ALU INSERTION POLYMORPHISMS IN ANATOLIA

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Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

ALU INSERTION POLYMORPHISMS IN ANATOLIA

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In the present study, five human specific *alu* insertion polymorphisms; YAP, ACE, A25, PV92, APO were examined in Anatolian Turkish population and partially in two Sicillian populations. The *alu* insertions are thougth to have arisen from the 7SL RNA by retroposition and the ancestral situation is hypotethically the absence of the *alu* element.

The frequencies of YAP element was found to be 0.069, 0.23, 0.17 for Turkish, Sciacca and Troina populations, respectively.

The frequencies of other *alu* insertions in Anatolian Turkish population were; 0.345, 0.03, 0.955, 0.175 for ACE, A25, APO and PV92, respectively. All systems were deviated from the Hardy-Weinberg equilibrium due to the deficiency in heterozygotes..

By combining the results of a previous study which was carried on 34 populations from all over the world with these data, the genetic distances between each pair of populations were calculated. The neighbour-joining tree was constructed, as expected Turkey positioned in a group composed of European populations and United Arab Emirates. The lowest genetic distances were observed between the populations of this branch of the tree. The principal component analysis was performed and same pattern of population groupings was obtained. The most discriminative markers for the differentiation of the populations were found to be ACE, PV92 and A25. The heterozygosity vs. distance from centroid graph was plotted and surprisingly the lowest heterozygosity value was observed in Anatolian Turkish population which may have a meaning of low effective population size and/or less gene flow.

Keywords: Polymorphism, ACE, APO, A25, PV92, YAP, *Alu* insertion, Genetic Distance, Human Evolution.

ÖZ

ANADOLU ' DA *ALU* İNSERSİYON POLİMORFİZMLERİ

Özbaş Gerçeker, Filiz

Yüksek Lisans, Biyolojik Bilimler Bölümü

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Bu çalışmada, beş polimorfik *alu* insersiyonu (YAP, ACE, A25, PV92, APO) Anadolu Türk populasyonunda ve kısmen de iki Sicilya populasyonunda incelenmiştir. *Alu* insersiyonlarının 7SL RNA' dan türedikleri ve başlangıçta hiç bulunmadıkları varsayılmaktadır.

Bu çalışmada YAP elementinin frekansı Türkiye için 6.9%, Sciacca (batı Sicilya) için 22% ve Troina (doğu Sicilya) için 16% olarak hesaplanmıştır. Diğer *alu* insersiyonlarının frekansları ACE için 0.345, APO için 0.955, PV92 için 0.175 ve A25

için 0.03 bulunmuştur. Ayrıca her lokusun Hardy-Weinberg dengesine uyup uymadığı test edilmiş ve bütün lokusların heterozigot eksikliğinden dolayı sapma gösterdiği belirlenmiştir..

Daha önce 34 populasyon için yapılan bir çalışmanın verileri bu çalışmanın sonuçları ile birleştirilerek populasyonlar arasındaki genetik uzaklıklar hesaplanmıştır. Ayrıca bu değerlerden yararlanarak evrim ağacı çizilmiş, Turkiye'nin Avrupa toplumları ve Birleşik Arap Emirlikleri ile aynı gruba düştüğü ve en düşük genetik uzaklıkların bu gruba ait populasyonlar arasında olduğu görülmüştür. Populasyonların genetik yakınlıklarını üç boyutlu düzlemde görebilmek için Principal Component Analizi yapılmış ve populasyonların ağaçtaki gibi gruplandığı görülmüştür. Populasyonları ayırmada en etkili lokusların ACE, PV92 ve A25 olduğu tesbit edilmiştir. Averaj heterozigotluk değerleri her populasyon için hesaplanmış ve "heterozygosity vs. distance from centroid" grafiği çizilerek populasyonların geçmişteki büyüklükleri ve/veya tecrübe ettikleri gen akışı tahmin edilmeye çalışılmıştır. Beklenenin aksine en düşük heterozigotluk yani en az gen akışı ve/veya geçmişteki en düşük populasyon büyüklüğü Anadolu Türk populasyonu için bulunmuştur. Ancak bu sonucun daha iyi örneklemenin yapıldığı ve daha çok lokusun çalışıldığı yeni araştırmalarla test edilmesine ihtiyaç vardır.

Anahtar kelimeler: Polimorfizm, ACE, APO, A25, PV92, YAP, *Alu* insersiyonu, genetik uzaklık, insan evrimi.

To my family

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LIST OF ABBREVIATIONS

DNA	Deoxiribonucleic acid
RNA	Ribonucleic acid
STR	Short Tandem Repeats
VNTR	Variable Number of Tandem Repeats
mtDNA	Mitochondrial DNA
RFLP	Restriction Fragment Length Polymorphism
HW	Hardy-Weinberg
PCR	Polymerase Chain Reaction
YAP	Y Alu Polymorphism
PCA	Principal Component Analysis
PC	Principal Component
NJ	Neighbour Joining
UAE	United Arab Emirates
N	Number of Sample
PNG	Papua New Guinea
M	Male
F	Female
EDTA	Ethylenediaminetatraacetic acid
SDS	Sodium Dodecyl Sulfate

TE	Tris-EDTA
UV	Ultra Violet
dNTP	Deoxyribonucleotide triphosphate
bp	Base Pair
EtBr	Ethidium Bromide
ml	mililiter
µl	microliter
ng	nanogram
mM	milimolar
KCl	Potassium Chloride
HCl	Hydrogen Chloride
min.	Minute (s)

CHAPTER I

INTRODUCTION

Development of new molecular biological techniques made it easier to define genetic structure of the populations. In humans these techniques can be used to identify the relatedness, to study the evolutionary history as well as to examine the genetic diseases of the populations. The early studies in populational genetics were based on immunogenetic markers such as blood groups (Haldane, 1940). With the establishment of protein electrophoresis (Simithies, 1955) protein polymorphisms were used to define the genetic structures of the populations. The improvement of molecular techniques which can directly examine DNA molecule enhanced the use of DNA markers in the studies of population genetics. Studying DNA has many adventages over the protein polymorphisms; very small amount of material is enough for the study by the help of PCR amplification, many potential markers are available, some loci are multi allelic, and some loci which are non-coding and not subject to direct selection and the mutations not resulting in an electrophoretic mobility changes are detectable by DNA techniques. In addition to these, the preservation of tissues in ethanol or by drying simplifies collection and storage (Ferguson *et al.*, 1995).

The genetic structure of Anatolian Turkish population has been studied by many scientists, some of these studies are Saatçioğlu (1979) Önde and Kence (1994 and 1995) on ABO blood groups, Atasoy *et al.*(1990) Togan and Ergüven (1994) , Togan *et al.* (1996) on red blood cell enzymes, Altay *et al.*(1978) on the proteins, Comas *et al.*(1996), Calafell *et al.* (1996) on mitochondrial DNA sequence variability.

1.1. DNA Markers Employed on Turkish Populations Previously

To study population genetics of Anatolain Turkish populations various types of DNA markers and various methods based on DNA examination have been used, recently. These can be grouped as fallows:

i) Short Tandem Repeats (STR) and Variable Number of Tandem Repeats (VNTR) polymorphisms

ii) Restriction Fragment Length Polymorphism (RFLP)

iii) Mitochondrial DNA (mtDNA) polymorphism by sequencing

1.1.1. STR and VNTR Polymorphisms

Short Tandem Repeats (STR) and Variable Number of Tandem Repeats (VNTR) loci are composed of tandemly repeated sequences of 1-7 bp in length, are highly polymorphic and are highly abundant in the human genome (Huang *et al.*, 1995). There have been many studies on different populations with these genetic markers and they all

suggested that these loci can be used to estimate the frequency of a polymerase chain reaction based DNA profile in a particular population (Hammond *et al.*, 1994, Martin *et al.*, 1995, Huang *et al.*, 1995, Gusmao *et al.*, 1995).

As the part of the study carried by Budowle and Monson (1994) three VNTR loci polymorphisms were studied in three ethnically distinct populations (Norwegians, Spanish,and Turks) and in Caucasians and Blacks from the United States. They have founded that the genetic diversity between subgroups within a race is greater than between races.

Allele distributions of two STR loci have been investigated in Turks and Germans (Alper *et al.*, 1995). The number of the observed alleles differed between these two populations.

Meyer *et al.* (1995) have studied six polymorphic STR systems in 7 human populations one of which were the Turks. No deviation from the Hardy-Weinberg equilibrium was observed in each population. It was observed that within major ethnic groups (Germans, Turks, Moroccans, Japanese, Chinese, Papuans, Ovambos) only minor differences in allelic frequencies were found.

In one of the other studies carried by Iwasa *et al.* (1997), five short tandem repeat systems HumTH01, HumVWA, HumCD4, HumF13B, HumFES were investigated in two subpopulations living in Turkey (Laz Turks and Kurds). The population genetic data

were compared to another Turkish population sampled from Adana. A closer genetic relationship was found to the Laz Turks than to the Kurdish sample which was also confirmed by phylogenetic tree reconstruction with seven populations from three major ethnic groups (Caucasian, Asian, African). In contrast to the Laz and Adana populations the Kurdish sample showed relatively low heterozygosity values and deviations from the Hardy-Weinberg equilibrium in four systems.

Jarjanazi *et al.* (1998) have studied two microsatellite repeat polymorphisms of the factor VIII gene in Turkish population. They stated that the Turkish population is slightly less polymorphic than Anglo-Americans but more polymorphic than Chinese, Slavs and Uzbekians.

1.1.2. RFLP

Restriction Fragment Length Polymorphism depends on the presence or absence of the restriction sites for endonucleases in a particular part of the DNA. Due to the specificity of the restriction enzymes these polymorphisms are thought to be reliable.

Schurmann *et al.* (1987) have studied four restriction fragment length polymorphisms on short arm of the X chromosome in German and Turkish populations (50 individuals for each). They stated that there was no significant difference in allele frequencies of these two populations.

1.1.3. Mitochondrial DNA (mtDNA) Polymorphisms by Sequencing

Maternal inheritance, lack of recombination, relatively small size and high rate of mutation make mtDNA a useful marker for population genetic studies (Avise, 1994).

According to the mtDNA sequence variability , Turks exhibit a stepping-stone position between the Middle East and Europe (Calafell *et al.*, 1996 and Comas *et al.*, 1996).

1.2.Some Other DNA Polymorphisms

1.2.1. *Alu* Insertion Polymorphisms

Alu elements are thought to be derived from 7SL RNA gene and mobilized through an RNA Polymerase III derived transcript by retroposition. They have been amplified in the last 65 million years in primates reaching to a copy number in excess of 500,000 (Stoneking *et al.*, 1997). Retroposition of an *alu* element is an ongoing process (Deininger and Batzer, 1995). Some of the *alu* elements being human specific have retroposed so recently that they have not been fixed in the human species.

The *alu* insertion elements are very useful genetic markers in population genetics studies having four big advantages over the other types of polymorphisms. Firstly, they are stable elements , i.e once they are inserted at a specific chromosomal location they

very rarely undergo deletion. *Alu* elements are subject to very limited amounts of gene conversion (Batzer *et al.*, 1995). Second, the presence of the *alu* element means identity by descent because the probability of the insertion at a same chromosomal location is negligible. Third, the typing of these insertions do not require complex, time-consuming methods. They can be typed by PCR- based simple and quick techniques. Another advantage of studying human specific *alu* insertions is that; the ancestral state is the absence of the *alu* elements in humans with certainity. Due to these positive features of the *alu* insertions they represent a very important role in human population genetic studies.

There have been many studies of *alu* insertion polymorphisms in different populations but none of them includes Turkish populations. In one of these studies, (Knight *et al.*,1996) 10 populations were examined for three *alu* insertions (ACE, APO, and α-Globin 2 *Alu* 1) ; ACE was found monomorphic in all populations including Greek Cypriots and Turkish Cypriots probably because of the very few sample sizes employed. However, some other studies indicated that ACE is polymorphic in all of those populations (Batzer *et al.*, 1996, Stoneking *et al.*,1997 and Batzer *et al.*, 1994).

In a recent study (Novick *et al.*, 1998), five polymorphic *alu* insertions were studied in totally 895 individuals of 30 populations from North, Central, and South America. They detected significant level of interpopulation variabilty but it was less than

that observed in a worldwide population survey. They suggested that close similarity between the Chinese and Native Americans is due to the recent gene flow from Asia.

Batzer *et al.* (1994) observed that the tree of population relationships based on *Alu* insertion polymorphisms is very similar to population trees based on classical blood protein markers (Cavalli-Sforza *et al.*, 1988), nuclear DNA RFLP loci (Bowcock *et al.*, 1991), microsatellite loci (Bowcock *et al.*, 1994) and mitochondrial DNA (Merriweather *et al.*, 1991). Stoneking *et al.*(1997) , have studied 1500 individuals from 34 worldwide populations for eight *alu* insertions. The heterozygousity v*alu*e for the world populations overall was found to be 0.426 $\pm$ 0.035 and the Gst v*alu*e (the measure of interpopulational genetic differences) was 0.128 which was comparable to other studies of nuclear DNA polymorphisms in human populations (Jorde *et al.*, 1995).

1.2.2. Y Chromosome Polymorphisms

Variations on Y chromosome are useful in understanding the evolution of the human paternal lineage and male gene flow. Y chromosome is haploid and do not recombine. In addition to that, it is transmitted paternally and thus is subject to only male specific mutation rates. Previous studies indicated that, the level of the polymorphism on Y chromosome is lower than that of the autosomal and X chromosome but Y chromosome has higher evolutionary rate than X chromosome.

Y *alu* polymorphism (YAP element) is present at nonrecombining portion of the long arm of human Y chromosome (Yq11) in some males and absent in others (Hammer, 1994). Therefore, YAP is useful for investigating human population history through male lineages during the last 200,000 years. It is thought that YAP (DYS287) is originated at a single time and the distribution of the polymorphism increases the possibility of tracing paternal lineages and male-mediated gene flow between largely seperated geographical regions (Hammer, 1995).

There have been many studies on Y *alu* polymorphism for many different populations but none for the Anatolian Turkish populations.

1.3.Some of the Uses of Genetic Polymorphisms in the Study of Population Genetics

Once the allele frequencies of the loci were determined for the populations they can be analyzed by various statistical methods. The presence of the Hardy-Weinberg equilibrium can be tested for each locus. Frequencies can be used to find the genetic similarities / dissimilarities of the individual populations (as an example see Nei and Roychoudhury, 1993). Genetic similarities, in turn, can be used to visualize the groups of genetically similar populations by constructing evolutionary trees.

Frequencies can be employed to find the relative heterozygosity levels of the populations which may indicate the presence of bottleneck and/or inbreeding in a

population (Nei and Roychoudhury, 1993) and may give some information about the effective size of the populations in the past (Stoneking *et al.*, 1997).

As rewieved by Calafell *et al.* (1996), it has been expected that during the evolution of modern humans a group of migrants came out of Africa passing Middle East and later Anatolia, arrived to Europe in paleolithic ages. Furthermore, it was suggested that later in neolithic ages after the settlement of hunter gatherers another migration towards Europe took place starting from Middle East (Sokal *et al.*, 1989) or Catalhoyuk (Renfrew, 1991). Since Anatolia was well populated throughout historic times by peoples of various origins the effective size of the Anatolian population is expected to be high. Because of the high effective population size and high degree of intermixing that took place in this region the level of heterozygosity is also expected to be high in Anatolia population.

Frequencies can also be subjected to Principle Component Analysis which reveals the genetic relatedness of the populations in the 3D space and can identify the power of discrimination of the genetic markers in differentiating the populations

1.4.The purpose of the study

To understand the genetic background of the Turkish population there is still need for new researches on populations having high sample sizes with the polymorphic genetic markers. The data about the *alu* insertion polymorphisms in many populations is

available now but there has been no data on the *alu* insertion polymorphisms in Turkish populations. In this study, the aim was to examine the genetic structure of the Turkish population with respect to the presence or absence of the five *alu* elements. In this context, a total of 100 healthy individuals from different regions of Turkey were examined for the presence / absence of ACE, APO, A25 and PV92 *alu* insertions. In addition to that, 72 males from Turkey, 39 males from Troina (east of Sicily) and 53 males from Sciacca (west of Sicily) were studied for Y *alu* polymorphism (YAP).

The purposes of this study can be summarized as fallows:

- To increase the knowledge about the genetic structure of the Turkish population by providing *alu* insertion polymorphism data.
- To have an idea about the relative effective population size of the Anatolian population in the past by the plot of heterozygosity vs. distance from centroid.
- To see the genetic relatedness of the Turkish population to the other populations by constructing a neighbour joining (NJ) tree using the available data for other populations based on the markers employed in the present study.
- To employ Principal Component Analysis (PCA) in order to identify the power of discrimination of the studied markers in differentiating the populations.

CHAPTER 2

MATERIAL AND METHODS

2.1. Materials

Sampling for this study was done in TUBITAK DNA/Cell Bank and Gene Research Laboratories.. During the sample collection, healthy individuals who did not share any common kinship were preferred. Additionally, sampling was done from different regions of Turkey so that Anatolian Turkish population is well represented in the samples. The individuals settled in one particular area during the last three generations were selected for this study. This way, the current distribution of the alleles which was formed at least three generations ago were studied. For Y *Alu* Polymorphism totally 164 males from which 53 were from Sciacca (west of Sicily), 39 from Troina(east of Sicily) and 72 from Turkey were studied and the regions of samples from Turkey were listed in Appendix B. Autosomal *alu* insertion polymorphisms; ACE, APO, PV92 and A25 were studied for only Turkish population which consists of 100 persons including both females and males (Appendix B).

2.2.DNA Isolation From Peripheral Blood Samples

The blood samples (≈ 10 cc) were collected into the Ethylenediaminetatraacetic acid (EDTA) tubes and stored at 4°C until the use. DNA was extracted from the pheripheric blood with high salt precipitation method . 10 cc blood was placed into the tubes and 40 ml. of cold and sterile water was added. Red blood cells were lysed by shaking the tube slowly. Then the tubes were centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended in 25 ml. of cold sterile water again. Tubes were centrifuged at 2000 rpm for 10 minutes and the supernatant was removed. 3 ml. of Nuclei Lysis Buffer (pH 8.2), 150 µl. Proteinase K (10 mg/ml) and 200 µl Sodium Dodecyl Sulfate (SDS) solution were added and mixed by short vortex . Then tubes were incubated at 37 °C overnight to lyse the white blood cells. The next day, 2 ml. of 10 M ammonium acetate was added to the tubes and incubated at room temperature for 10 minutes. After that, they were centrifuged at 3500 rpm for 15 minutes and the supernatant was collected into another tube by a transfer pipette. 2 volumes of 99% cold ethanol was added to collect DNA. DNA was then transferred to eppendorf tube containing 500 µl TE buffer (pH 7.4) and stored at –80°C.

2.3.PCR Amplification

The oligonucleotide primer sequences together with annealing temperatures were listed in Table 1. For autosomal *alu* insertion polymorphisms (ACE, APO, PV92 and A25) amplification of DNA samples was carried out in 25 µl reactions using;

50 ng of target DNA

375 ng of each primer

0.2 mM of each dNTPs in 50 mM KCL, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.4

0.5 u Taq polimerase (Promega).

The amplification conditions for these oligonucleotides were;

- denaturation at 94 °C for 2 min. 1 cycle
- denaturation at 94 °C for 1 min.
  Primer annealing at appropriate temperatures for 1 min. 32 cycles
  extension at 72 °C for 1 min.
- Final extension at 72 °C for 3 min. 1 cycle

The amplification of the YAP was carried out in a slightly different way such that; 0.12 µM from each primer were used for amplification. The PCR conditions for YAP element was as follows;

- denaturation at 94 °C for 2 min. 1 cycle
- denaturation at 94 °C for 1 min

  primer annealing at 51°C for 1 min. 30 cycles

  extension at 72 °C for 1 min.
- Final extension at 72 °C for 3 min. 1 cycle

2.4.Analysis of the PCR products

10 µl of each PCR product was mixed with 5 µl of Orange G dye and analysed by electrophoresis through a 2% agarose gel containing 0.5 µg/ml ethidium bromide (EtBr) and reaction products were directly visualised by UV fluorescence. The length of the PCR products (filled and empty sites) were given in table 2. The photographs of the gels were obtained by a gel image system (Illustrations 1, 2, 3, 4, 5).

2.5.Statistical Analysis

The allele frequencies of the insertions were calculated according to the formula given below:

$$\text{Frequency of an allele} = \frac{(2x \,\#\text{ of homozygotes}) + (\#\text{ of heterozygotes})}{(2x \text{ total } \#\text{ of individuals})}$$

The Hardy-Weinberg equilibrium (expected) frequencies (p^2, $2pq$, q^2) were also calculated and Goodness of fit test was done (Sokal and Rohlf, 1995) to see if there is

any deviation from the Hardy-Weinberg equilibrium or not. The Hardy-Weinberg Law states that in a randomly mating infinitely large population both the gene and genotype frequencies will be in the equilibrium in the absence of mutation, migration and natural selection. If a population is highly inbred or substructured (having Wahlung effect) a deviation from the HW equilibrium can be observed in that population. Wahlung principle states that when there is a large difference in allele frequencies of the subsamples, there will be a net deficiency of heterozygotes and excess of homozygotes in whole sample even if the Hardy-Weinberg equilibrium exists within each subsample.

The observed heterozygosity can be calculated as fallows:

$$\text{Observed Het.} = \frac{\text{number of heterozygotes}}{\text{Total number of individuals}}$$

However, the expected heterozygosity is defined by Hardy-Weinberg Law as :

$$\text{Expected Het.} = 2pq$$

In this case, p and q are the frequency of the presence of the insertion and the frequency of the absence of the insertion, respectively.

Table 1. Oligonucleotide primers, annealing temperatures and chromosomal locations of the polimorphic *alu* insertions

Locus	Primer Sequences		Ann. Temp. (°C)	Chrom. Location
	5′ primer	3′primer		
ACE	5′-CTGGAGACCACTCCCATCCTTTCT-3′	5′-GATGTGGCCATCACATTCGTCAGAT-3′	58	17
APO	5′-AAGTGCTGTAGGCCATTTAGATTAG-3′	5′-AGTCTTCGATGACAGCGTATACAGA-3′	50	11
A_{25}	5′-CCACAAATAGGCTCATGTAGAAC-3′	5′-TATAATATGGCCTGGCCTGGATTATACC-3′	63	8
PV92	5′-AACTGGGAAAATTTGAAGAGAGAAAGT-3′	5′-TGAGTTCTCAACTCCTGTGTGTTAG-3′	54	16
YAP	5′-CAGGGGAAGATAAAGAAATA-3′	5′-ACTGCTAAAAGGGGATGGAT-3′	51	Y

Table 2. Sizes of the PCR products in the presence and absence of the insertion

Locus name	**Insertion Positive (bp)**	**Insertion Negative (bp)**
ACE	490	190
APO	437	122
A25	552	268
PV92	416	101
YAP	455	150

By combining the results of the present study with those of the previous one (Stoneking et al., 1997) ;

a) Genetic similarities (Genetic Distances) between the pairs of the populations were determined.
b) Heterozygosity vs. distance from centroid was plotted in order to be able to asses the relative gene flow experienced by each population and to estimate the relative effective population sizes of the populations in the past.
c) Overall relatedness of the populations were visualized with the help of tree construction method (Neighbour-Joining Tree).
d) Relative importance of the variables (different alu insertions) for the discrimination of the groups were studied by means of the Principle Component Analysis (PCA).

The genetic distance between two populations can be defined in terms of the allele frequencies for the studied loci. In this study, Nei's standart genetic distances (1972) and standart errors of standart genetic distances were calculated and the Neighbour- Joining tree was constructed by the help of the computer program called "DISPAN: Genetic Distance and Phylogenetic Analysis" (Ota T., 1993). This program also made it possible to get the multilocus Gst which is the measure of the amount of differentiation among the populations.

To see the relative positions of the populations in the 3 dimensional space the Principal Component Analysis was performed by the help of computer program called "NTSYS: Numerical Taxonomy and Multivariate Analysis System" (Rohlf ,1993).

The average heterozygosity and its standart errors for each population were also calculated. The plot of heterozygosity vs. distance from centroid was constructed according to the model by Harpending and Ward (1982).

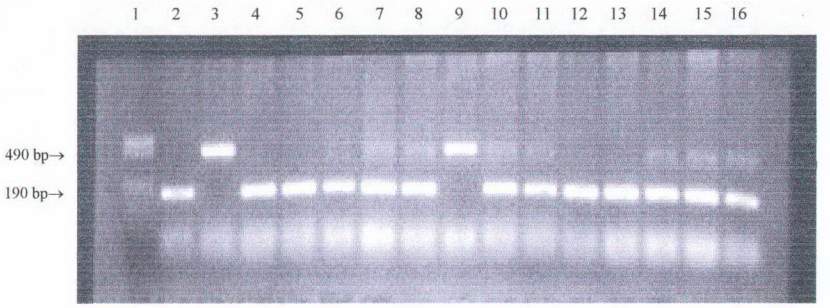

Illustration 1.. The sample photograph for the amplified products of ACE
Lane 1: pBR322 DNA size marker
Lanes 2, 4, 5, 6, 12, 13 : insertion negative
Lanes 3, 9: insertion homozygote
Other lanes: insertion heterozygote

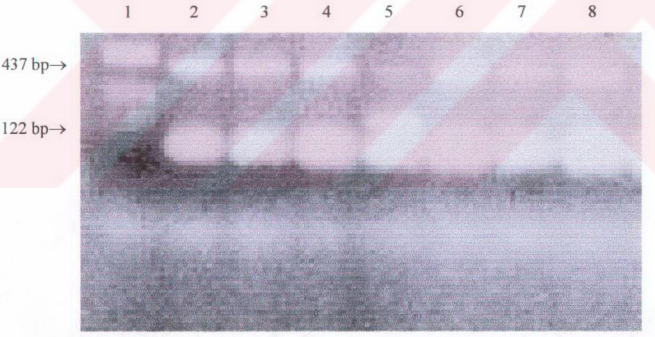

Illustration 2. The sample photograph for the amplified products of APO
Lane 1: pBR322 DNA size marker
Lane 2,3,4,6,7,8: insertion homozygote
Lane 5: insertion heterozygote

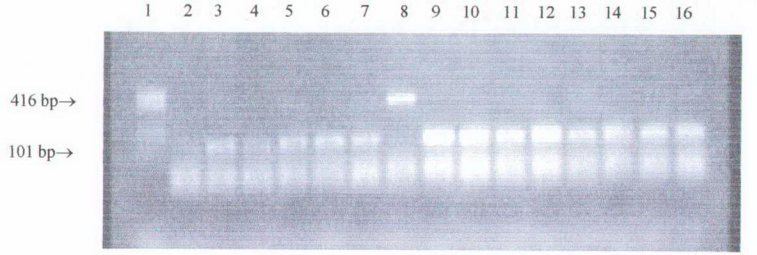

Illustration 3 . The sample photograph of the amplified roducts of PV92
Lane 1: pBR322 DNA size marker
Lane 8: insertion homozygote
Lanes 5,7,13,14,16 :insertion heterozygotes
Other lanes: insertion negative

Illustration 4. The sample photograph of the amplified products of A25
Lane 1: pBR322 DNA size marker
Lane 17: insertion homozygote
Other lanes: insertion negative

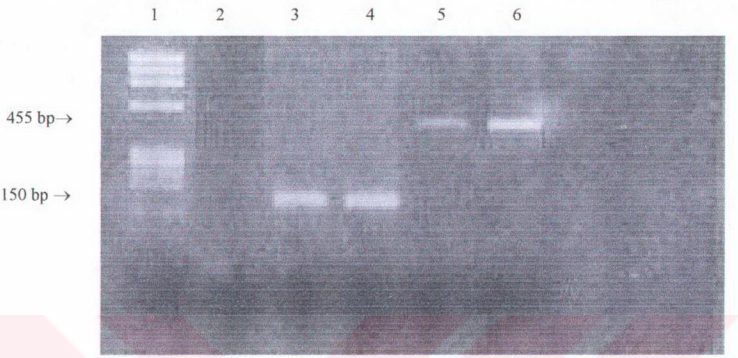

Illustration 5. The sample photograph for the amplified products of YAP
Lane 1: pBR322 DNA size marker
Lane 3,4: insertion negative
Lane 5,6:insertion positive

CHAPTER 3

RESULTS

3.1. Distribution of the *Alu* Insertions

Allele frequencies for the five systems were calculated, all loci were polymorphic in Anatolian Turkish population. The observed allele frequencies of the *alu* insertions were found to be 0.345 for ACE, 0.175 for PV92, 0.03 for A25 and 0.955 for APO. The frequencies of these four insertions in Turkish population were summarized in table 3. The frequency of YAP element was found to be 6.9 % for Anatolian Turks and 23 % for Troina and 17 % for Sciacca populations (Table 4)

The presence of Hardy-Weinberg equilibrium was tested for the four systems: ACE, APO, A25 and PV92. All systems were deviated from the HW equilibrium ($p < 0.05 - p< 0.001$). For the A25 and APO insertions the frequencies were very extreme; being 0.03 and 0.955, respectively. The expected genotype frequencies and χ^2 values were calculated and shown in table 3 together with the observed genotypic frequencies. The average expected heterozygosity value for Turkish population was found to be 0.22 with respect to the four *alu* loci.

To visualize the presence of the distribution patterns of the markers in Turkey the sampled individuals and their genotypes for ACE, A25, APO, PV92 and YAP were shown in figures 1-5. A25 seemed to be mostly confined in the southeast of Turkey while ACE seemed to be distributed more homogenously in Turkey, perhaps not present on the Aegean coast. The last observation could be the result of sampling error due to the low sample size from this region. The frequency for APO was very high (0.955) and the presence of this *alu* insertion seemed to be distributed in all over the Turkey. PV92 was again present in every region studied in Turkey.

The frequency of the insertion of the YAP element was determined and tabulated in table 4 for Anatolian Turkish and two Sicillian populations (Troina and Sciacca). The frequency of the *alu* insertion for the Y chromosome was found to be 6.9 % in Anatolian Turkish population while 23 % in Sciacca (west of Sicillia) and 17 % in Troina (east of Sicillia) population. The freqeuncy of YAP differed very much between Anatolian Turkish population and two Sicillian populations. YAP insertion exhibited to have a distribution limited to the central and western Anatolia.

3.2. Present (Anatolian Turks) data together with the previous results

Considerable data of four *alu* loci (ACE, APO, PV92 and A25) frequencies for various populations were published before (Stoneking et al., 1997). In Appendix A, as well as the frequencies of the insertions in the populations the Gst values (a measure of the amount of subpopulation differentiation) were given.

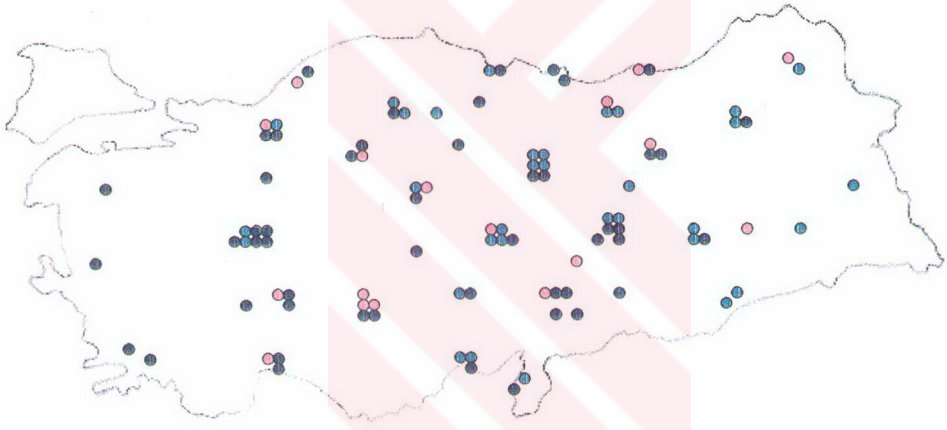

Figure 1. The distribution of ACE insertions in Turkey

: insertion negative

: insertion heterozygote

: insertion homozygote

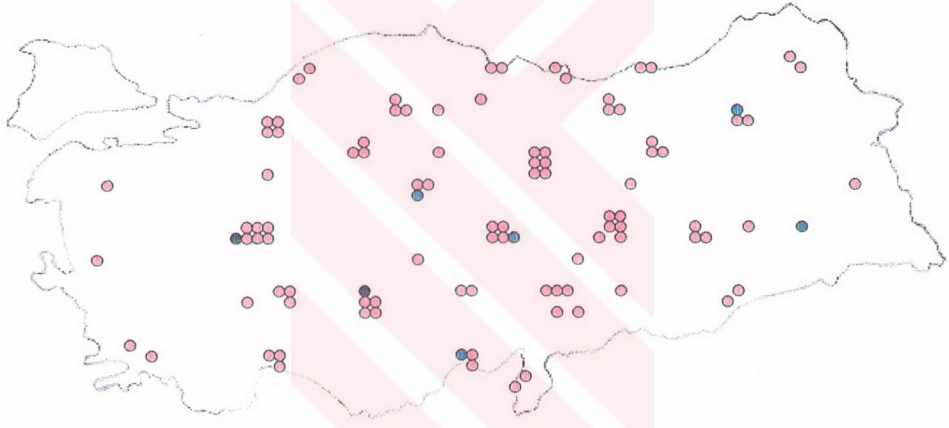

Figure 2. The distribution of APO insertions in Turkey

- : insertion negative
- : insertion heterozygote
- : insertion homozygote

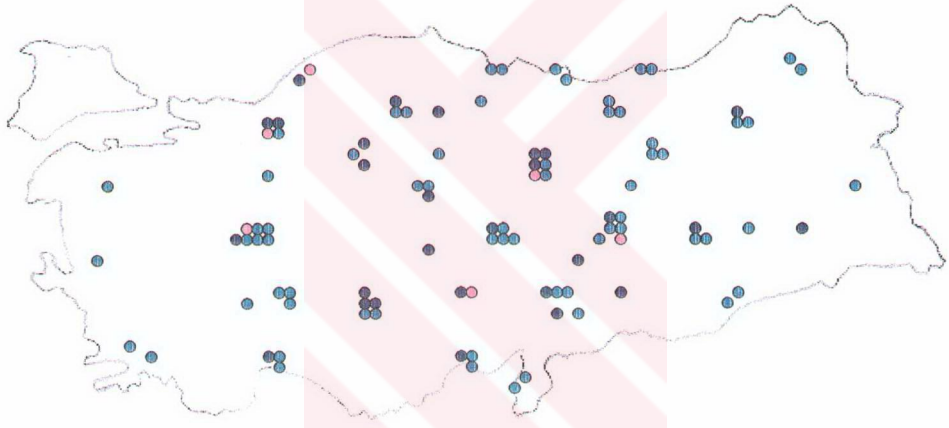

Figure 3. The distribution of the PV92 insertions in Turkey

: insertion negative

: insertion heterozygote

: insertion homozygote

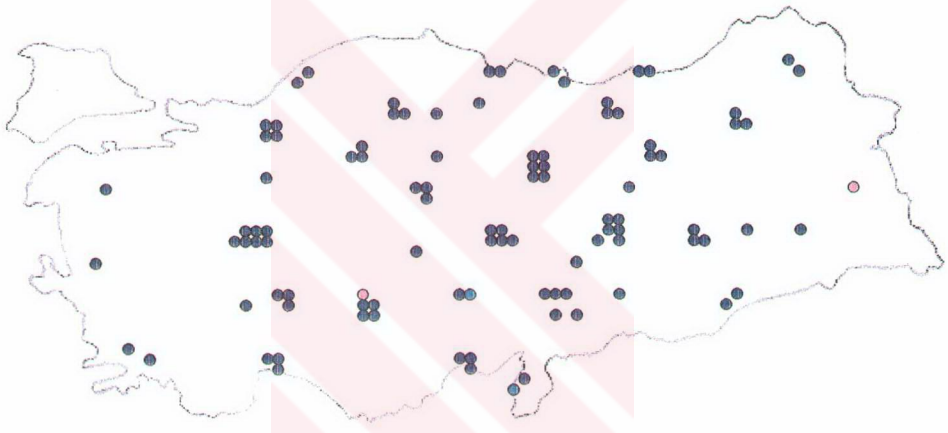

Figure 4. The distribution of the A25 insertions in Turkey

: insertion negative

: insertion heterozygote

: insertion homozygote

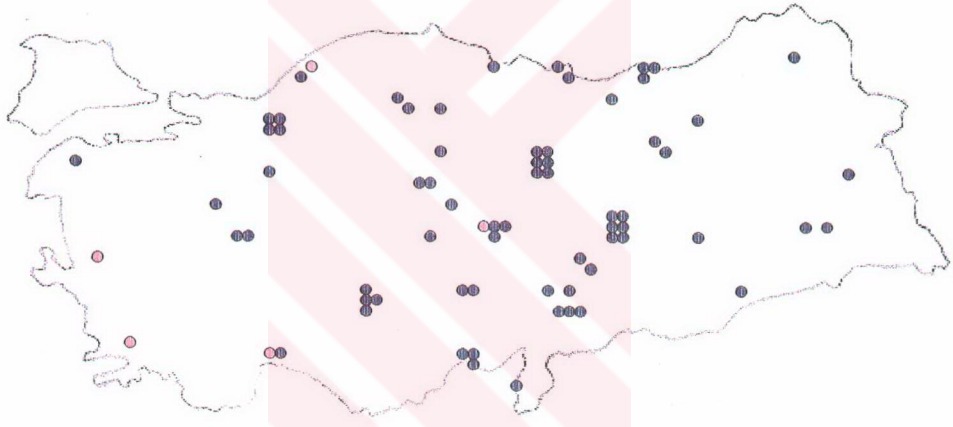

Figure 5. The distribution of YAP insertions in Turkey

: insertion negative

: insertion positive

Table 3. Allele frequencies, expected and observed frequencies of the genotypes, χ^2 values and average heterozygosities of *alu* insertions in Anatolian Turkish population.

Alu	Genotypes	Freq.of The insertion	Expected Freq.	Observed Freq.	χ^2	Heterozygosity Observed	Expected
ACE							
	+		11.9	17			
	-	0.345	42.9	48	5.092	0.35 ± 0.03	0.45 ± 0.03
	+/-		45.2	35			
APO							
	+		91.2	93			
	-	0.955	0.2	2	17.73	0.05 ± 0.01	0.08 ± 0.02
	+/-		8.6	5			
PV92							
	+		3.06	7			
	-	0.175	68.06	72	7.4	0.21 ± 0.03	0.28 ± 0.03
	+/-		28.8	21			
A25							
	+		0.09	2			
	-	0.030	94.09	96	43.04	0.02 ± 0.01	0.06 ± 0.03
	+/-		5.82	2			
Average Expected Heterozygosity						0.22 ± 0.09	

Table 4. Frequencies of the Y *Alu* insertion (%) in Anatolian Turkish population and two Sicillian populations

Population	N	YAP (+)
Turkey	72	6.9
Sciacca(west of Sicily)	53	17
Troina (east of Sicily)	39	23

N: Number of individuals studied.

3.2.1. The Genetic Distances Between Populations

By combining the results of the present study with the previous results the genetic distances (Nei, 1972) between pairs of the populations were calculated and given as a matrix in table 5. According to this matrix, no genetic distance was observed between the Turkish population and the Swiss population and also between the Turkish population and Greek Cypriot population (values below 0.09 were accepted as zero). Very small genetic distances were observed between Turkish population and the geographically close populations (Turkish Cypriot, UAE, Bretons, French).

3.2.2. The plot of Heterozygosity vs. Distance from Centroid

It is known that in a structered population a simple relationship is expected between the mean heterozygosity of a population and the genetic distance of that population from the centroid (Harpending and Ward, 1982). Heterozygosity vs. distance from centroid for 29 populations including Turkey was plotted in this study (Figure 6). The African populations were removed from this analysis because in the previous studies they were distributed very far from other populations due to having large heterozygosity values and large effective population size. The plot showed a good fit between the observed relationship and that predicted by the model. Among 29 populations Turkey was below the line among the similar ones it was the most deviant from the expectation. This has a meaning of having low heterozygosity and perhaps low

effective population size and/or admixture which was surprising for the Anatolian Turkish population.

3.2.3. Neighbour – Joining Tree Construction

To observe the relative position of Turkey on the network of genetic relationships formed by other 34 countries a neighbour-joining tree was constructed (Figure 7). In order to root the tree an ancestral population was added to the analysis with insertion frequencies of zero. In this tree various grouping can be observed such as Africans and non-Africans ; Sahul populations (Australia, Papua New Guinea Coastal and Papua New Guinea Highland). Each population or group was connected to the tree by a branch. Branch lengths are proportional to the genetic distances (differences).

As expected, Turkish population was found in a group composed of European (French, French Acadian, Bretons, Greek Cypriot and Turkish Cypriot) and UAE populations. This group was denoted as group A in the figure. Group A exhibits smallest genetic differences between the group members compared to those of the other groups.

The numbers at the branching nodes denoted the bootstrap numbers. These numbers can be taken as the percent confidence levels for the groupings (Li, 1997). Number were generally low on the tree but the group A which holds Turkey had the lowest values on the internal branch nodes once more indicating the presence of nonsignificant genetic differences between the group members.

Table 5. The genetic distance matrix and average heterozygosities of the populations with respect to the alu insertions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.Alaska Natives	0.34													
2.Ausrtalia	0.134	0.28												
3.Bretons	0.040	0.080	0.34											
4.China	0.017	0.239	0.145	0.29										
5.Europ.-Amer.	0.066	0.062	0.000	0.193	0.31									
6.Filipino	0.002	0.234	0.100	0.009	0.139	0.28								
7.French	0.052	0.081	0.000	0.170	0.000	0.113	0.29							
8.French-Acadian	0.064	0.071	0.000	0.184	0.000	0.136	0.000	0.29						
9.Greek-Cypriot	0.054	0.119	0.000	0.171	0.001	0.111	0.000	0.000	0.29					
10.Greenland Natives	0.000	0.136	0.036	0.024	0.061	0.002	0.045	0.059	0.047	0.35				
11.India-Christian	0.012	0.095	0.025	0.046	0.049	0.057	0.049	0.041	0.047	0.016	0.43			
12.India-Hindu	0.000	0.133	0.016	0.038	0.042	0.023	0.029	0.032	0.024	0.000	0.000	0.35		
13.India-Muslim	0.026	0.071	0.000	0.116	0.000	0.085	0.000	0.000	0.000	0.024	0.005	0.000	0.35	
14. Java	0.042	0.205	0.178	0.004	0.223	0.045	0.206	0.210	0.219	0.054	0.056	0.065	0.141	0.25
15.Kung	0.187	0.169	0.086	0.360	0.074	0.257	0.085	0.100	0.087	0.168	0.175	0.181	0.106	0.451
16.Malaysian	0.009	0.195	0.094	0.001	0.137	0.019	0.119	0.122	0.116	0.016	0.011	0.009	0.066	0.011
17.Mayan	0.005	0.180	0.113	0.003	0.147	0.002	0.127	0.148	0.138	0.008	0.051	0.036	0.094	0.018
18.Moluccan	0.011	0.179	0.090	0.006	0.129	0.027	0.115	0.114	0.113	0.019	0.008	0.008	0.061	0.011
19.Mvskoke	0.000	0.085	0.046	0.033	0.063	0.022	0.053	0.064	0.068	0.000	0.022	0.013	0.032	0.043
20.Nguni	0.134	0.123	0.049	0.251	0.052	0.218	0.069	0.061	0.063	0.127	0.056	0.101	0.048	0.297
21.Nigerian	0.205	0.189	0.063	0.352	0.069	0.315	0.087	0.064	0.064	0.199	0.085	0.127	0.061	0.411
22.Pakistan	0.053	0.123	0.005	0.138	0.020	0.116	0.020	0.010	0.009	0.053	0.005	0.011	0.000	0.169
23.PNG-Coastal	0.050	0.089	0.035	0.098	0.053	0.115	0.056	0.039	0.057	0.057	0.000	0.016	0.021	0.093
24.PNG-Highland	0.083	0.055	0.041	0.152	0.048	0.169	0.058	0.036	0.067	0.091	0.015	0.046	0.020	0.133
25.Pushtoon	0.023	0.070	0.000	0.111	0.000	0.079	0.000	0.000	0.001	0.021	0.007	0.005	0.000	0.138
26.Pygmy-CAR	0.149	0.251	0.053	0.292	0.064	0.206	0.065	0.070	0.041	0.133	0.111	0.108	0.064	0.398
27.Pygmy-Zaire	0.093	0.144	0.042	0.216	0.044	0.140	0.048	0.065	0.046	0.079	0.091	0.094	0.054	0.294
28.Sotho	0.096	0.121	0.029	0.206	0.035	0.164	0.045	0.044	0.039	0.089	0.044	0.071	0.030	0.259
29.Swiss	0.071	0.122	0.000	0.200	0.001	0.135	0.000	0.000	0.000	0.064	0.057	0.036	0.000	0.250
30.Taiwan	0.022	0.295	0.148	0.011	0.196	0.000	0.166	0.196	0.161	0.021	0.082	0.053	0.131	0.053
31.Tamil	0.000	0.086	0.042	0.023	0.065	0.029	0.059	0.061	0.068	0.001	0.000	0.002	0.023	0.029
32.Tenggarras	0.009	0.105	0.033	0.042	0.057	0.047	0.050	0.046	0.052	0.014	0.000	0.000	0.012	0.049
33.Turkish-Cypriot	0.043	0.159	0.002	0.148	0.014	0.085	0.003	0.010	0.000	0.036	0.051	0.017	0.001	0.206
34.UAE	0.051	0.150	0.001	0.163	0.010	0.097	0.001	0.008	0.000	0.043	0.054	0.023	0.001	0.222
35.Turkish	**0.087**	**0.149**	**0.007**	**0.217**	**0.012**	**0.151**	**0.007**	**0.005**	**0.000**	**0.079**	**0.071**	**0.043**	**0.006**	**0.268**

Table 5. continued

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
15.Kung	0.36													
16.Malaysian	0.322	0.32												
17.Mayan	0.272	0.618	0.31											
18.Moluccan	0.328	0.000	0.022	0.31										
19.Mvskoke	0.185	0.028	0.008	0.027	0.34									
20.Nguni	0.036	0.188	0.221	0.188	0.144	0.46								
21.Nigerian	0.092	0.242	0.347	0.238	0.238	0.000	0.38							
22.Pakistan	0.132	0.074	0.135	0.070	0.074	0.038	0.023	0.37						
23.PNG-Coastal	0.230	0.043	0.107	0.034	0.056	0.085	0.085	0.010	0.35					
24.PNG-Highland	0.229	0.086	0.149	0.073	0.075	0.091	0.091	0.026	0.000	0.32				
25.Pushtoon	0.092	0.068	0.085	0.064	0.028	0.044	0.069	0.002	0.022	0.032	0.37			
26.Pygmy-CAR	0.023	0.229	0.257	0.236	0.188	0.013	0.004	0.049	0.152	0.180	0.061	0.38		
27.Pygmy-Zaire	0.000	0.192	0.154	0.200	0.100	0.010	0.073	0.075	0.152	0.169	0.040	0.000	0.44	
28.Sotho	0.026	0.155	0.174	0.157	0.108	0.000	0.008	0.028	0.078	0.090	0.025	0.002	0.000	0.45
29.Swiss	0.083	0.137	0.164	0.133	0.085	0.058	0.052	0.011	0.063	0.070	0.005	0.036	0.047	0.037
30.Taiwan	0.279	0.034	0.007	0.045	0.045	0.244	0.364	0.160	0.160	0.228	0.119	0.230	0.153	0.188
31.Tamil	0.199	0.007	0.014	0.005	0.000	0.112	0.182	0.047	0.022	0.043	0.022	0.173	0.108	0.088
32.Tenggarras	0.222	0.010	0.044	0.006	0.017	0.111	0.142	0.023	0.005	0.026	0.017	0.156	0.130	0.088
33.Turkish-Cypriot	0.100	0.101	0.120	0.101	0.066	0.081	0.083	0.015	0.069	0.090	0.007	0.040	0.049	0.050
34.UAE	0.086	0.114	0.132	0.113	0.071	0.071	0.074	0.015	0.071	0.089	0.007	0.033	0.042	0.043
35.Turkish	**0.116**	**0.147**	**0.187**	**0.142**	**0.105**	**0.084**	**0.061**	**0.015**	**0.069**	**0.077**	**0.018**	**0.051**	**0.079**	**0.061**

	29	30	31	32	33	34	35
29.Swiss	0.28						
30.Taiwan	0.189	0.29					
31.Tamil	0.084	0.051	0.38				
32.Tenggarras	0.064	0.080	0.000	0.35			
33.Turkish-Cypriot	0.000	0.128	0.068	0.052	0.28		
34.UAE	0.000	0.141	0.074	0.058	0.000	0.29	
35.Turkish	**0.000**	**0.212**	**0.102**	**0.073**	**0.002**	**0.001**	**0.22**

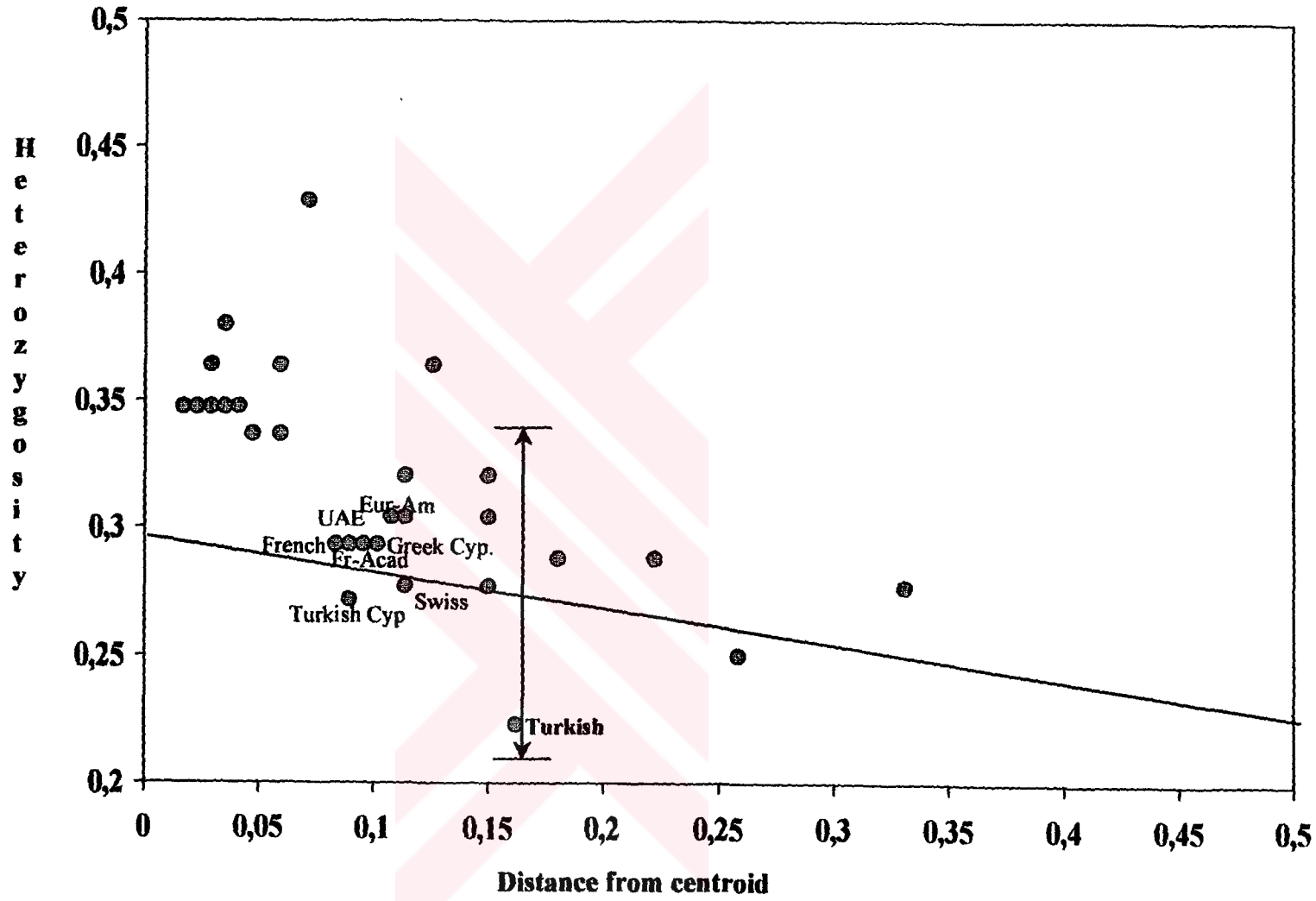

Figure 6 . The plot of heterozygosity vs. distance from centroid

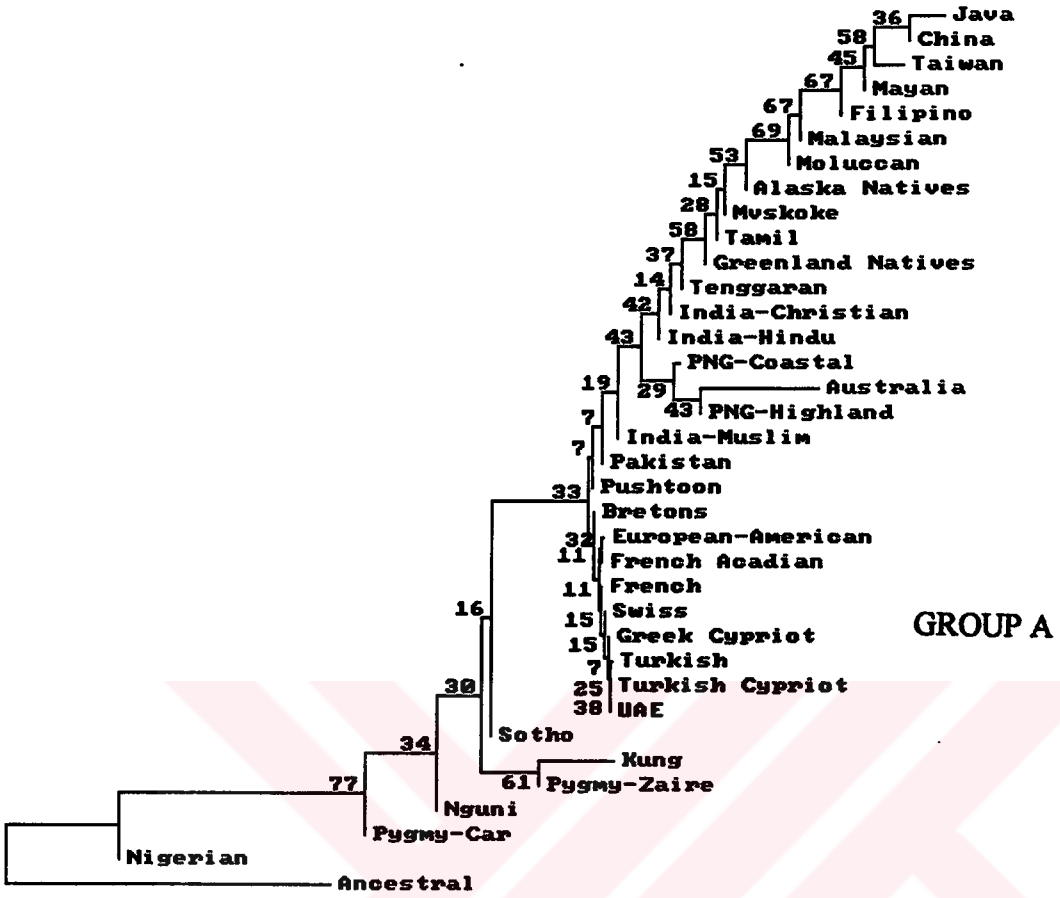

Figure 7. The Neighbour-Joining Tree

Numbers on the branch nodes indicate the bootstrap values.

3.2.4. Principal Component Analysis (PCA)

Relative positions of the populations based on their four *alu* insertion frequencies were observed in the space generated by the 3 principle component axes (Figure 8). The equations of these components were as follows:

PC1= 0.81957 ACE + 0.17292 APO + 0.78564 PV92 - 0.69045 A25

PC2= 0.22219 ACE - 0.97359 APO - 0.08536 PV92 - 0.07721 A25

PC3= 0.17101 ACE – 0.05620 APO + 0.45107 PV92 + 0.70217 A25

The PC1 covers 75.58 % of the overall variation while 18.45 and 5.96 % of the total variation were covered by PC2 and PC3, respectively. From the weights of the variables one can deduce that ACE, PV92 and A25 contributed almost equally for the differentiation of the populations on the first axis. On the first axis African populations having relatively large genetic differences among each other occupied one end of the axis. Java and China being the most distant pair to the African populations occupied the opposite end of the first PC axis. This pattern is quite parallel to the results of Neighbour – Joining Tree. After PCA, it was understood that this clustering is basically due to the differences of the frequencies of ACE, PV92, A25 in these populations. APO, on the other hand, seperated the populations on the second axes. The two populations occupying the most distant positions were French and Nigeria, their frequencies for APO were 0.99 and 0.50, respectively. Hence the order for the importance of the variables in discrimination of the populations was as fallows : ACE, PV92 and A25 were almost

equally important, APO was less important than the others. Third axis was weighted first by A25 and second by PV92. Turkey having low values for insertion on both of these loci occupied one end of this axis. Hence combination of values of these two loci differentiated Turkey from Swiss, European-American, Greek-Cypriot, Turkish-Cypriot, French, French-Acadian, Bretons and UAE.

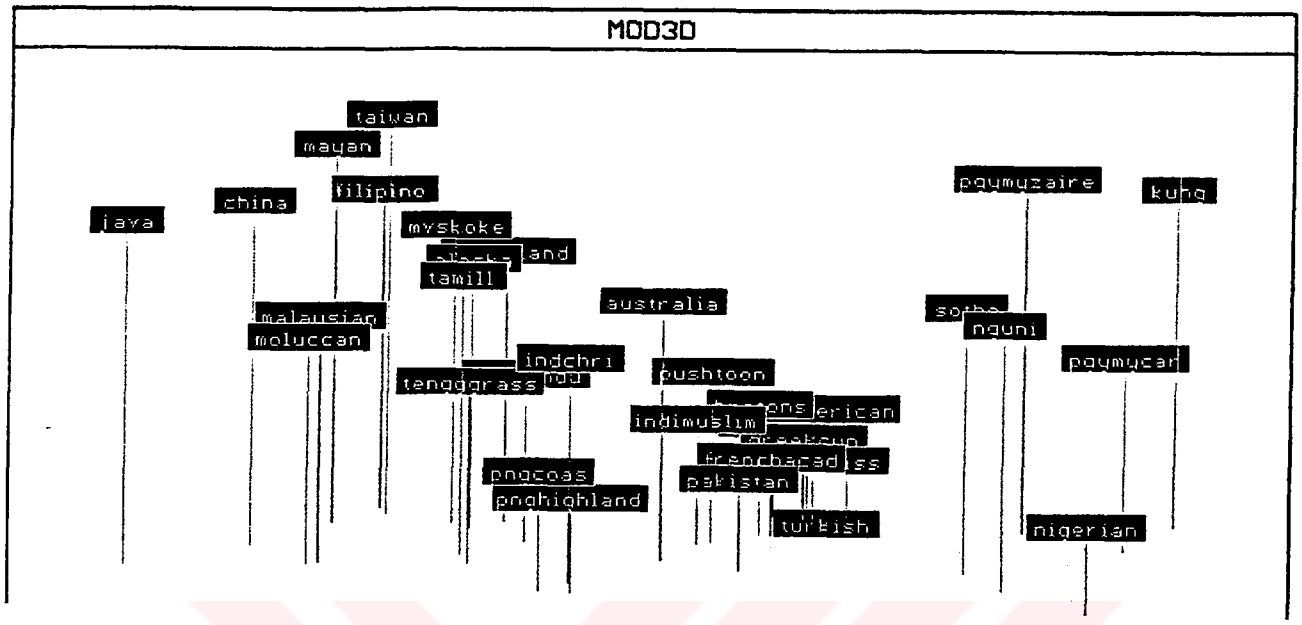

Figure 8 . Plot of the three principal coordinates for the allele frequencies of four polymorphic *alu* insertions.

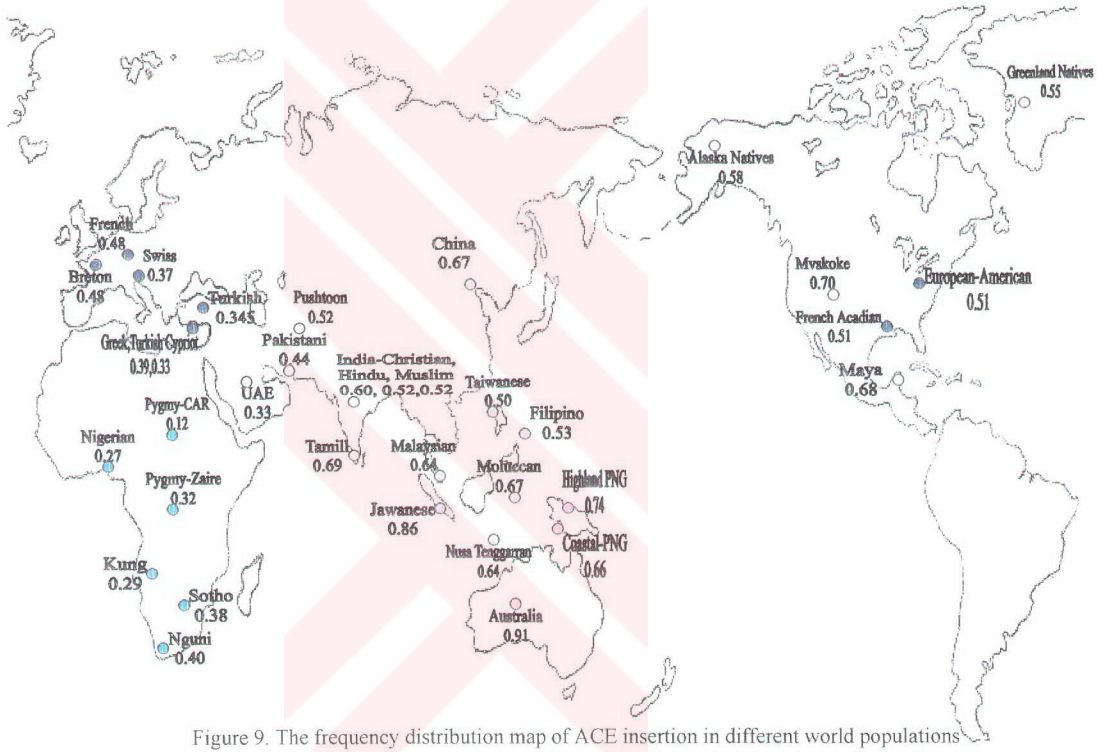

Figure 9. The frequency distribution map of ACE insertion in different world populations

The frequencies for other populations were taken from Stoneking et al. (1997)

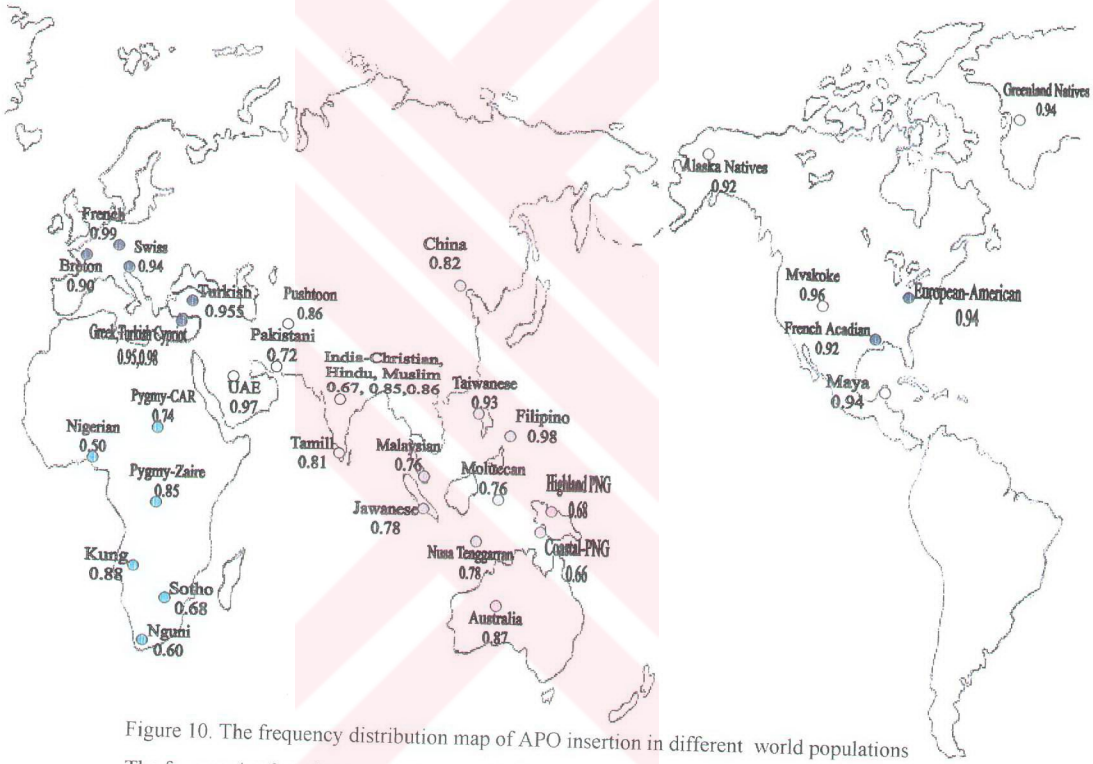

Figure 10. The frequency distribution map of APO insertion in different world populations

The frequencies for other populations were taken from Stoneking et al. (1997)

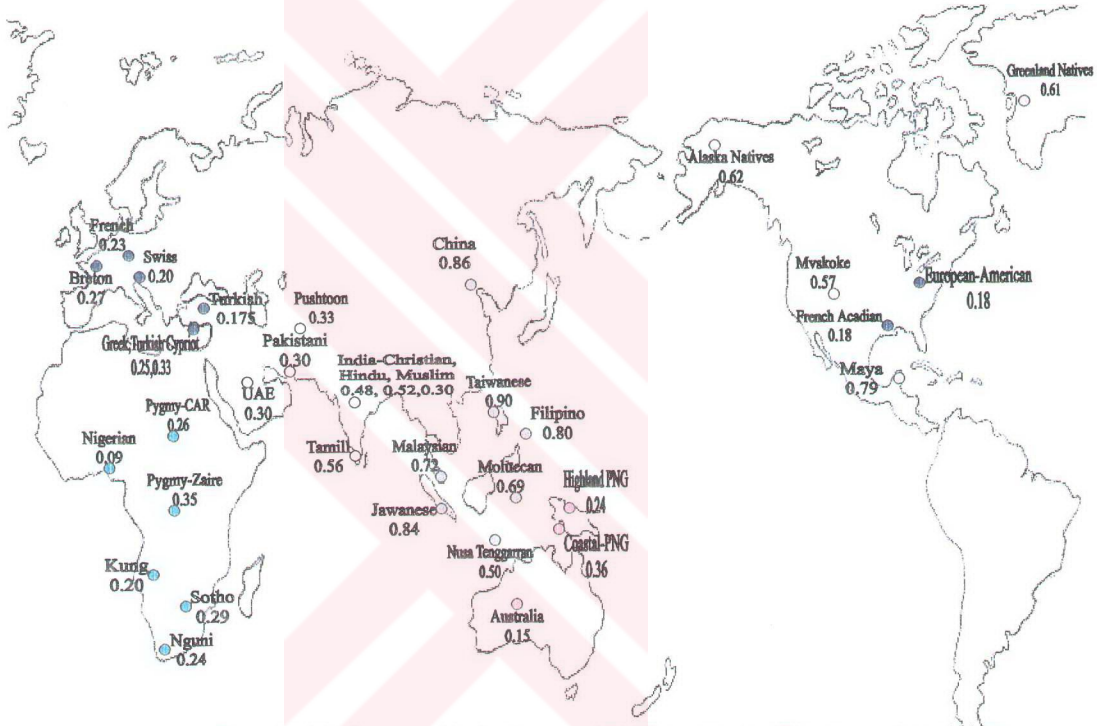

Figure 11. The frequency distribution map of PV92 insertion in different world populations

The frequencies for other populations were taken from Stoneking et al. (1997)

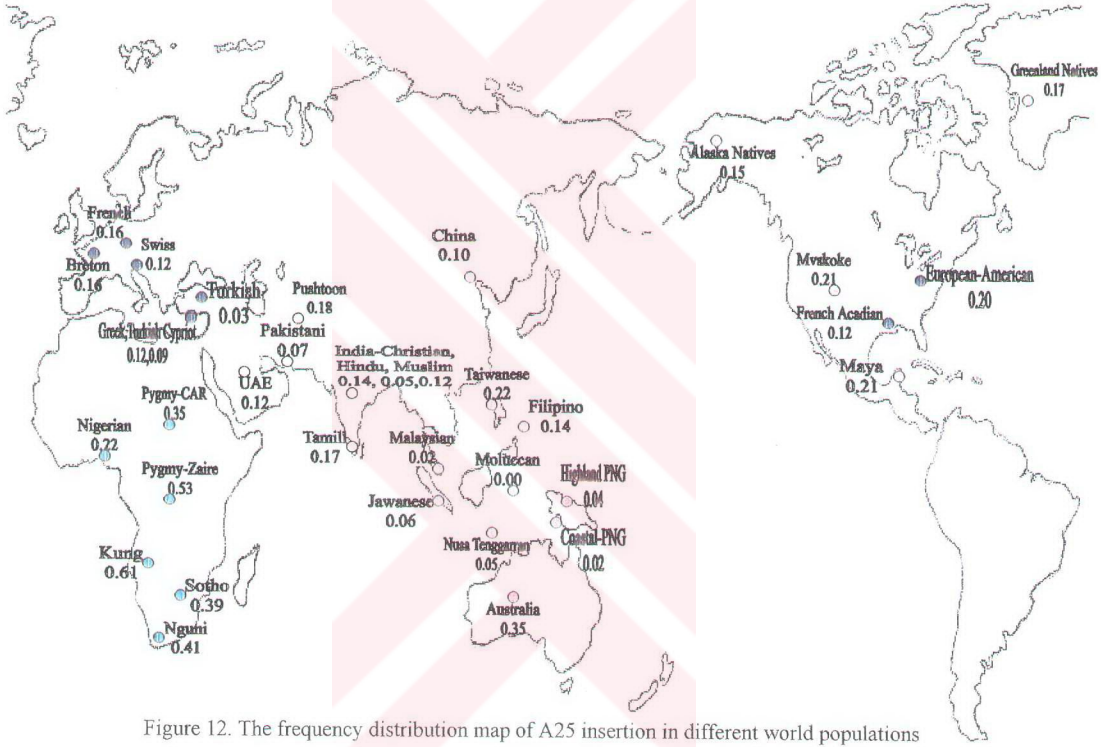

Figure 12. The frequency distribution map of A25 insertion in different world populations

The frequencies for other populations were taken from Stoneking et al. (1997)

CHAPTER 4

DISCUSSION

4.1. Distribution of the *Alu* Insertions

Polymorphic *alu* insertions in humans have been used in the studies of evolutionary history of human populations very recently (Batzer *et al.*, 1991, Batzer and Deininger 1991, Perna *et al.* 1992).

In the present study, four autosomal *alu* insertions; ACE, APO, PV92 and A25 and one Y chromosomal *alu* insertion ;YAP were examined in the Anatolian Turkish population and partially in two Sicillian populations; Sciacca and Troina. Among the studied *alu* insertions two of them suggested geographically limited distributions in Anatolia : A25 insertion was perhaps absent in the western Anatolia, YAP was present only in the Western and Central Anatolia. The other insertions were distributed almost equally in every studied region of Anatolia.

In order to see the distribution of frequencies of the insertions in the world, the data presented by Stoneking *et al.* (1997) was combined with the results of the present study and these distributions were given in figures 9-12.

When the frequency of ACE in Turkey was compared with those of the frequencies obtained from Turkish Cypriots, Greek Cypriots, United Arab Emirates, and Swiss (Stoneking et al., 1997) which were the geographically closest ones to Turkey among the countries having data for ACE frequencies, very high resemblences in their frequencies between all these countries were observed. For ACE, frequency from Anatolia was 0.345 the range for the others was 0.33-0.39. Whereas, the range from all over the world was 0.12-0.91 (Figure 9). The same pattern between the countries mentioned above was observed for APO freqeuncies (Figure 10). For PV92 and A25 Anatolian Turkish population frequencies were less than the minimum of the frequency range defined by the geographically close regions (Turkish and Greek Cypriots, UAE, and Swiss) (Figure 11 and 12). There have been no data from Caucasus , Northern Black Sea region, Balcans and South of Turkey. Perhaps there may be areas around the Turkey exhibiting low frequency values for these loci (PV92 and A25) and hence explain the low frequency values observed in Anatolia. It is also possible that when the sample sizes less than 50 are increased there will be smoother gradients of the freqeuncies covering Anatolia. For example, there seems to be a continiuum in the low frequency of A25 on the line of Jawanese (0.06), Malaysian (0.02), Hindu (0.05), Pakistani (0.07) and finally Anatolian Turks (0.03). The sample sizes along this path were; 32, 47, 28, 42, 100, respectively.

Again with a more extensive sampling a homogenous PV92 distribution for Europe starting from Anatolia including French Acadians and European Americans may come up.

The frequency of the presence of the YAP element was found to be 6.9 % in Anatolian Turkish population. It was present much higher frequencies in Sciacca (west of Sicily) ; 23 % and in Troina (east of Sicily) populations; 16 %. On the average, the YAP frequency for Europe was 7 % (Hammer, 1994). Both of the Sicillian populations exhibited higher frequency than Europe average possibly because of the geographical position being closer to Africa. Hammer et al. (1994) have studied 340 individuals from 14 different populations and revealed that there exists significant heterogeneity among populations and a clear pattern in the frequency such that ; sub-Saharan Africans > northern Africans > Europeans > Oceanians > Asians.

In one of the studies, Spurdle *et al.* (1994) have examined 889 individuals from 23 different African population groups and observed a similar trend in frequency of the insertion, with the insert largely absent in Caucasoid populations, at intermediate frequency in the Khoisan and at high frequency in Negroids. Combining the other studies, the maximum frequency for the YAP element was observed in Wolof population by 100 % (Passarino *et al.*, 1998) and the minimum was 0 % for Taiwan and Korean (Hammer and Horai, 1995). However, since the sample number was very low for these two populations this result may be due to the sampling error.

Table 6 . Distribution of the YAP element in different populations

Population	(N)	YAP^+ (%)	Reference (s)
Ethiopian	64	50	Passarino et al., 1998
Senegalase			
Mandenka	56	98.2	Passarino et al.,1998
Wolof	31	100	Passarino et al.,1998
Mixed African	44	59.1	Seielstad et al.,1994
Bantu	442	78	Hammer, 1994
			Spurdle et al.,1994
Khoisan			
Nama+Sekele San	68	46	Spurdle et al.,1994
Tsumkave San	38	11	Spurdle et al.,1994
Egyptian	64	53	Hammer,1994
Suudi Arabian	21	10	Hammer,1994
European	192	7	Hammer,1994
Japanese	132	42	Hammer and Horai,1995
Taiwan	21	0	Hammer and Horai,1995
Korean	13	0	Hammer and Horai,1995
Amerindians	NA	4	Bianchi et al., 1997
Caucasians	NA	12	Bianchi et al., 1997

NA: not available

The frequencies of Y *alu* element on different world populations were given in figure 13. YAP frequencies were very high (>98 %) in some African countries and absent in some Eastern Asian countries. These observations were interpreted as this insertion is relatively recent, , it occured in between successive migrations out of Africa (Batzer et al., 1994). It is possible that among the first invaders of Europe this insertion was absent , it was brougth to the continent by more recent successive migrations from Africa through southern European countries such as Sicily. This insertion perhaps also entered to Europe by the diffusion from Africa through Middle East and Anatolia. However, since the YAP(+) samples were mostly located on the western and Central

Anatolia and not on the south East of Anatolia, this diffusion may be basically before the arrival of Turks to Anatolia and it pushed already existing population in the Anatolia towards the western part of Anatolia when arrived to Middle East and Anatolia from eastern part of Caspian sea (≈1200 years ago). Hammer et al. (1997) have stated that African populations have greater haplotypic diversity than others and only subsets of the YAP haplotypes were observed in outside of Africa which is competable with the multiple human migrations and range expansions hypotheses.

In the present study YAP haplotypes of the YAP(+) individuals could not be studied. Further studies on the YAP (+) haplotypes could allow to locate the geographic origins of the individuals possesing this marker.

All the scenarios need to be tested with better sampling from Anatolia and in accordance with the history of Anatolia. It is worth noting that comparisons of the Y chromosome data with mitochondrial DNA and beta-globin data revealed different patterns of inferences for males and females concerning the relative roles of population history and population structure (recurrent gene flow) (Hammer *et al.*, 1998). Hence, by studying Y chromosome and mtDNA markers it is possible to examine the evolutionary history of maternal and paternal lines seperately in Anatolia.

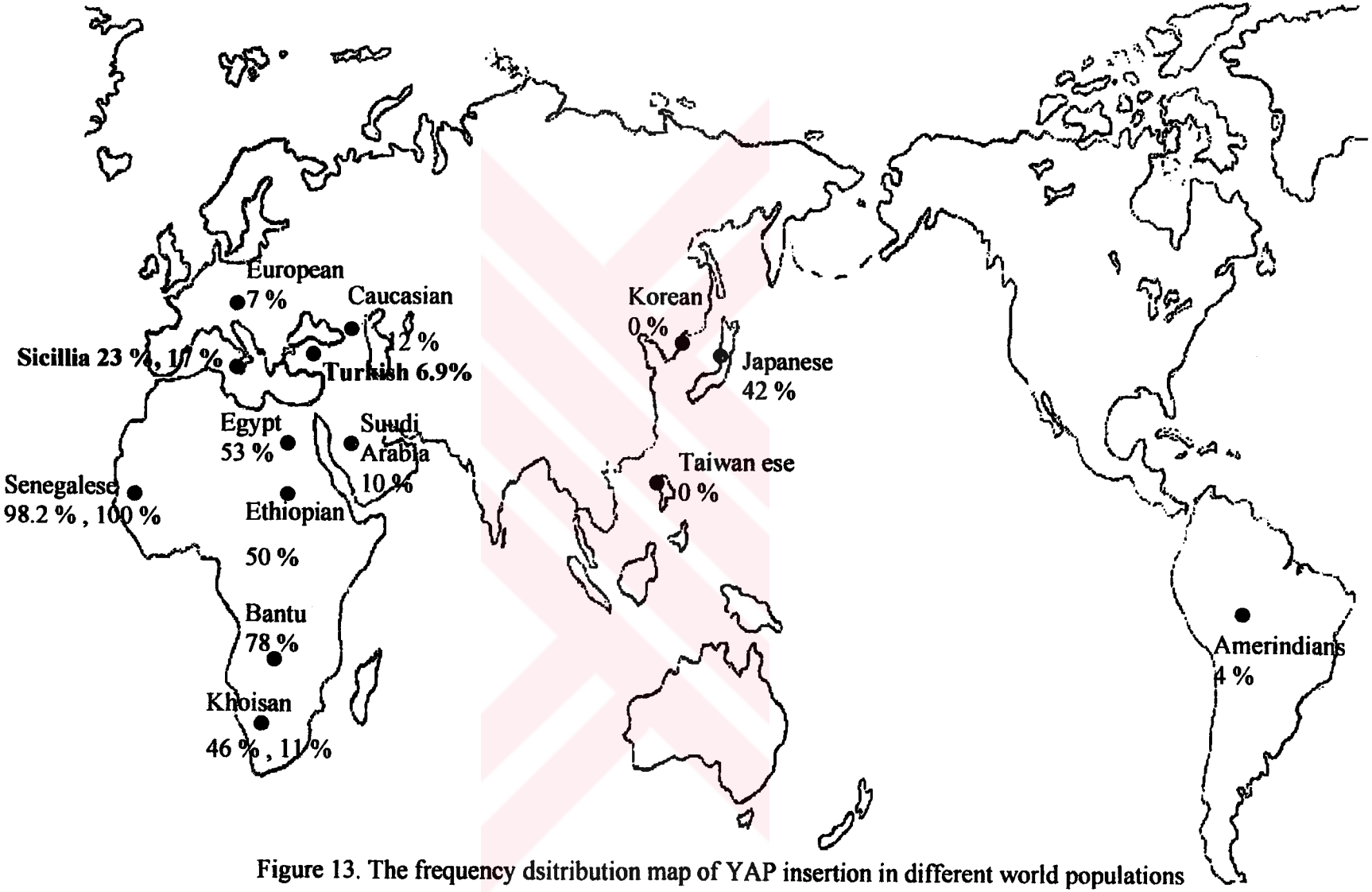

Figure 13. The frequency dsitribution map of YAP insertion in different world populations

The references for the frequencies were given in table 6

The frequency of YAP element was found to be higher in both Sicillian populations than Anatolian Turkish population and also higher than the average value for Europe.The observation of higher YAP (+) frequencies in Sicily may suggest higher admixture of this population with the Africans due to the geographical position (very close to Africa) and the difference between east and west of Sicily can be explained by the regional differences in this admixture in Sicily.

When Hardy-Weinberg equilibrium frequencies were calculated for ACE, APO, PV92 and A25, significant deviations ($p<0.05$– $p<0.01$) were observed. However, these deviations were due to the deficiency in heterozygotes. In a previous study for 34 populations and eight *alu* insertion markers only 10 of them were deviant from the expectations (Stoneking et al.,1997). To explain the observed deviations in Anatolian population presence of inbreeding was invoked. In Anatolia, average degree of inbreeding was known as 0.01 (Tuncbil0*ek and Ulusoy, 1989). When expected heterozygosity deficiencies due to inbreeding were calculated it was observed that frequencies of hetrozygotes in inbred Anatolian population were as fallows: 0.351, 0.05, 0.22 and 0.039 for the markers ACE, APO, PV92 and A25, respectively. When these numbers were compared with the observed ones (Table 3) it could be seen that inbreeding effect was far from explaining the deficiencies in heterozygotes. Therefore, presence of substructuring in Anatolian population and hence the Wahlung effect was accepted as the reason of this deviation. In other populations perhaps, individuals were the representatives of nonstructered populations or in some regions there are no substructuring.

Frequencies of A25 (0.03) and APO (0.955) was very close to 0 and 1 , respectively in Anatolia and based on these frequencies which are within the limits of the ranges, Anatolia was expected to posses a relatively lower value in expected heterozygosity compared to those of the other countries. When the expected heterozygosities of the populations (the diagonal elements of the distance matrix in table 5) were examined the minimum value (0.22) belongs to Anatolian Turks. Since observed heterozygosity is even lower than the expected heterozygosity based on these markers Anatolians seems to be the least heterozygote among the studied populations.

This was visualized once more on the graph of heterozygosity vs. distance from centroid. This graph which was drawn in accordance with the Harpending and Ward 's (1982) method was expected to differentiate those populations having high effective population sizes and/or those populations receiving high amount of gene flow from those populations having low effective population sizes and/or recieving low amount of gene flow. In Stoneking et al. (1997), the study was based on eight *alu* insertion frequencies and it was observed that African populations were all above the expected line and this was interpreted as the high effective population sizes possesed by these populations. Similarly, Batzer et al. (1996) have used the same plot and observed that Hispanic, African-American and British Afro-Caribbean groups were above the expected line. This observation was explained by the consequence of relatively high admixture present in these groups.

One of the most widely accepted scenario for the evolutionary history of modern humans is the origination in Africa ≈ 200,000 years ago, movement out of Africa ≈ 100,000 years ago, migration towards Asia 50-70,000 years ago (Nei and Roychoudhury, 1993). Modern humans are known to move to Europe through Anatolia ≈ 40,000 years ago in the upper paleolithic ages (Nitecki and Nitecki, 1994). After the settlement of hunter gatherers in Middle East and Anatolia in Neolithic ≈ 10,000-5,000 years ago population sizes expanded and farming spread towards Europe. Analysis of the genetic data suggested that migrations towards Europe from (Renfrew, 1991) or through Anatolia spread the farming in Europe (Sokal *et al.*, 1989).

Throughout the ancient times, Anatolia has been the place of various civilizations established by peoples of various origins (Akurgal, E., 1998) and different migration waves have crossed the Bosphorus in both directions. Because of the above mentioned facts or highly probable scenarios, Anatolian population was expected to be more heterozygous compared to European populations. If not more population sizes as European populations, with respect to gene flow should have higher influx then those of the other European populations.

The lowest heterozygosity value observed in Anatolia point on the heterozygosity vs. distance from centroid plot can only be explained by employement of few markers and choice of A25 and PV92 by chance. To drive at a conclusion on admixture level of the populations and/or relative effective population sizes of the populations high number of markers and better sampling must be utilized.

4.2. Genetic Relatedness of the Populations and the Markers to Resolve Them

When these four markers were used to construct neighbour joining tree for the 34 previously studied populations and the Anatolian Turks, pattern of genetic relatedness , trees built by eight *alu* insertions (Stoneking *et al.*, 1997) and four *alu* insertions (present study) were basically the same.

Geographic clusters of African, European, and Sahulian populations distinct in both of the trees. Southeast Asian and American populations were intermingled in one cluster again in both of the trees. As it was in the tree of eight markers (Stoneking *et al.*, 1997) ancestral population was closest to the African populations in the tree built based on four markers (present study). Within the above mentioned clusters minor differences in the pairwise groupings were observed but they were between very close groups and do not effect the major groups. Bootstrap numbers were equally low in both. When these two trees were compared to the one given in Nei and Roychoudhury 's (1993) study almost the same major clusters (4 out of 5) were observed. In the latter study 29 polymorphic loci (blood groups, protein, HLA-A, HLA-D and PTC loci) were used , North Amerindian, South Amerindian and Eskimos formed a distinct cluster and the bootstrap values were considerably higher than those of the trees constructed by the *alu* insertion markers.

In the tree obtained by the present study, Anatolian Turkish population was very close genetically to Swiss, Greek and Turkish Cypriots, French, French Acadians, Europaen Americans and UAE populations perhaps slightly closer to the former populations. The tree constructed by using protein loci and blood group loci revealed the same relationship (Triantophyllidis *et al.*, 1986). As in the study of Comas *et al.* (1996) this study denoted that Turkey has a stepping stone position between the Middle East and Europe.

The multilocus Gst value for four alu loci was calculated as 0.15 which implies that 15 % of the total variance is between populations and the rest 85% was within the populations. The Gst value for eight alu loci was determined in one of the previous study (Stoneking *et al.*, 1997) as 0.128 which was comperable to the nuclear data obtained by RFLP (Gst =0.107) and STR (Gst = 0.034) analysis (Jorde *et al.*, 1995). This indicated that the markers that were selected for this study have comperative power of differentiation of the populations as the ones used by Stoneking *et al.*(1997)..

After these comparisons, it can be said that *alu* insertions, even with a few number of markers, can be useful to visualize the genetic relatedness of the populations in the crude level.

On the Principal Component axes relatedness of the populations can be visualized in three dimensional space. One can assume that PC analysis yields a more detailed visualization of the genetic relatedness of the populations. However, if the

number of the variables is n, then the number of the principal components is n-1. If first tree components do not account high proportion of the total genetic variation present between the populations then positions of the populations on the first three components may not give sufficient information about the genetic relatedness of the populations. In the present study, four insertions were used hence first three components were accounting all the genetic variability. The groupings in these axis were very similar to the one shown by neighbour joining tree (Figure 7).

PC Analysis has another very important property ; it manifests the most effective variables in differentiating the populations. Thereby, some variables can be suggested for future studies. Variables with high Fst (Gst of a single variable) values are also candidates for the differentiation of the populations. However, they may have redundant information. Principle Component Analysis forming independent principle components reveal independent non redundant composed variables in the form that one can choose the best variables to differentiate the populations. The present study suggested that the order for the importance of the variables (ACE, APO, PV92 and A25) in differentiation of the populations is as fallows : ACE, PV92, A25 and APO. When the same analysis was carried on the Stoneking et al. 's data : First component exhibited 40 % of the variability; FXIIIB had highest discriminatory power; ACE, PV92, A25 followed the FXIIIB; APO had the lowest discriminatory power. Therefore, if four *alu* insertions are going to be employed on these populations perhaps instead of APO, FXIIIB should be used.

4.3. Conclusion

In the present study, the frequency and distribution of APO, PV92 and A25 were examined in Anatolian Turkish population. Distribution of YAP (+) seemed to be confined to Western and Central Anatolia and the frequency was lower than both the frequencies in west of Sicily and in east of the Sicily.

Comperative studies revealed that frequency of ACE and APO were within the range of values of the populations which are geographically close. However, A25 and PV92 were unexpectedly low in the region. New data from the neighbouing countries is needed to confirm and explain these frequencies. Similarly, origin of YAP (+) in Anatolia may shed light on as the part of evolutionary history of Anatolian population.

On the contrary of expectation based on the knowledge of peopling and history of Anatolia heterozygosity level of Anatolian Turkish population was the lowest among the studied populations in terms of the four *alu* insertions. This suggests that few randomly chosen *alu* insertions may not reflect heterozygosity correctly.

Anatolian Turkish population clustered tightly with the European populations on the neighbour joining tree. The general pattern of this tree was the same as the one constructed by eight *alu* insertions. These four *alu* markers seem to give reliable information on the general pattern of genetic relatedness of the populations. Among the employed markers ACE, PV92 and A25 were seemed to be equally important to

differentiate the populations. Among the known ones however, FXIIIB seems to be more powerful than any other known marker. This information must be taken into account in the future studies.

REFERENCES

Alper B., Meyer E., Schurenkamp M.., Brinkmann B., 1995. " HumFES/FPS and HumF13B: Turkish and German population data''. Int J Legal Med, 108 (2), pp.93-95.

Akurgal, E., 1998. " Anadolu Kültür Tarihi (in Turkish)". TUBITAK Popüler Bilim Yayınları, Mart.

Altay C., Yetgin S., Ozsoylu S., Kutsal A., 1978. " Hemoglobin S and some other hemoglobinopathies in Eti-Turks''. Hum Hered, 28 (1), pp.56-61.

Atasoy S., Abaci E., Turkoglu M., Cenani A., Ozturk M., Kiymetli U., 1990. "Erythrocyte glyoxalase-I polymorphism in Turkey''. Adli Tıp Dergisi, 6, pp.65-68.

Avise JC., 1994. "Molecular Markers, Natural History and Evolution". Chapman & Hall, Inc.

Batzer MA., Deininger PL., 1991. " A human specific subfamily of Alu sequences". Genomics, 9, pp 481-487.

Batzer MA., Gudi VA., Mena JC., Foltz DW., Herrera RJ., Deininger PL., 1991." Amplification dynamics of human-specific (HS) Alu family members". Nucleic Acid Res. 19, pp 3619-3623.

Batzer MA., Arcot SS., Phinney JW., Alegria-Hartman M., Kass DH., Milligan SM., Kimpton C., Gill P., Hochmeister M., Ioannou PA., Herrera RJ., Boudreau DA., Scheer WD., Keats BJB., Deininger PL., Stoneking M, 1996. " Genetic variation of recent alu insertions in human populations''. J Mol Evol, 42, pp. 22-29.

Batzer MA., Rubin CM., Hellmann-Blumberg U., Alegria-Hartman M., Leeflang EP., Stern JD., Bazan HA., Shaikh TH., Deininger PL., Schmid CW., 1995. "Dispersion and insertion polymorphism in two small subfamilies of recently amlified human alu repeats''. J Mol Biol , 247 (3), pp. 418-427.

Batzer MA., Stoneking M., Alegria-Hartman M., Bazan H., Kass DA., Shaikh TH., Novick GE., Ioannou PA., Scheer WD., Herrera RJ., Deininger PL., 1994. "African origin of human-specific polymorphic Alu insertions''. Proc Natl Acad Sci USA, 91, pp. 12288-12292.

Bianchi NO., Bailliet G., Bravi CM., Carnese RF., Rothhammer F., Martinez-Marignac VL., Pena SD., 1997. " Origin of Amerindian Y-chromosomes as inferred by the analysis of six polymorphic markers''. Am J Phys Anthropol, 102 (1), pp. 79-89.

Bowcock AM., Kidd JR., Mountain JL., Hebert JM., Carotenuto L., Kidd KK., Cavalli-Sforza LL., 1991. " Drift, admixture, and selection in human evolution: a study with DNA polymorphisms''. Proc Natl Acad Sci USA ,88, pp. 839-843.

Bowcock AM., Ruiz-Linares A., Tomfohrde J., Minch E., Kidd J.R.,Cavalli-Sforza LL., 1994. "High resolution of human evolutionary trees with polymorphic microsatellites''. Nature, 368 (6470), pp.455-457.

Budowle B., Monson KL., 1994. " Greater differences in forensic DNA profile frequencies estimated from racial groups than from ethnic subgroups.''. Clin Chim Acta, 228 (1), pp. 3-18.

Calafell F., Underhill P., Tolun A., Angelicheva D., Klaydjieva L., 1996. " From Asia to Europe: mitochondrial DNA sequence variability in Bulgarians and Turks''. Ann Hum Genet, 60, pp. 35-49.

Cavalli-Sforza LL., Piazza A., Menozzi P., Mountain J., 1988. "Reconstruction of human evolution: bringing together genetic, archaeological, and linguistic data''. Proc Natl Acad Sci USA, 85 (16), pp. 6002-6006.

Comas D., Calafell F., Mateu E., Perez-Lezaun A., Bertranpetit J., 1996. "Geographic variation in human mitochondrial DNA control region sequence: The population history of Turkey and its relationship to the European populations''. Mol Biol Evol, 13(8), pp.1067-1077.

Deininger PL., Batzer MA., 1995. " SINE master genes and population biology. In Maraia RJ (ed) The impact of short interspersed elements (SINEs) on the host genome''. RG Landes,Georgetown,TX, pp.43-60.

Ferguson A., Taggart JB, Prodöhl PA., Mcmeel O., Thompson C., Stone C., Mcginnity P., Hynes RA., 1995. " Population and Conservation". Journal of Fish Biology, 47, pp 103-126.

Gusmao L., Prata MJ., Amorim A., 1995. ''The STR system hTPO: population and segregation data''. Int J Legal Med ,108, pp.167-169.

Haldane, JBS., 1940. " The blood group frequencies of European peoples of racial origins". Human Biology, 12, pp. 457-480.

Hammer MF., 1994. "A recent insertion of an alu element on the Y chromosome is a useful marker for human population stuidies''. Mol Biol Evol, 11 (5), pp. 749-761.

Hammer MF., 1995. " A recent common ancestry for human Y chromosomes''. Nature, 378 (6555) , pp.376-378.

Hammer MF., Horai S., 1995. "Y chromosomal DNA variation and the peopling of Japan''. Am J Hum Genet , 56, pp. 951-962.

Hammer MF., Spurdle AB., Karafet T., Bonner MR., Wood ET., Novelletto A., Mlaspina P., Mitchell RJ., Horai S., Jenkins T., Zegura SL., 1997. "The geographic distribution of human Y chromosome variation''. Genetics, 145 (3), pp. 787-805.

Hammer MF., Karafet T., Rasanayagam A., Wood ET., Altheide TK., Jenkins T., Griffiths RC., Templeton AR., Zegura SL., 1998. "Out of Africa and back again: nested cladistic analysis of human Y choromosome variation''. Mol Biol Evol, 15 (4), pp. 427-441.

Hammond HA., Jin L., Zhong Y., Caskey CT., Chakraborty R., 1994. "Evaluation of 13 short tandem repeat loci for use in personal identification applications''. Am J Hum Genet, 55, pp.175-189.

Harpending HC., Ward RH., 1982. "Chemical systematics and human populations. In Biochemical aspects of evolutionary biology, pp 213-256. University of Chicago Press, Chicago, IL.

Huang NE., Schumm J., Budowle B., 1995. " Chinese population data on three tetrameric short tandem repeat loci – HUMTHO1, TPOX, and CSF1PO –derived using multiplex PCR and manual typing''. Forensic Science International, 71, pp.131-136.

Iwasa M., Wiegand P., Rand S., Schurenkamp M., Atasoy S., Brinkmann B., 1997. " Genetic variation at five STR loci in subpopulations living in Turkey''. Int J Legal Med, 110 (3), pp. 170-172.

Jarjanazi H., Timur AA., El-Maarri O., Caglayan SH., 1998. "Analysis of the two microsatellite repeat polymorphisms of the factor VIII gene in the Turkish population''. Br J Haemotol,100 (3), pp. 589-593.

Jorde LB., Bamshad MJ., Watkins WS., Zenger R., Fraley AE., Krakowiak PA., Carpenter KD., Soodyall H., Jenkins T., Rogers AR., 1995. "Origins and affinities of modern humans : a comparison of mitochondrial and nuclear genetic data''. Am J Hum Genet, 57, pp. 523-538.

Knight A., Batzer MA., Stoneking M., Tiwari HK., Scheer WD., Herrera RJ., Deininger PL., 1996. "DNA sequences of Alu elements indicate a recent replacement of the human autosomal genetic complement''. Proc Natl Acad Sci USA, 93, pp.4360-4364.

Li, WH., 1997. " Molecular evolution". Sinauer Associates, Inc. Publishers Sunderland Massuchusetts.

Martin P., Alonso A., Budowle B., Albarran C., Garcia O., Sancho M., 1995. "Spanish population data on 7 tetrameric short tandem repeat loci''. Int J Legal Med, 108, pp.145-149.

Merriwether DA., Clark AG., Ballinger SW., Schurr TG., Soodyall H., Jenkins T., Sherry ST., Wallace DC., 1991. "The structure of human mitochondrial DNA variation''. J Mol Evol , 33 (6), pp. 543-555.

Meyer E., Wiegand P., Brinkmann B., 1995. "Phenotype differences of STRs in 7human populations''. Int J Legal Med ,107 (6), pp.314-322.

Nei M., 1972. "Genetic distance between populations". Am. Nat., 106, pp 283-292.

Nei M., Roychoudhury AK., 1993. "Evolutionary relationships of human populations on a global scale''. Mol Biol Evol ,10 (5), pp.927-943.

Nitecki MH., Nitecki DV., 1994. "Origins of Anatomically modern humans". New York and London : Plenum Press.

Novick GE., Novick CC., Yunis E., Antunez de Mayolo P., Scheer WD., Deininger PL., Stoneking M., York D.S., Batzer MA., Herrera RJ., 1998. Polymorphic Alu insertions and the Asian origin of Native American populations''. Hum Biol ,70 (1), pp. 23-39.

Ota T., 1993. DISPAN: Genetic distance and phylogenetic analysis. Pennsylvania State University.

Onde S. , Kence A., 1994. " Distribution of ABO and Rh gene frequencies over 67 provinces of Turkey''. Tr J of Biology, 18, pp.133-139.

Onde S., Kence A., 1995. " Geographic variation analysis of the ABO and RH systems in Turkey''. Gene Geogr , 9 (3), pp.211-220.

Passarino G., Semino O., Quintana-Murci L., Excoffier L., Hammer M., Santachiara-Benerecetti S., 1998. " Different genetic components in the Ethiopian population, identified by mtDNA and Y-chromosome polymorphisms''. Am J Hum Genet ,62, pp.420-434.

Perna NT., Batzer MA., Deininger PL., Stoneking M., 1992. " Alu insertion polymorphism : a new type of marker for human population studies''. Hum Biol, 64 (5), pp. 641-648.

Renfrew C., 1991. " Before Babel: Speculations on the Origins of Linguistic Diversity". Cambridge Archaelogical Journal, 1 (1), pp 3-23.

Rohlf, FJ., 1993. "NTSYS-pc :Numerical Taxonomy and Multivariate Analysis System" version 1.80., App. Biostatistics, Inc.

Saatcioglu A., 1979. " Analysis of the ABO gene frequencies in Turkey''. J Hum Evol, 8, pp.367-373.

Schurmann M., Warneke R., Schwinger E., 1987. "Four DNA polymorphisms on the short arm of the X chromosome : allele frequencies in a German and in a Turkish population''. Hum Hered , 37 (6), pp. 329-333.

Seielstad MT., Hebert JM., Lin AA., Underhill PA., Ibrahim M., Vollrath D., Cavalli-Sforza LL., 1994. " Construction of human Y-chromosomal haplotypes using a new polymorphic A to G transition''. Hum Mol Genet ,3 (12), pp. 2159-2161.

Simithies, O., 1955. "Zone electrophoresis in starch gels : group variation in the serum proteins of normal individuals". Biochemical Journal, 61, pp. 629-641.

Sokal RR., Harding RM., Oden NL., 1989 (a). " Spatial patterns of human gene frequencies in Europe". Am. J. Phys. Anthropol., 80, pp 267-294.

Sokal RR., Rohlf FJ., 1995. Biometry, 3rd ed. New York: W.H. Freeman and Co.

Spurdle AB., Hammer MF., Jenkins T., 1994. " The Y Alu polymorphism in southern African populations and its relationship to other Y-specific polymorphisms''. Am J Hum Genet ,54 (2), pp.319-330.

Stoneking M., Fontius JJ., Clifford SL., Soodyall H., Arcot SS., Saha N., Jenkins T., Tahir MA., Deininger PL., Batzer MA., 1997. "Alu insertion polymorphisms and human evolution : Evidence for a larger population size in Africa''. Genome Research ,7, pp.1061-1071.

Togan I., Erguven A., 1994. "Red cell enzyme and blood group polymorphisms in Turkey''. Gene Geography ,8, pp.75-80.

Togan I., Yılmaz A., Erguven A., Dereli I., Ozbas F., 1996. " Glyoxalase-I (GLO) polymorphism in eight geographic regions of Turkey''. Anthropol Anz ,54, pp.122-124.

Triantaphyllidis CD., Kouvatsi L., Kaplanoglou L., Natsiou T., 1986. " Genetic Relationships among the inhabitants of nine Mediterranean Countries". Hum Hered., 36, pp 218-221.

Tuncbilek E., Ulusoy M., 1989. " Consanguinity in Turkey in 1988 ". The Turkish Journal of Population Studies, 11, pp 35-46.

APPENDIX A

Table 7. The data of Stoneking et al. (1997) together with the present data

Population	N	ACE	APO	PV92	A25
Alaska Natives	41	0.580	0.920	0.620	0.150
Australia	69	0.910	0.870	0.150	0.350
Bretons	54	0.480	0.900	0.270	0.160
China	49	0.670	0.820	0.860	0.100
European-American	57	0.510	0.940	0.180	0.200
Filipino	47	0.530	0.980	0.800	0.140
French	53	0.480	0.990	0.230	0.160
French Acadian	46	0.510	0.920	0.180	0.120
Greek Cypriot	48	0.390	0.950	0.250	0.120
Greenland Natives	41	0.550	0.940	0.610	0.170
India-Christian	27	0.600	0.670	0.480	0.140
India-Hindu	28	0.520	0.850	0.520	0.050
India-Muslim	26	0.520	0.860	0.300	0.120
Java	32	0.860	0.780	0.840	0.060
Kung	40	0.290	0.880	0.200	0.610
Malaysian	47	0.640	0.760	0.720	0.020
Mayan	51	0.680	0.940	0.790	0.210
Moluccan	48	0.670	0.760	0.690	0.000
Mvskoke	50	0.700	0.960	0.570	0.210
Nguni	43	0.400	0.600	0.240	0.410
Nigerian	11	0.270	0.500	0.090	0.220
Pakistan	42	0.440	0.720	0.300	0.070
PNG-Coastal	48	0.660	0.660	0.360	0.020

Table 7 (cont'd)

PNG-Highland	68	0.740	0.680	0.240	0.040
Pushtoon	50	0.520	0.860	0.330	0.180
Pygmy-CAR	17	0.120	0.740	0.260	0.350
Pygmy-Zaire	17	0.320	0.850	0.350	0.530
Sotho	48	0.380	0.680	0.290	0.390
Swiss	43	0.370	0.940	0.200	0.120
Taiwan	46	0.500	0.930	0.900	0.220
Tamill	47	0.690	0.810	0.560	0.170
Tenggaras	90	0.640	0.780	0.500	0.050
Turkish Cypriot	33	0.330	0.980	0.330	0.090
UAE	42	0.330	0.970	0.300	0.120
Turkish*	**100**	**0.345**	**0.955**	**0.175**	**0.030**
Gst		0.115	0.109	0.223	0.137
All loci Gst	≈ 0.15				

N: Number of individuals studied.

* : **The results of the present study.**

APPENDIX B

Table 8. Distribution of the samples for ACE, APO, A25 and PV92 polymorphisms according to the provinces and sexes.

SAMPLE NO.	PROVINCE	SEX
1	SIVAS	M
2	YOZGAT	M
3	CANKIRI	M
4	ESKISEHIR	M
5	KIRIKKALE	M
6	AKSARAY	M
7	KAYSERI	M
8	KONYA	M
9	NIGDE	M
10	KIRIKKALE	F
11	SIVAS	F
12	ANKARA	F
13	KAYSERI	F
14	ANKARA	F
15	BOLU	M
16	MUGLA	M
17	ERZINCAN	M
18	ELAZIG	M
19	BOLU	F
20	ORDU	M
21	AFYON	F
22	MARMARIS	F
23	G.ANTEP	M

Table 8 (cont'd)

24	D.BAKIR	M
25	BURDUR	F
26	K.MARAS	F
27	MERSIN	M
28	ISPARTA	F
29	TARSUS	M
30	AFYON	M
31	BALIKESIR	F
32	AFYON	F
33	ANTALYA	M
34	AFYON	M
35	SAMSUN	M
36	ERZINCAN	F
37	ELAZIG	F
38	ELAZIG	M
39	MARDIN	F
40	TRABZON	M
41	BOLU	M
42	GUMUSHANE	M
43	AFYON	F
44	K.MARAS	M
45	ELAZIG	M
46	D.BAKIR	F
47	TRABZON	M
48	MANISA	M
49	BATMAN	F
50	ISKENDERUN	F
51	ERZURUM	F
52	ZONGULDAK	F
53	SIIRT	M
54	KAYSERI	M
55	KAYSERI	M

Table 8 (cont'd)

56	SIVAS	M
57	KONYA	M
58	SIVAS	M
59	NIGDE	M
60	KAYSERI	M
61	SIVAS	M
62	SIVAS	M
63	KONYA	M
64	ANTALYA	M
65	BOLU	M
66	KIRIKKALE	M
67	ELAZIG	M
68	KARS	M
69	K.MARAS	M
70	ORDU	M
71	MERSIN	M
72	CANKIRI	M
73	ERZURUM	F
74	D.BAKIR	F
75	AFYON	F
76	SAMSUN	M
77	KONYA	F
78	URFA	M
79	ERZURUM	M
80	CORUM	F
81	ISPARTA	M
82	ANKARA	F
83	CANKIRI	F
84	MALATYA	M
85	ZONGULDAK	M
86	KARS	F
87	ERZINCAN	M

Table 8 (cont'd)

88	G.ANTEP	M
89	AMASYA	F
90	ISPARTA	F
91	GUMUSHANE	F
92	KONYA	M
93	GUMUSHANE	F
94	ANTAKYA	F
95	MARDIN	M
96	AFYON	F
97	ELAZIG	M
98	VAN	F
99	TUNCELI	F
100	ANTALYA	F

Table 9. Distribution of the samples from Turkey for YAP element according to the provinces.

SAMPLE NO.	PROVINCE
1	SIVAS
2	YOZGAT
3	CANKIRI
4	ESKISEHIR
5	KIRIKKALE
6	AKSARAY
7	KAYSERI
8	KONYA
9	NIGDE
10	BOLU
11	MUGLA
12	ERZINCAN
13	ELAZIG
14	ORDU
15	G.ANTEP
16	D.BAKIR
17	MERSIN
18	TARSUS
19	AFYON
20	ANTALYA
21	AFYON
22	SAMSUN
23	ELAZIG
24	TRABZON
25	BOLU
26	GUMUSHANE
27	K.MARAS
28	ELAZIG

Table 9 (cont'd)

29	TRABZON
30	MANISA
31	SIIRT
32	KAYSERI
33	KAYSERI
34	SIVAS
35	KONYA
36	SIVAS
37	NIGDE
38	KAYSERI
39	SIVAS
40	SIVAS
41	KONYA
42	ANTALYA
43	BOLU
44	KIRIKKALE
45	ELAZIG
46	KARS
47	K.MARAS
48	ORDU
49	MERSIN
50	CANKIRI
51	SIIRT
52	CORUM
53	ZONGULDAK
54	ELAZIG
55	MALATYA
56	BOLU
57	G.ANTEP
58	BAYBURT
59	KAYSERI
60	KIRSEHIR

Table 9 (cont'd)

61	TRABZON
62	VAN
63	CANAKKALE
64	HATAY
65	KUTAHYA
66	MALATYA
67	ZONGULDAK
68	ERZINCAN
69	G.ANTEP
70	KONYA
71	MARDIN
72	ELAZIG

APPENDIX C

SOLUTIONS USED IN THIS STUDY

1. Nuclei Lysis Buffer pH:8.2

   10 mM Tris-HCl

   400 mM NaCl

   2 mM Na_2EDTA

2. 1X TE Buffer

   1 M Tris-HCl pH:7.4

   0.1 M EDTA pH:8.0

3. Amonium Acetate Solution

   10 M ammonium acetate

4. 10XTBE Buffer

   0.9 M Tris-Base

   0.9 M Boric acid

   0.02 M EDTA- Na_2

5. Agarose gel (2%)

   2 % (w/v) Agarose

   10 % (v/v) 1XTBE Buffer pH: 8.2

6. SDS

   10 % (w/v) Sodium Dodecyl Sulfate