantity is less, SNP analyses indicate much more of successful results compared to STR analyses [17-20].

52 SNP-plex suitable for forensic analysis were identified in 2003 by SNP for ID Consortium set by five forensic science laboratories out of a group of researchers by being examined from as the aspects of validation, polymorphism, sequence quality, SNP typing technologies, and binding sites [9;21]. The selection criterion for SNPs to be used for 52SNP-plex were set to be as; in the distal regions of the p and q arms of each autosome, with a distance from known genes and commonly used STRs as 100kb, amplicon size in the range of 59 to 115 base pairs, polymorphic as in three main population group (Europe, Asia, and Africa), in the non-coding region of DNA, 30% heterozygosity in at least one population and at least 20% heterozygosity in all three populations [22].

Following the publication of 52 SNP-plex by the consortium, researches were conducted by many researchers to identify and validate autosomal SNP loci for citizens of their own countries. The first autosomal SNP study in Turkey was conducted in 2009 with in Istanbul University, Institute of Forensic Sciences [8;23]. A similar study was carried out by Greek researchers in the year of 2015 by studying 49 autosomal SNP loci selected out of 52 SNP-plex in 2015 for their own populations. Moreover, in order to assay the efficiency of SNPs, 30 autosomal insertion-deletion polymorphisms (InDels), 12 X-STRs, 23 Y-STRs, and 16 autosomal

STRs studies were also carried out [24]. In an ethnic group study conducted on Iranian Turkmen, the F_{ST} (fixation index) value, which is the criteria of genetic discrimination between races, is calculated by using 49 autosomal SNP loci selected out of 52 SNP-plex. The proximity of the F_{ST} value isindicative of high genetic similarity. The lowest F_{ST} values are observed among the Pakistani population and Iranian Turkmen, and then the closestF_{ST} value was found to belong to the population of Turkey [25].

SNP locus studies as an alternative to 52 SNP-plex studies and set by the SNPforID Consortium, are ongoing worldwide. For example, in a study conducted in Japan, researchers found an SNP-plex with 60 loci which they call 'Japaneseplex'; includes entirely Japanese alleles to differentiate their population from other Eastern Asians and to use it in forensic cases [26]. Moreover, the study, which researchers called 'Pacifiplex', was aimed to extract the ancestry of individuals belonging to Oceania populations [27]. Researchers using SNPs obtained as a result of forensic analyses in the study demonstrated a successful genotyping in serum samples obtained from the Oceania region at the end of the study.

In this study, although 23 SNP loci that were not previously studied for the Turkish population out of the 52 SNP loci set by the SNPforID Consortium were asked to be studied, due to lack of budget it is decided to study over the 13 SNP loci. In the study, samples of 50 volunteers were used and genotype information was determined for 13 SNP loci. Along with the data obtained as a result of the study showing parallelism with the values published by the SNPforID Consortium, appropriateness of the discrimination power of the studied loci for Turkish population was proved as well. A population whose allele frequency intact from one generation to another and the genotype frequency can be calculated from allele frequency is expressed to be in the state of "Hardy-Weinberg Equilibrium". One of the results of the Hardy-Weinberg equilibrium is that the expected frequencies can be determined, albeit estimated for the next generation over allele frequencies calculated on individuals in depopulation. With the help of the calculated genotype frequency, it is necessary that the "expected genotypes" and "observed genotypes" to be compatible with each other. Hardy-Weinberg Equilibrium was tested for the loci studied and it is determined that Hardy-Weinberg Equilibrium was found to exist in all loci except for one of them (rs876724). It is anticipated that the reason why a locus does not comply with the Hardy-Weinberg Equilibrium could be caused by a wide scale ranging from population size to random matching rule.

Conclusion

While the number of samples made it possible for the evaluation of the discrimination power of 13 loci statistically for the Turkish population, it was deemed as scarce for regional studies. Therefore, when data regarding loci wished to be evaluated in a regional context, no statistically significant difference was observed except for the significant results obtained for rs729172 and rs740910 loci in the regional context. Observing regional differences in the loci of rs729172 and rs740910 demonstrated that it is possible to evaluate SNP's regional discrimination power to be evaluated from a statistical point of view in case it is studied with more samples. Although enough samples are not available to comment on the discrimination power of geographical regions of the studied loci, the data obtained are deemed as significant for them to give an insight. 29 loci outof 52 SNP-plex in 2009 and 13 loci were evaluated by assessing Turkish population in this study, thus studies of a total of 42 SNP points were completed. It is believed that 52 SNP-plex will be ready for use within the Turkish population in case of 10 loci to be also studied in future researches.

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