Emine KALEİNTZİ

M.S. Thesis In Genetics and Bioengineering

July - 2012

MITOCHONDRIAL DNA ANALYSIS in ANATOLIAN POPULATIONS

by

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APPROVAL PAGE

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Halil Rıdvan ÖZ

Head of Department

This is to certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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M. S. Thesis – Genetics and Bioengineering
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Supervisor: Assoc. Prof. M. Fatih ABASIYANIK

ABSTRACT

Turkey has a special geographic location, which is between Asia and Europe. Thank to this location, Turkey has been a popular place to live for many populations in the past. Moreover, many populations used the Anatolia for the way of the migration in both directions. All these mobilities affected the Anatolia not only culturally but also genetically. In this study, 130 samples' (110 from Turkey and 20 from Turkish minority from Western Thrace) mitochondrial DNA control regions were analyzed by sequencing.

The haplogroup analysis showed that the most common mitochondrial cluster in Turkey is haplogroup R0 and it is also the most popular haplogroup in Turkish people from Western Thrace. According to the results of this study, Turkey's genetic structure is closer to Europe. There are also many Asian haplogroups in addition to Europe. Phylogenetic tree analysis showed that Turkey has heterogeneous genetic structure like it culture

Keywords: Mitochondrial DNA, haplogroup, polymorphism, gene diversity, population genetics, Geographical Regions, Turkey

ANADOLU TOPLUMLARINDA MİTOKONDRİAL DNA ANALİZİ

Emine KALEİNTZİ

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ÖZ

Türkiye, Asya ve Avrupa arasında olması sebebiyle özel bir coğrafik öneme sahiptir. Türkiye'nin coğrafik yapısı, geçmişte birçok topluluklara ev sahipliği yapmasına ve Avrupa ve Asya arasında göç yolu olarak kullanılmasına sebep olmuştur. Bu hareketlilik, Türkiye'yi kültürel ve genetik olarak etkilemiştir. Bu çalışmada, 110'si Türkiye ve 20'si Batı Trakya'dan olmak kaydıyla, 130 örneğin mitokondrial DNA'sının kontrol bölgesi dizi analizi yapılarak incelenmiştir.

Haplogurup analizlerine göre R0, Türkiye ve Batı Trakya'dan alınan örneklerde en sık görülen haploguruptur. Bu çalışmanın sonucunda, Türkiye'nin mitokondrial DNA anlamında Avrupa'ya daha yakın olduğu görülmüştür. Bunun yanında Asya haplogurupları da Türk populasyonunda görülmektedir. Filogenetik ağaç analizleri Türkiye'nin çeşitliliğini göstermektedir.

Anahtar Kelimeler: Mitokondrial DNA analizi, haplogurup frekansı, polimorfizm, genetik çeşitlilik, popülasyon genetiği, Coğrafi Bölgeler, Türkiye

To my sons, husband and mom for all their support and patient

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INTRODUCTION

1.1 ANTHROPOLOGY

Anthropology is a science that focused on humankind. Because of complexity of humans, the methodologies employed to investigate them are also complex. Basically, anthropology has four main fields: cultural anthropology, archaeological anthropology, physical (biological) anthropology and linguistic anthropology. Overlapping between fields is common and main fields also contain several related sub-fields. Cultural anthropology studies cultural variations among humans and depends mainly on ethnography. Archaeological anthropology focuses on remaining materials such as ancient weapons etc.

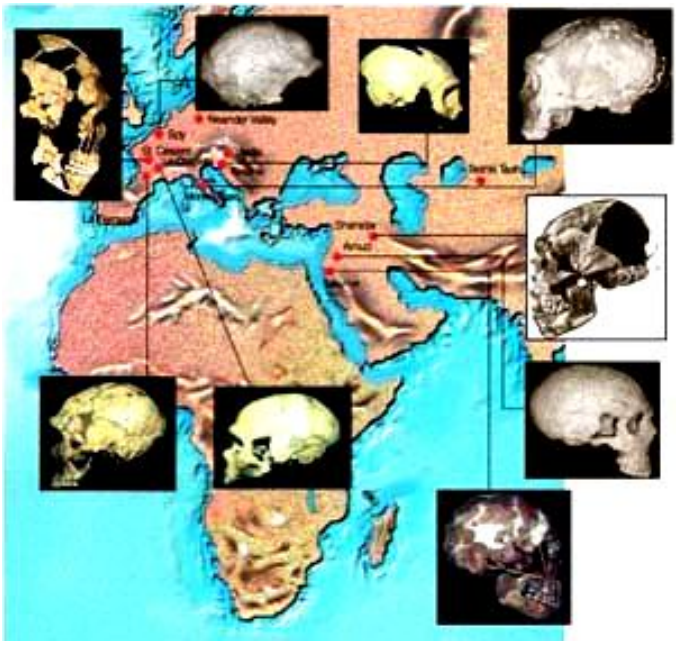

Figure 1.1 Neandental Remainings [31].

Linguistic anthropology tries to understand the processes of communication between humans, verbally and non-verbally and also work with languages. And finally physical anthropology is concerned with biological evolution and variation of the human species [61].

Molecular anthropology is a sub-field of physical anthropology. Molecular analysis and genetic markers are used to study evolutionary links between ancient and modern human populations. Genetic markers in human are one of the important investments in understanding of evolutionary relationships between human populations. The study with genetic markers in determining differences between populations starts with Hirszfeld in 1919 with determination of A and B blood antigen frequency differences in different ethnic groups. After Hirszfeld research, many scientists started to look the relations between populations at the molecular level. For example, ABO blood system, immunoglobulins and proteins were used to detect differences between populations. These types of markers were named classical polymorphisms and there were many documented data about them. With the invention of the technology, anthropologies or population geneticists started to use DNA and molecular markers for the population studies. For instance, restriction fragment length polymorphism (RFLP) was used to detect genetic linkages. After the polymerase chain reaction (PCR) discovery, population genetic studies focused on DNA and the differences or common points of genomic material among people.

1.2 TYPES of POLYMORPHISMS

Although most of the human's genetic materials are the same (99.7%), small unique parts make people different from each other. These unique parts are basically constituted by single nucleotide changes, variable number of tandem repeats and deletions and insertions to the sequence. These types of polymorphisms can be both in coding regions and also in non-coding regions [32].

Polymorphisms in coding regions can be silence or they can be harmful by causing genetic diseases. Polymorphisms especially in noncoding parts are very helpful for population analysis. Single nucleotide changes and repeats in genome are useful differences among people.

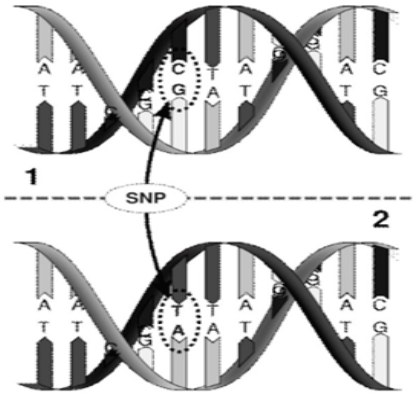

Figure 1.2 Single nucleotide change [29].

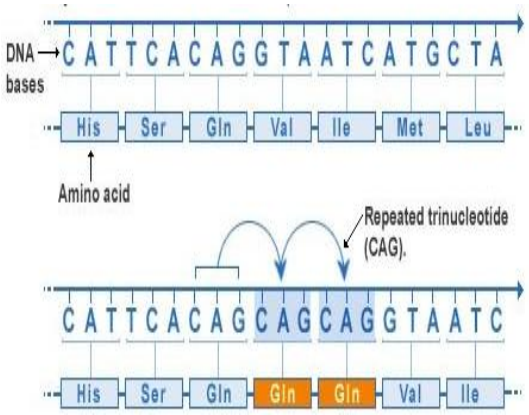

Figure 1.3 Variable repeats in sequences [30].

1.1.2 Repeats in Genome

In the human genome, there are many repeating short DNA sequences. These repeats can be seen in different parts of the genome. For example, long repeat regions are seen around the centromere of a chromosome, abundantly. Repeats in the genome can be divided into two; Variable number tandem repeats (VNTR) which are also called minisatellite markers and simple sequence repeats (SSRs) or microsatellite markers. They are highly polymorphic because of high mutation rate so they are useful markers for population genetics [33,34].

1.2.1.1 VNTRs

The number of VNTR is changing from person to person and the length of the repeating unit is between 10 to 100 bases [35]. In the figure below, one individual has 12 repeating units and the other one has 17 repeating VNTR units.

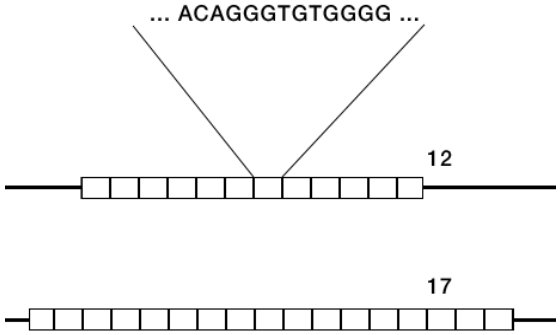

Figure 1.4 VNTR example [37].

1.2.1.2 SSRs

SSRs have two to six base pairs in length and they are first identified in the late 1980's ([38], figure 1.5). Because of their codominance character, both homozygotes and heterozygotes can be identified by using SSRs markers. SSRs are also called short tandem repeats (STR). STR sequences are characterized in two ways; i) according to the length of the repeat unit and ii) the repeat pattern. For example, **tetranucleotide repeats** have four repeating nucleotides and in **the simple repeats,** all the repeating units have the same length and the sequence [40, 41].

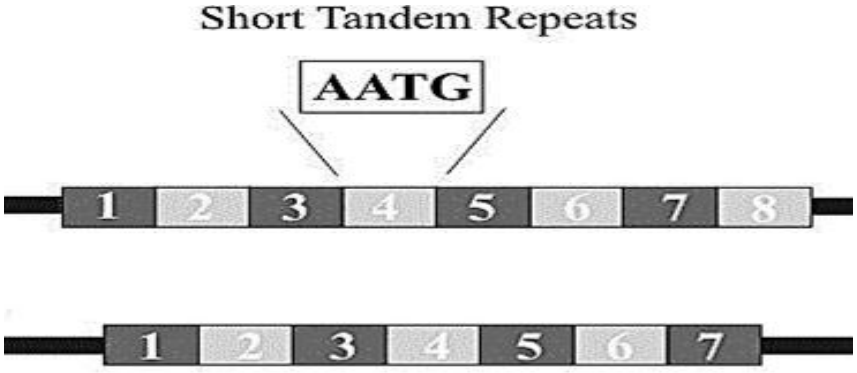

Figure 1.5 STRs [39].

1.2.1.3 DNA Repeats in Population Genetics

Although both STR and VNTR repeats can be used in identification of people, STR markers are more popular than the VNTR markers and they have many advantages over VNTR markers. The sizes of the STR alleles are smaller and it makes them easily to study especially in degraded samples [40]. And also the small sizes of the STR markers give advantages to them while their amplification; the STR markers can be amplified by using polymerase chain reaction (PCR) easily. Because of these reasons and their identification power, STR markers are frequently used in forensic cases. In figure 1.6, most widely used STR markers and their position on the chromosomes can be seen.

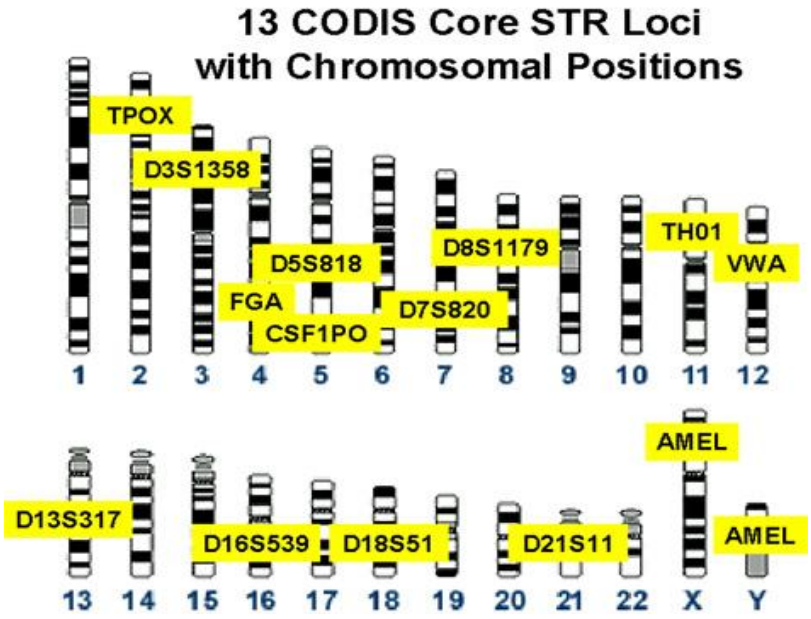

Figure 1.6 13 STR loci [39].

1.2.2 Single Nucleotide Polymorphisms

SNP markers are abundant in the human genome. They are single nucleotide polymorphism and they have lower mutation rate than STR markers. So the point mutation in the genome became fixed in a population [42]. Thank to identification

power and lower mutation rate, SNP markers can be used to identify the ethnic origin of the individuals.

The classification of SNPs is made according to their effects of the genome. After point mutation, if both alleles synthesize the same polypeptide sequence then it is called **synonymous polymorphism**. If different polypeptide sequences are produced then the name is **replacement polymorphism**. And also when the replacement polymorphism changes the amino acid then the name is missense and when it leads to premature stop codon, its name is **nonsense polymorphisms**.

The basic techniques to detect the SNPs are DNA sequencing, restriction fragment length polymorphisms and single-strand conformation polymorphism (SSCP) analysis. And also capillary electrophoresis, hybridization analysis, mass spectrometry and electrochemical analysis can be used to find the SNP sites [44, 40].

When comparing the STR and SNP markers, STRs are more useful and informative in forensic cases with the high mutation rate. They have high identification power. On the other hand, SNP markers are more informative in **ethnic origin analysis** and can also be used in forensic cases when the samples are degraded or ancient.

1.2.3 Lineage and Polymorphisms

Polymorphisms or markers on chromosomes 1-22 are referred as autosomal markers because there is a recombination between chromosomes during meiosis. However, on the Y chromosome, X chromosome and mitochondrial DNA, there is a passage from one parent to offspring without any recombination so markers on these chromosomes and mitochondrial DNA are called lineage markers [40].

Y-STR and Y-SNP markers are used to observe paternal lineages and on the other hand, mitochondrial DNA is informative for maternal lineages (figure 1.7).

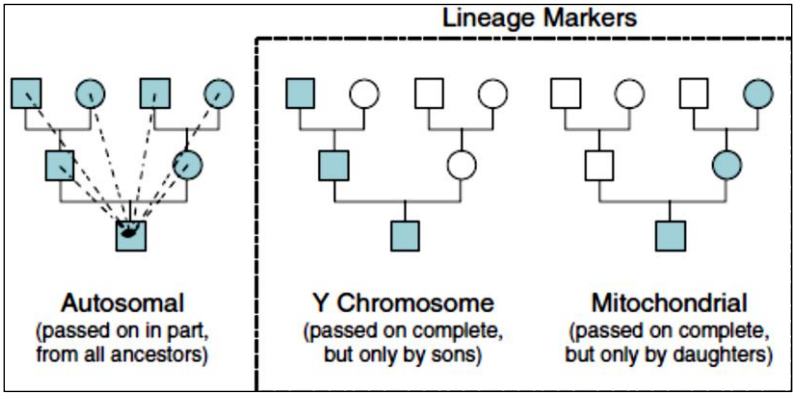

Figure 1.7 Inheritance pattern of autosomal and lineage markers.

1.2.4 DNA Markers

1.2.4.1 X-STR

Besides their autosomal chromosomes everyone has at least one X chromosome (men only one from mother, women two: one from mother and the other one from father). The markers on X chromosomes are used in forensic genetics especially in women suspect case [45]. And they are also used together with autosomal markers to increase the power of test. Paternity tests are the other site of the X-STR markers usage.

Some loci that are used in X-STR analysis are DXS8378, DXS6795, DXS7132, DXS6803, DXS9898, DXS6801, DXS7133, GATA165B12, HPRTB, DXS8377 and DXS7423 [46].

1.2.4.2 Y-STR

Y-STRs are short tandem repeats on Y chromosomes that pass from father to son so paternally. It is an important identification method for men in forensic cases, for example in sexual assault cases. It is also used in population studies but because of its high polymorphisms, SNPs are more convenient in population cases. There are more than 200 Y-STR loci which can be found from Genome Database. On the figure 1.8, there are some Y-STR loci.

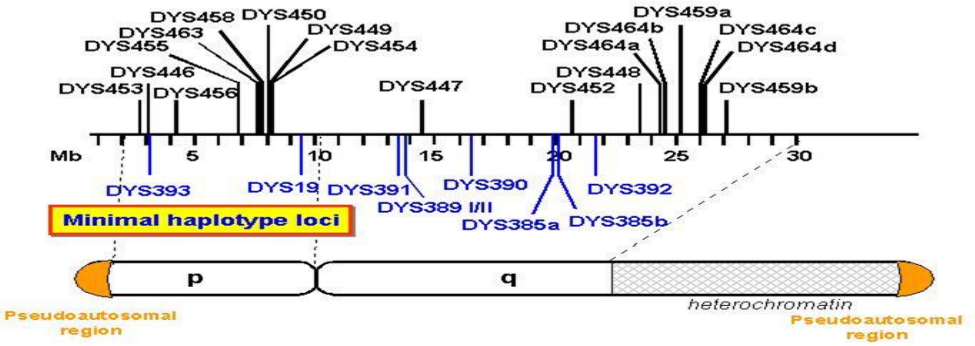

Figure 1.8 Y-STR loci example.

1.2.4.3 Mitochondrial DNA

Mitochondria are double-membrane intracellular organelles that responsible for energy production in all nucleated mammalian cells. In addition to energy production, they have roles in intracellular signaling and apoptosis. There are hundreds copies of mitochondria in each cell. Mitochondrial DNA (mtDNA), which is different from nuclear DNA, is a circular molecule and the length is 16,569 base pairs (bp). Basically, the human mitochondrial DNA is divided in to two region; coding region and control region. Coding region is about 15,000 bp long and encodes 37 different genes; 22 transfer RNAs encoding genes, 13 protein encoding genes and 2 ribosomal subunits encoding genes [62].

NDI-ND6 and ND4L encode seven subunits of complex I of the respiratory chain. Cyt b encodes complex III subunit. COI-COIII encode three subunits of complex IV. And two subunits of complex V are encoded by ATP6 and ATP8 genes.

Control region unlike coding region does not encode any protein. It has roles in mitochondrial DNA replication and gene expression. Control region or displacement loop (D-loop) is divided into three segments: hypervariable region I (16024-16400, HVS-I), hypervariable region II (40-400, HVS-II) and hypervariable region III (430-576 ,HVS-III).

Mitochondrial DNA is very important for evolutionary and population studies. The importance comes from some special features of mitochondria. Firstly, mitochondrial DNA is inherited through the maternal line so it gives maternal ancestor of the individual [1]. Although paternal recombination in mitochondrial DNA has recently been challenged, according to many articles, it is exceptionally rare [2]. Paternal mitochondria enter the egg cell but it is destroyed by an active ubiqutination mechanism [3].

Secondly, because of high mutation rate when compared to nuclear DNA, mtDNA is a good sample for studying evolution [4]. mtDNA has five to ten times faster mutation rate than nuclear DNA. And within the mtDNA, control region has ten times higher mutation rate than coding region. Through the coding region, polymorphic sites are not distributed. They mainly found on HVS-I and HVS-II, [62].

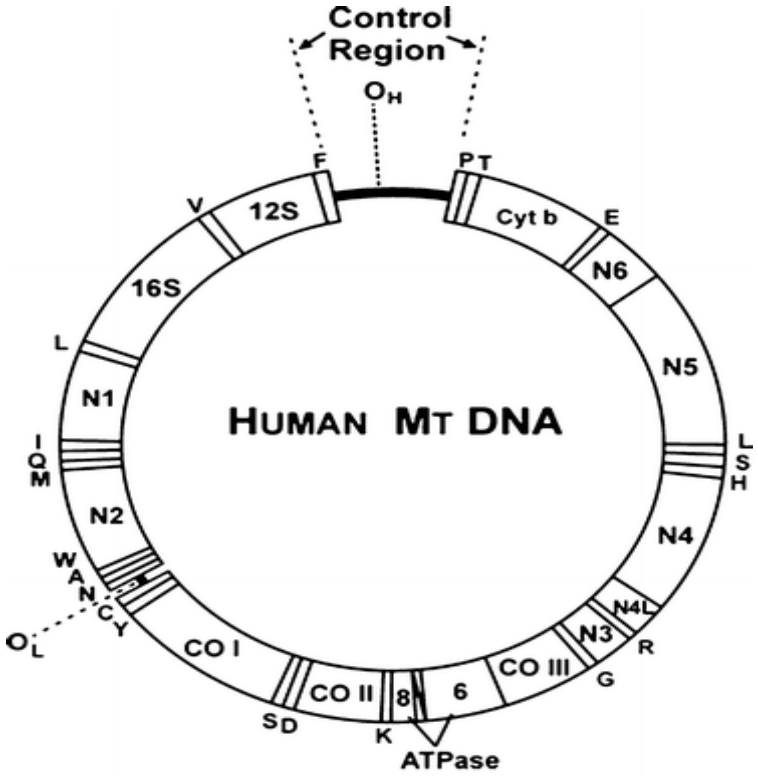

Figure 1.9 mtDNA [61].

1.2.4.3.1 Segregation of Mitochondrial DNA and Heteroplasmy

Heteroplasmy, which is mixture of more than one type genome, is present in mtDNA. The level of heteroplasmy is influenced by many factors. According to many studies, random genetic drift through a hypothetical bottleneck determines the process of transmission of mtDNA heteroplasmy. Thanks to the bottleneck, most of the novel mutations are lost and some of them are fixed. If these mutations weakly pathogenic, advantageous or truly neutral, they will subscribe to the molecular evolution of the mitochondrial genome [66].

1.2.4.3.2 Haplogroups in mtDNA

Haplogroups are identified from each other according to defining mutations in mtDNA which can be both in coding region and control region. During determination of haplogroups for better phylogenetic resolution, both coding region and control region information is needed. Control region have high mutation rate and there are C-stretches in positions 16189 and 310, respectively. These sites are excluded from mtDNA phylogenetic tree. The example of some haplogroups and their defining mutations are on the table 1.1. The present mtDNA phylogenetic tree is developed by using both high-resolution RFLP data and control region data of the mtDNA. If they are considered alone, both techniques have same weaknesses. For example, by using only high-resolution RFLP analysis, haplogroup R cannot be resolved and on the other hand, some members of haplogroup M from N and many members of haplogroup H from HV or U cannot be distinguished by using only control region sequence analysis [65].

Table 1.1 Some haplogroups and their defining mutations.

HAPLOGROUP	DEFINING MUTATIONS
Haplogroup H	263
Haplogroup HV	263, 2706, 7028, 14766
Haplogroup J	295, 462, 489, 10398, 12612, 13708, 16069
Haplogroup N	73, 263, 2706, 7028, 11719, 12705, 14766, 16223

The Nomenclature of mtDNA haplogroups was started with Torroni [5]. He used the restriction fragment length polymorphism(RFLP) data set of Native Americans to define the four basic branches in the tree, whose names are A,B,C and D. And with addition of new population data from other countries to the phylogenetic tree of the mtDNA, the tree started to grow. Then the cladistic rules of the haplogroups and subhaplogroups hierarchy were determined [6]. The names of the haplogroups are started with A and go until Z. They are named according to the discovery not to the genetic relationship.

To better understand the hierarchical labeling, the example of haplogroup H can be used. In figure 1.10, some subhaplogroups of the H can be seen. The nomenclature of haplogroups goes from root to tips with the gain of extra mutation or identifying point like H>H1>H1e> H1e1>H1e1a>H1e1a1.

Each branch is identified by one or more mutations. The mutation points are determined according to difference from Cambridge reference sequence (CRS). It is a first sequenced mtDNA in 1981 [8]. In 1999, its correction was published and it is also used by the scientist for the reference sequence [9]. The haplogroup of the CRS is H2b which is a subclade of haplogroup H [10]. The star symbol, which can be seen in phylogenetic tree of the mtDNA , means different subclade that do not belong to any named subhaplogroups (figure 1.10).

1.2.4.3.3 Mitochondrial Eve

As it is told before, in evolutionary studies, mitochondrial DNA has been used by the scientist. Searching human genetic history through mtDNA starts with Wesley Brown in 1980s. Brown's group studied 21 samples whose ethnic and geographic origins were different. In these pre-PCR days, only restriction enzyme cleavage pattern was used [11]. According to their results, there was a central RFLP haplotype which was shared by the individuals from all over the world. After the publication of first complete sequence of mtDNA, another scientist showed an RFLP site which separated most modern sub-Saharan Africans from Europeans and Asians [12].

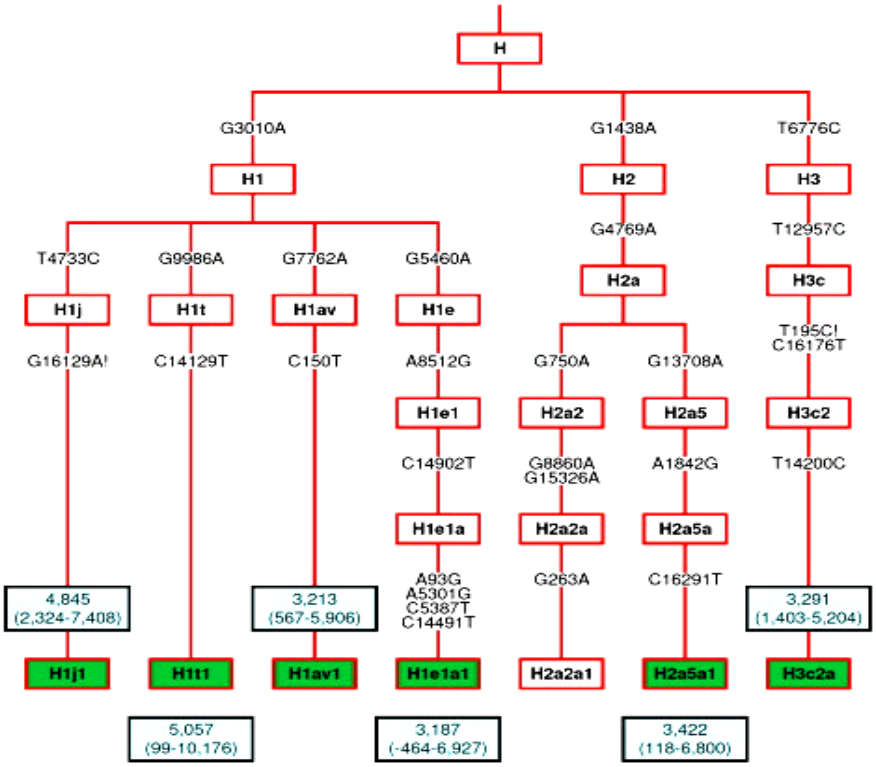

Figure 1.10 Haplogroup H [47].

In January 1987, Rebecca Cann, Mark Stoneking, and Allan Wilson made a publication with high resolution RFLP analysis of mtDNA with 12 restriction enzymes [13]. They used 147 individuals from European Americans, African Americans, East Asians, New Guineans and Aboriginal Australians. The phylogenetic tree they presented had two basal clades. One of them was found only in African Americans, the other one was found not only in African Americans but also in the other population samples. They calculated the time of the most recent common ancestor of modern humans as between 140 000 and 290 000 years ago. Therefore, according to their interpretation of the study, modern human ancestor come from the Africa and which means ‘ Mitochondrial Eve’ was African ([62], figure 1.12).

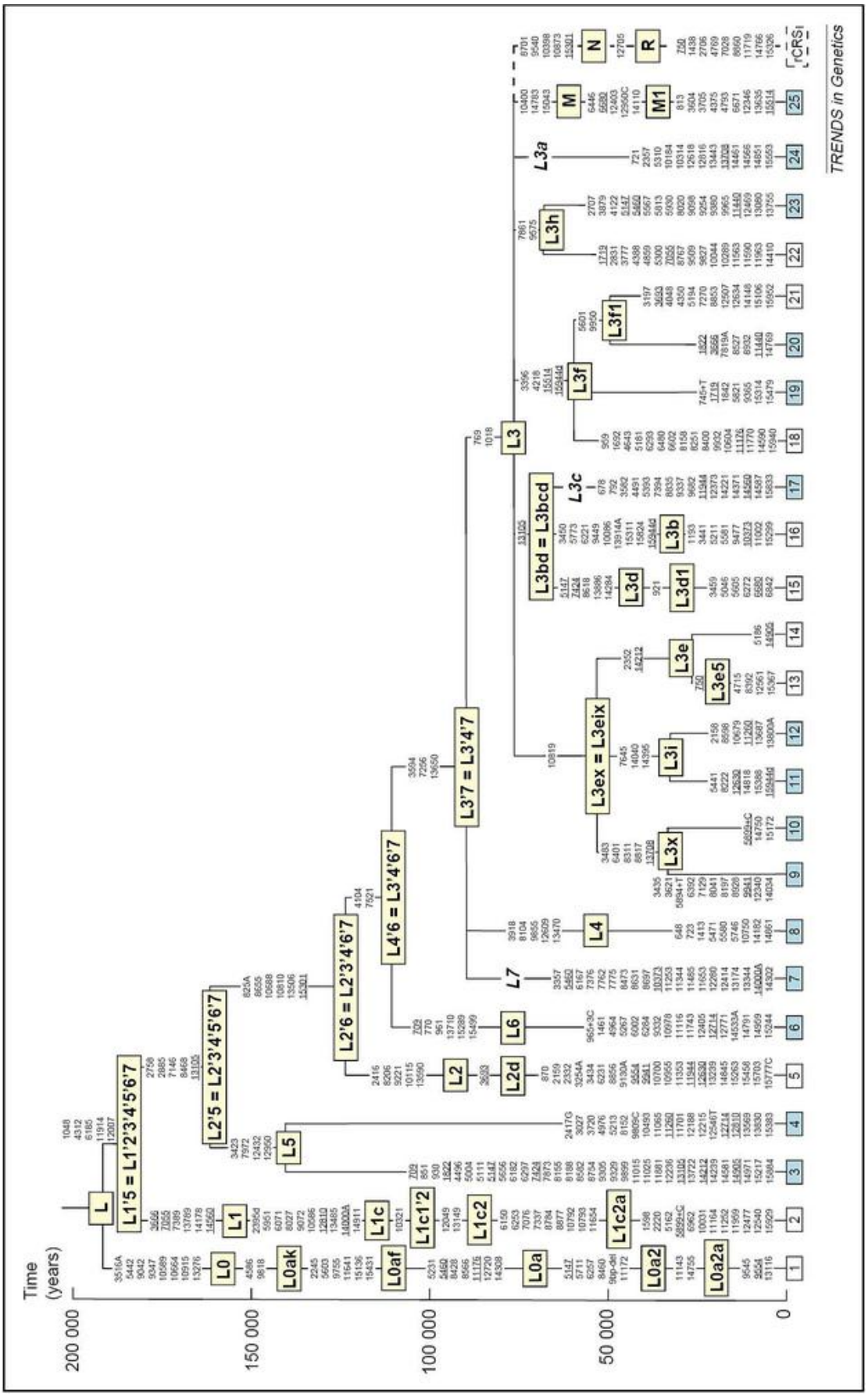

Figure 1.11 mtDNA Phylogenetic tree, [68].

Vigilant published a supportive paper for African origin [14]. In this paper, the sequence of the control region was used. According to the results, there were two clades at the base of the tree like Cann's results and there was long-term evolution within Africa before spreading the world. Although there were some mistakes in these studies [15,16], new researches also supported the conclusion of the Cann's paper which was African origin, whose results were based on more sophisticated phylogenetic tools, [17].

1.2.4.3.4 Haplogroups in the World

Most of the African individuals' mtDNA enter haplogroup L which is found at the deepest point of the phylogenetic tree. L is a macrohaplogroup whose subclades are L1, L2 and L3. African people show great diversity and its deepest phylogenetic branches are sub-Saharan [14]. In modern humans, all non-African people come from haplogroup L3 [18]. L3 gave rise to macro haplogroups M and N. Approximately, 60,000 years ago with the effect of external factors such as climate changes, people started to emigrate from Africa to South Asia, then Australia and Papua New Guinea about 46,000 years ago [19].

In Asia, the distributions of haplogroups are more complicated. It is a region that had been a birth place for many empires. The present Asian mtDNA haplogroups are a mixture of both Western and Eastern Eurasian haplogroups [22, 23]. The complex gene pattern was produced by the help of the continental trade routes and empire-buildings. The main haplogroups of East Asia are A, B, C, D, F, G, M, Y and Z [20, 21].

The haplogroups of America are A, B, C, D and X which are the subclades of Asian haplogroups. The haplogroup types support the common believe about Native American population originated from Asia [24].

In Europe, the haplogroups were descendant from macrohaplogroups M and N about 30 to 60 thousand years ago [25]. Migrations and gain of mutations from Asia to Europe were a result of harsher conditions such as climate change and terrain. The basic haplogroups in Europe are H, I, J, K, T, U, V, W and X.

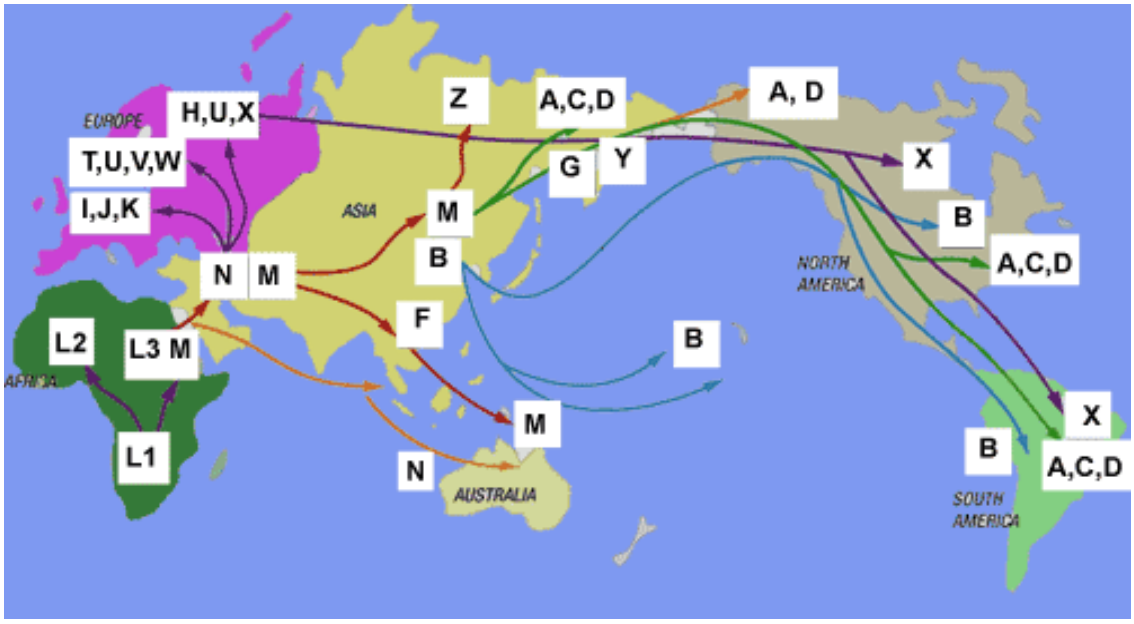

Figure 1.13 mtDNA migration map [48].

By using statistical analysis the ages of the haplogroups can be calculated and they are used to estimate the migration ways through mtDNA. Some haplogroups and their ages can be seen on the table.

Table 1.2 Example of ages of Haplogroups [60].

N	75,000 years ago
R	70,000 years ago
U	60,000 years ago
Pre-HV(R0)	50,000 years ago
J	45,000 years ago
T	17,000 years ago
K	16,000 years ago
H	35,000 years ago

1.2.4.3.5 Haplogroups in Turkey

Turkey or Anatolian peninsula locates between Europe and Asia. Through the history, it was homeland for many civilizations such as Hattis, Hittites, Phrygians,

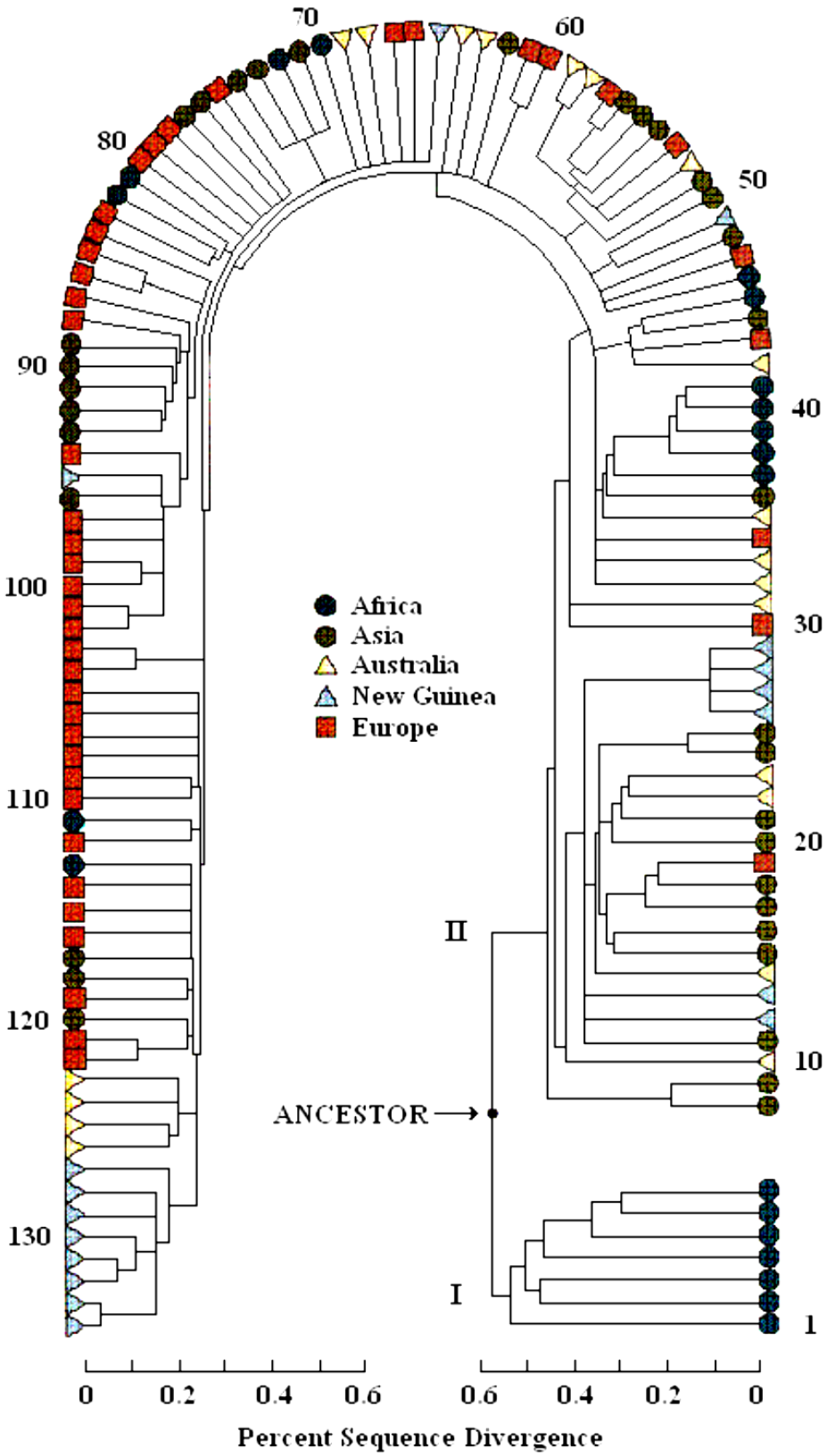

Figure 1.12 mtDNA Genealogical Tree [49].

Urartians, Lycians, Lydians, Ionians, Persians, Macedonians, Romans, Byzantines, Seljuks and Ottomans since 6500 B.C [26]. Genetic variations of present Anatolian or Turkey population come from many different populations who lived in Anatolia in the past.

The haplogroup data and also scientific papers about Turkish mtDNA are very limited when compared to other countries. Because of its geographic position, mtDNA information from Turkey is very valuable to better understand the migrations from Asia to Europe or vice versa.

The first mtDNA studies about Turkey were made by the Calafell et al, [27, 28]. In 1996, the paper about Turks and Bulgarians was published [27]. They used 29 Turks mtDNA control region sequences to compare 30 Bulgarians sequences. They collected samples from different parts of the Turkey and sequenced only HVSI and HVSII regions. According to their results, samples from Turkey were higher and more polymorphic than Bulgarians and Turks were close to Middle Eastern populations in terms of levels of differentiation and genetic distance. They did not make any haplogroup determination.

On the second study of Calafell [28], 45 unrelated samples from different parts of the Turkey were used to compare different populations (Middle Easterners, Sardinians, British and Basques). Genetic distance and also population tree were used to make the comparison. Their analysis showed that Turkish samples were between Europe and Middle East with respect to mtDNA sequences ([28], figure 1.14).

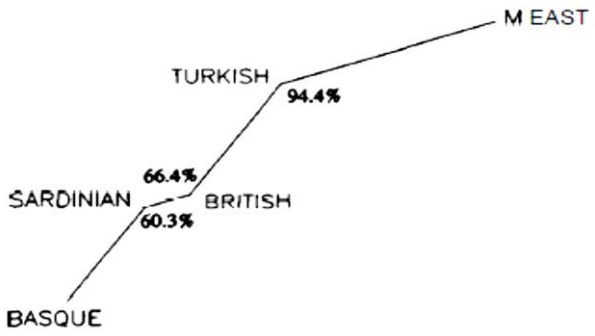

Figure 1.14 Neighbor- joining tree between populations [28].

In 2004, Mergen at al published a research for mtDNA analysis of Turkey, [50]. Their sample size is 75 individuals and they sequenced HVSI and HVSII regions of mtDNA. They made haplogrouping of samples and the results are on the table 1.1. According to the results, the cluster U has the highest frequency in Turkish samples which is a cluster that is seen in both European and African people. They interpreted that the U cluster spreading from Africa to Europe was happening through Northern Anatolia [50].

Table 1.3 mtDNA clusters frequency [50].

	Clusters; no. (%) of sequences								
Regions	H	U	K	U2	U5	J	T	M	W
Northern Anatolia	3(17.6%)	9(52.9%)	–	–	1(5.8%)	1(5.8%)	–	2(11.7%)	1(5.8%)
Southern Anatolia	6(40%)	5(33.3%)	1(6.6%)	–	–	–	–	3(20%)	–
Western Anatolia	4(28.5%)	6(42.8%)	1(7.1%)	–	1(7.1%)	–	–	2(14.2%)	–
Central Anatolia	8(57.1%)	3(21.4%)	1(7.1%)	1(7.1%)	–	1(7.1%)	–	–	–
Eastern Anatolia	4(26.6%)	4(26.6%)	1(6.6%)	–	–	4(26.6%)	1(6.6%)	1(6.6%)	–
Total	25(33.3%)	27(36%)	4(5.3%)	1(1.3%)	2(2.6%)	6(8%)	1(1.3%)	8(10.6%)	1(1.3%)

By calculating the genetic distance, they showed that Turkish people are closer to Central Asian and British people and their results supported the location of Anatolia between Europe and Asia (figure1.15).

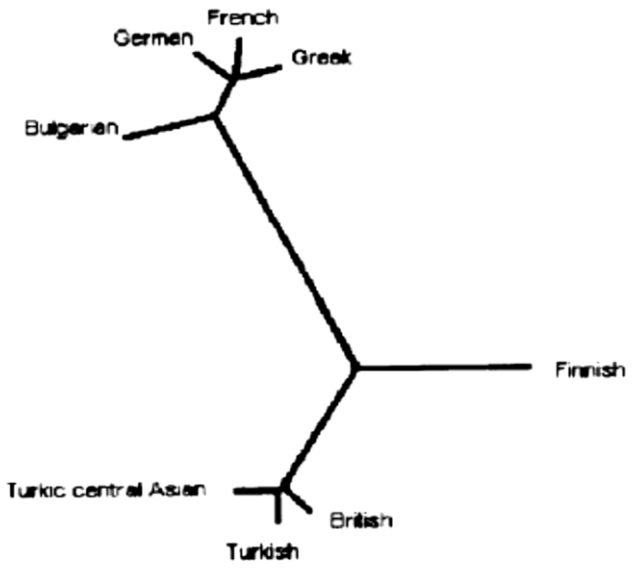

Figure 1.15 Neigbour-joining tree between populations [50].

According to many studies, the haplogroup H has the highest frequency among Turkish people. For example, a study with the 50 sample size showed that 26 % of Turkish people were in haplogroup H [51] and also in another paper with 494 samples from Turkey, the frequency of haplogroup H is 0.979 [52].

1.2 THE STUDY OBJECTIVE

The number of studies about mtDNA analysis is very limited in Turkey and the present studies did not cover the whole regions of Turkey. In this study, the sample collection was made according to population distribution among regions so although the sample size is small to make exact or assertive results, the results of this work could give important information for maternal ancestry of Turkey. In evolutionary point of view, Turkey has a crucial geographic position. It is a bridge between Europe and Asia. Better understanding the present Turkey's genetic structure could give advantages while studying history of migrations.

Turkey still does not have any mtDNA database which can be very helpful in forensic cases, population genetics and anthropology. The results of this work would contribute the future database of Turkey. Briefly, the aims of this study are;

a) to determine haplogroups in Turkish samples
b) to calculate their frequencies (for both regional and whole Turkey)
c) to contribute internal genetic databases
d) to try to understand the genetic sources of Turkish people in maternal linage.

CHAPTER 2

MATERIALS & METHODS

2.1 SAMPLE COLLECTION

Blood samples of 130 healthy individuals with the annotations about the ethnic origins and family histories of samples were collected from different geographical regions of Turkey and other countries. Before collection, a *Request for Review by the Ethic Committee of Fatih University Medical School, Ankara for the Protection of Human Subjects* was submitted and approved for the project. Special care was taken to avoid sampling from related individuals. A copy of the questionnaire with *The Informed Consent to Participate in a Research Study* is provided in Appendix A. The populations included in this study are the Marmara (*n*=21), Black Sea (n=22), Aegean (n=14), Central Anatolia (n=19), Mediterranean (n=12), Eastern Anatolia (n=12), Southeastern Anatolia (n=10), and other countries (n=20) (Figure 2.1). This research was supported by Fatih University Scientific Research Projects Fund (BAP), under the project number P50091001.

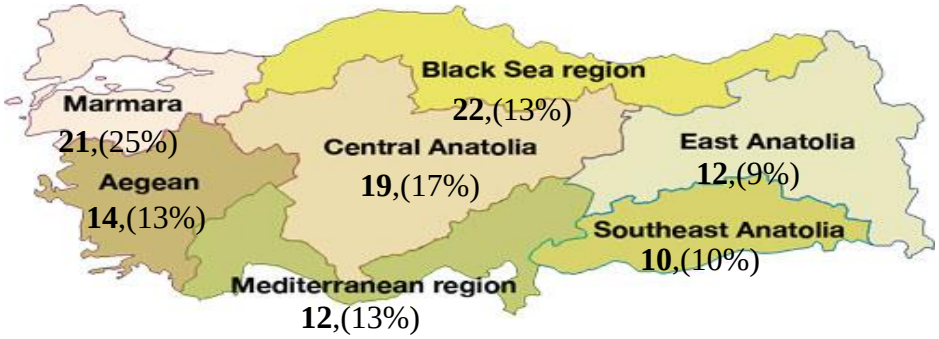

Figure 2.1 Regions of Sampling (collection numbers; the percentages of the population distribution) [67].

2.2EXPERIMENTAL PROCESSES

2.2.1 DNA isolation and quantification

The whole blood was collected into EDTA vacutainer tubes by using venipuncture. DNA was isolated from blood using Gentra® Puregene® Blood Kit according to the manufacturer's protocol [63].

The quantity of extracted DNA was measured by using a Nanodrop® ND-100 spectrophotometer [64]. Then, extracted DNA samples were stored at -20° C.

2.2.2 Amplification of DNA

The polymerase chain reaction (PCR) is an amplification technique used to amplify DNA. The copy number can vary from thousands to millions. Many factors can affect the efficiency of this amplification reaction. One of these factors is the concentration of template DNA. That is why before PCR, the DNA concentrations of all extracted DNA samples were adjusted to 1-10 ng/µl.

In this study, a partial (1357 bp) sequence of human mitochondrial genome was analyzed. This region covers hypervariable region I (16024-16400, HVS-I), hypervariable region II (40-400, HVS-II) and hypervariable region III (430-576 ,HVS-III). Primers for amplification reaction were F15851 for forward primer and R639 for reverse primer. The master mix for PCR is shown in the following table (Table 2.1):

Table 2.1 Master mix for PCR.

Reagent	Concentration	Volume
Buffer	10X	2,5µl
BSA	2,5mg/ml	2,5µl
dNTPs	10mM	2 µl
Forward primer	10mM	0,5 µl
Reverse primer	10mM	0,5 µl
Advantage® Taq polymerase	50X	0,2 µl

To prepare each tube, 24µl PCR master mix and 1µl template DNA were used. PCR amplification was performed on the *TC-412 Techne*® Thermal Cycler according to the conditions given in the table below (Table 2.2) and the products were stored at an appropriate temperature.

Table 2.2 PCR conditions.

Initial Incubation Step	**Cycles (35 cycles)**			**Final Extension**	**Final Hold**
	Denature	**Anneal**	**Extend**		
HOLD	CYCLE			HOLD	HOLD
95°C 2 min	95°C 15 sec	56°C 30 sec	72°C 90 sec	72°C 10 min	4°C ∞

Amplification reactions were checked for PCR products on a 1,5% agarose gel using electrophoresis at 100 volts for approximately 50 min. A total of 5 µl of the PCR product and 2µl of loading dye were mixed and added to each well.

Before sequencing, the PCR products were purified by using ExoSAP-IT [53] purification method as follows: Initially, 5µl from PCR product and 2µl ExoSAP-IT were put into the tubes. Then, they were put into the thermal cycler mentioned above to denature excess nucleotides, primers and proteins except amplicons. The conditions of the reaction are given in the following table:

Table 2.3 Purification conditions.

Temperature	Time
37°C	15 min
80°C	15 min
4°C	forever

2.2.2.1 Mitochondrial DNA Sequencing

The HVS-I, HVS-II and HVS-III regions of the mtDNA control region were sequenced on an ABI 3110 capillary sequencer by using Applied Biosystems BigDye® terminator v1.1 cycle sequencing kit. The prepared master mix was:

Table 2.4 Cycle Sequencing Mastermix.

Chemicals	Volume
Big Dye v1.1	1 µl
Big Dye Buffer	3 µl
H_2O	3,7 µl
Primer	0,3 µl

To make 10µl final volume, 2 µl purified PCR product and 8 µl cycle-sequencing master mix was used for sequencing PCR. The primer list, their sequences and conditions of sequencing PCR are on the following tables:

Table 2.5 Primer List.

F15851	5´ ATC TCC CTA ATT GAA AAC AAA ATA CTC AAA 3´
F15971	5' TTA ACT CCA CCA TTA GCA CC 3'
F16268	5' CAC TAG GAT ACC AAC AAA CC 3'
F15	5' CAC CCT ATT AAC CAC TCA CG 3'
R484	5' TGA GAT TAG TAG TAT GGG AG 3'
R16	5' TGA TAG ACC TGT GAT CCA TCG TGA 3'
R159	5' AAA TAA TAG GAT GAG GCA GGA ATC 3'
F29	5' CTC ACG GGA GCT CTC CAT GC 3'
R639	5´ GGG TGA TGT GAG CCC GTC TA 3´
R484	5' CCG CTT CTG GCC ACA GCA CT 3'

Table 2.6 Sequencing conditions.

Initial Incubation Step	**Cycles (25 cycles)**			**Final Hold**
	Denature	**Anneal**	**Extend**	
HOLD	CYCLE			HOLD
96°C 1 min	96°C 10 sec	50°C 5 sec	60°C 4 sec	4°C ∞

The purification of PCR products to remove unused primers and dyes were made by Sephadex Columns which were prepared manually. To prepare the columns, sephadex powder (from pharmacia fine chemicals)) and water were mixed on the columns and waited for 3 hours to preparation. After putting the products on the columns, the samples were centrifuged to get pure PCR products for loading the sequencing machine.

Before loading on the machine, 5µl formamid and pure PCR products were mixed to keep the two strands of DNA separately. The samples were loaded to ABI 3110 capillary sequencer. Chromatogram data which contain mtDNA sequence were recorded on a computer and used for mtDNA analysis.

2.2.3 Statistical and Computational Analysis

2.2.3.1 Analysis of Sequences

Sequencher 4.9 software was used to analyze and compare the mtDNA sequences.

2.2.3.2 Neighbor-Joining (NJ) Tree

Neighbor- joining tree is a method that shows distance between samples. Matlab software is used to make neighbor-joining analysis with the sequences. It is eliminate the unequal rates of evolution between branches.

2.2.3.3 Frequency of Haplogroups

Haplogroup frequency is a measure of the relative frequency of a haplogroup in a population.

$$\text{Haplogroup frequency} = \frac{\text{Total number of individuals in the haplogroup}}{\text{Total number of Individual}}$$

2.2.3.4 Phylogenetic tree

The trees were drawn by using Matlab software.

2.2.3.5 Analysis of Molecular Variance (AMOVA)

Differences between populations were assessed by using Amova analysis. Amova test calculates the difference according to genotype distance. It is made by using Arlequin software [54].

CHAPTER 3

RESULTS

Sample's haplogroups and the variable positions from Reference sequence are on table 3.2 and 3.3.

3.1 MARMARA REGION OF TURKEY

The frequencies of haplogroups and the number of people in marmara region can be seen on the table 3.1.

Table 3.1 Haplogroups in marmara region.

Haplogroup	Number of people	Percentage
R0	7	33%
H5	2	10%
U3	2	10%
K	1	5%
H11a2	1	5%
M	1	5%
T2	1	5%
J2a	1	5%
T1a	1	5%
H1a	1	5%
R2	1	5%
U4b2a	1	5%
Z1a	1	5%

3.2 MEDITERRANEAN REGION OF TURKEY

Haplogroup frequencies are on table 3.4.

Table 3.2 Variable positions of mtDNA control regions in Turkish samples with respect to Cabridge Reference Sequence [9].

Sample	Haplogroup	Profile
TK001	R0	263G-309.1C-315.1C----------
TK002	J2a2a	16069T-16126C-16214T-16362C-73G-150T-195C-235G-263G-295T-309.1C-315.1C-489C
TK003	R0	16129A-16294T-16311C-16519C-44.1C-151Y-263G-309.1C-315.1C----
TK004	K1a	16224C-16240G-16311C-16519C-73G-247A-263G-315.1C-497T-524.1A-524.2C--
TK005	J1d2	16069T-16126C-16172C-16186T-16193T-16519C-73G-152C-200R-263G-295T-309.1C-315.1C-462T-489C
TK006	R0	16260T-16304C-16399G-263G-309.1C-315.1C-------
TK008	A4	16214T-16223T-16234G-16284G-16290T-16311G-16319A-16362C-16525C-73G-152C-235G-263G-315.1C-523DEL-524DEL
TK009	C	16129A-16223T-16298C-16327T-16519C-73G-249DEL-263G-309.1C-315.1C-489C--
TK010	R0	16203G-16357C-186T-263G-309.1C-309.2C-315.1C-524.1A-524.2C----
TK013	R0	16261T-16311C-16519C-263G-315.1C--------
TK014	K1a	16093C-16224C-16311C-16519C-73G-263G-315.1C-497T-----
TK015	R0	16519C-263G-315.1C----------
TK016	C4a2	16167T-16171G-16223T-16298C-16327T-16344T-16357C-16519C-47A-73G-249DEL-263G-309.1C-315.1C-489C-
TK017	U4	16356C-16362C-16519C-73G-195C-263G-315.1C-499A-----
TK018	U1b	16111T-16214A-16249C-16327T-16519C-73G-146C-152C-263G-285T-315.1C--
TK019	R0	16311C-16519C-263G-309.1C-315.1C--------
TK020	J1d	16069T-16126C-16193T-16519C-73G-152C-263G-295T-309.1C-315.1C-462T-489C-573.1C
TK021	I1	16129A-16223T-16311C-16391A-16519C-73G-199C-204C-250C-263G-315.1C-455.1T-573.1C-573.2C-573.3C-573.4C

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK022	HV0	16129R-16257T-16298C-16519C-72C-263G-309.1C-315.1C-----
TK023	G2a	16169T-16223T-16227G-16262T-16278T-16294T-16318G-16362C-16526A-73G-152Y-185A-189G
TK024	H5	16304C-150T-263G-309.1C-309.2C-315.1C-456T-523DEL-524DEL----
TK025	I1	16129A-16223T-16264T-16311C-16319A-16362C-16391A-16519C-73G-199C-204C-250C-263G-315.1C-455.1T-573.1C
TK026	U3	16092C-16256T-16311C-16343G-73G-150T-263G-315.1C-----
TK027	R0	16172C-16293G-93G-263G-315.1C--------
TK028	R0	16067T-16260T-16261T-56.1A-263G-315.1C-482C-523DEL-524DEL----
TK029	R6	16093C-16129A-16179T-16227R-16245T-16266T-16278T-16362C-16519C-73G-195C-246C-263G-315.1C-524.1A-524.2C
TK030	R0	16093C-197G-263G-309.1C-315.1C-524.1A-524.2C------
TK031	R0a	16126C-16168T-16266T-16362C-60.1T-64DEL-66T-263G-309.1C-315.1C-523DEL-524DEL-
TK032	T2c1b	16126C-16292T-16294Y-16519C-73G-146C-152C-263G-279C-309.1C-315.1C-523DEL-524DEL
TK033	U5a1	16192T-16256T-16264T-16270T-16399G-73G-263G-315.1C-----
TK034	U3	16343G-73G-150T-263G-309.1C-315.1C-523DEL-524DEL-----
TK035	R0	16189C-16519C-153R-204C-263G-309.1C-309.2C-315.1C-----
TK037	T1b	16126C-16163G-16189C-16243C-16294T-16519C-73G-263G-309.1C-315.1C-524.1A-524.2C-524.3A-524.4C--
TK038	R0	16519C-263G-309.1C-315.1C---------
TK039	R0	16126C-16192T-263G-315.1C---------
TK040	R0	151T-152C-263G-309.1C-315.1C-482C-523DEL-524DEL-----

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK042	R0	16220G-16519C-195C-263G-309.1C-309.2C-315.1C------
TK043	R0	16051G-16312G-16519C-195C-263G-309.1C-315.1C------
TK044	F1b	16129A-16174T-16182C-16183C-16189C-16232A-16249C-16304C-16519C-73G-152C-249DEL-263G-309.1C-309.2C-309.3C
TK045	J1b	16069T-16126C-16145A-16222T-16261T-16519C-73G-143A-263G-295T-315.1C-462T-489C
TK046	U7	16239T-16309G-16318T-16519C-10C-73G-146C-152C-153G-263G-315.1C-523DEL-524DEL
TK047	D	16223T-16362C-16526A-73G-263G-298T-315.1C-489C-523DEL-524DEL---
TK048	U1a1	16161C-16182C-16183C-16189C-16249C-16288C-73G-263G-285T-310C-315DEL-385G-523DEL-524DEL--
TK049	R0	16172C-73G-263G-309.1C-315.1C--------
TK051	H1a	16162G-16519C-73G-263G-309.1C-309.2C-315.1C------
TK052	J1d	16069T-16126C-16153A-16193T-16519C-73G-152C-263G-295T-315.1C-335G-462T-489C
TK053	U1a1	16182C-16183C-16189C-16249C-73G-263G-285T-309.1C-309.2C-315.1C-385G-523DEL-524DEL
TK054	T2	16126C-16140C-16189C-16294T-16311C-16519C-73G-146C-195C-263G-309.1C-309.2C-315.1C-524.1A-524.2C-
TK055	K1a	16224C-16246T-16311C-16519C-73G-152C-263G-315.1C-497T-524.1A-524.2C--
TK056	R0	16093C-16519C-152C-263G-315.1C--------
TK058	U4b2a	16136C-16278T-16356C-16519C-73G-195C-263G-309.1C-315.1C-499A-524.1A-524.2C-
TK060	U7	16168Y-16309G-16318T-16519C-73G-152C-153G-195C-263G-315.1C-523DEL-524DEL-
TK061	H5	16301T-16304C-263G-309.1C-315.1C-456T-------
TK062	R0	16129A-16145A-152C-195C-263G-309.1C-309.2C-315.1C-----

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK063	J2b1a	16069T-16126C-16193T-16278T-16311C-73G-150T-152C-263G-295T-309.1C-315.1C-489C
TK064	T2	16126C-16294T-16519C-73G-263G-309.1C-315.1C------
TK065	W6	16192T-16223T-16292T-16325C-16519C-73G-189G-194T-195C-200G-204C-207A-263G-295T-309.1C-315.1C
TK066	N1c	16111T-16201T-16223T-16265G-16291T-16325C-16519C-73G-189G-195C-204C-207A-210G-256T-263G-315.1C
TK066		256T-263G-315.1C----------
TK067	R0	16218T-16519C-263G-309.1C-315.1C--------
TK068	N1c	16201T-16223T-16265G-16519C-73G-189G-195C-204C-207A-210G-263G-315.1C-482C
TK069	J2b	16069T-16126C-16193T-73G-150T-152C-185A-263G-295T-315.1C-489C-524.1A-524.2C
TK070	R0	16166DEL-16519C-151Y-152C-263G-315.1C-------
TK071	R0	16189C-199C-263G-315.1C-523DEL-524DEL-------
TK072	K1a	16224C-16311C-16519C-73G-263G-309.1C-315.1C-497T-524.1A-524.2C-524.3A-524.4C-
TK073	R0	16519C-143A-228A-263G-309.1C-315.1C-------
TK074	I5a	16086C-16129A-16148T-16223T-16311C-16391A-16519C-73G-199C-204C-250C-263G-315.1C-573.1C-573.2C-573.3C
TK075	Z1a	16129A-16185T-16223T-16224C-16260T-16298C-16519C-73G-151T-152C-249DEL-263G-309.1C-309.2C-315.1C-489C
TK076	R0a	16093C-16126C-16183C-16189C-16193.1C-16224C-16266T-16362C-58C-60.1T-64T-263G-309.1C
TK077	J1b1b1	16069T-16126C-16145A-16261T-16519C-73G-146C-152C-182T-263G-271T-295T-315.1C-524DEL
TK079	U3	16093Y-16168T-16343G-73G-150T-183G-263G-315.1C-----
TK080	N1b	16145A-16176A-16223T-16390A-16519C-73G-152C-263G-309.1C-315.1C-523DEL-524DEL-

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK081	T2	16051G-16126C-16294T-16296T-16519C-73G-263G-315.1C-----
TK083	J2a	16069T-16126C-16231C-73G-150T-195C-263G-295T-315.1C-489C-524.1A-524.2C-524.3A
TK084	H5	16172C-16304C-16375T-16519C-309.1C-315.1C-456T------
TK085	R0	16038G-16311C-263G-309.1C-309.2C-315.1C-513A------
TK086	R0	16354T-16399G-194T-263G-309.1C-315.1C-------
TK087	H5	16304C-16519C-263G-309.1C-309.2C-315.1C-456T-513A-----
TK088	U3	16093C-16311C-16343G-73G-150T-152C-263G-315.1C-523DEL-524DEL---
TK089	T2b	16126C-16189C-16258C-16294T-16296T-16304C-16309G-16519C-73G-263G-309.1C-315.1C-573.1C
TK090	U5b	16189C-16192T-16270T-73G-150T-263G-309.1C-315.1C-499A----
TK091	R0	16311C-16527T-263G-315.1C---------
TK092	R0	16129A-152C-195C-263G-309.1C-309.2C-315.1C------
TK093	T2c	16126C-16292T-16294T-16519C-73G-263G-315.1C-573.1C-573.2C-573.3C-573.4C-573.5C-
TK094	H11a2	16092C-16140C-16265G-16293G-16311C-195C-263G-315.1C-----
TK095	H5	16256T-16304C-263G-309.1C-315.1C-456T-------
TK096	R0	195C-263G-315.1C-456T-523DEL-524DEL-------
TK097	K1a	16189C-16224C-16311C-16519C-73G-263G-315.1C-497T-----
TK098	R	16051G-16172C-16183C-16189C-16234T-16294T-16519C-73G-152C-199C-309.1C-309.2C-315.1C
TK100	K	16224C-16311C-16519C-73G-146C-152C-263G-309.1C-315.1C----

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK101	U3b1a	16086C-16343G-73G-150T-263G-315.1C-------
TK102	T1a	16126C-16163G-16186T-16189C-16294T-16519C-73G-263G-309.1C-315.1C---
TK103	R0	16242T-16356C-263G-309.1C-309.2C-315.1C-524.1A-524.2C-524.3A-524.4C---
TK104	R0	263G-309.1C-315.1C----------
TK105	T2	16126C-16294T-16519C-73G-195C-263G-309.1C-315.1C-----
TK106	X2	16183C-16189C-16193.1C-16223T-16248T-16278T-16519C-73G-153G-195C-263G-309.1C-315.1C
TK107	D	16223T-16362C-16519C-73G-263G-309.1C-309.2C-315.1C-489C----
TK108	R0	16189C-16259T-16263C-263G-309.1C-315.1C-------
TK109	Z1a	16129A-16185T-16223T-16224C-16260T-16294T-16298C-16519C-73G-151T-152C-249DEL-263G
TK110	T1a	16126C-16163G-16186T-16189C-16294T-16519C-73G-152C-186T-263G-315.1C-471C-
TK111	A4a1	16223T-16249C-16290T-16319A-73G-152C-235G-263G-315.1C-523DEL-524DEL—
TK112	M	16129A-16223T-16311C-16519C-73G-146C-263G-315.1C-489C
TK113	T2c	16126C-16292T-16294T-16519C-73G-263G-309.1C-315.1C-573.1C-573.2C-573.3C
TK114	R0	16519C-263G-309.1C-315.1C
TK115	C4a1	16093C-16129A-16223T-16298C-16327T-16362C-16519C-73G-152C-195C-249DEL-263G-309.1C-315.1C-489C-573.1C
TK116	R0	152C-263G-309.1C-309.2C-315.1C
TK119	R2	16071T-16278T-16519C-16568C-73G-152C-195C-263G-315.1C
TK120	J1b1b1	16069T-16126C-16145A-16184T-16209C-16218T-16258C-16261T-16290T-16295T-16519C-73G-152C
TK121	K1a	16093C-16224C-16311C-16519C-73G-263G-309.1C-315.1C-497T-524.1A-524.2C-524.3A-524.4C-263G-271T-295T-309.1C-315.1C-462T-489C-513A-523DEL-

Table 3.2 (cont).

Sample	Haplogroup	Profile
TK121		524DEL
TK122	U5a1	16192T-16256T-16270T-16355T-16399G-73G-152C-263G-309.1C-315.1C
TK123	R0	16183C-16189C-16265C-16291T-16519C-75A-263G-309.1C-315.1C
TK124	R0	16172C-16293G-93G-263G-315.1C

Table 3.3 Werstern Trace Samples Differences from Cambridge[9].

Sample	Haplogroup	Profile
TK125	K	16224C-16256T-16311C-16519C-73G-146C-152C-195C-263G-309.1C-315.1C-524.1A-524.2C-524.3A-524.4C
TK126	R0	16287T-16519C-152C-263G-309.1C-309.2C-315.1C
TK127	K1a	16224C-16293R-16311C-16519C-73G-195C-263G-315.1C-497T
TK128	R0	16287T-16519C-195C-263G-315.1C
TK129	R0	16287T-16519C-152C-263G-309.1C-309.2C-315.1C
TK130	U5a1b1	16192T-16256T-16270T-16291T-16399G-73G-263G-315.1C
TK131	R0	16209C-16519C-189G-263G-315.1C
TK132	R0	16311C-16390A-131C-263G-309.1C-315.1C
TK133	R0	16284G-16519C-263G-309.1C-315.1C
TK134	R0	16209C-16287T-16519C-189G-263G-315.1C
TK135	H5	16304C-263G-315.1C-456T-523DEL-524DEL
TK136	R0	16104T-16176T-16311C-16519C-263G-315.1C
TK137	U3	16168T-16343G-73G-150T-152C-263G-309.1C-315.1C
TK138	T	16126C-16287T-16294T-16519C-73G-263G-309.1C-309.2C-315.1C

Table 3.3 (cont).

Sample	Haplogroup	Profile
TK139	R0	16325C-16519C-252C-263G-309.1C-309.2C-315.1C
TK140	R0	16209C-16519C-189G-263G-315.1C
TK141	R0	16519C-263G-315.1C
TK142	U2e1	16051G-16129C-16183M-16189C-16193.1C-16256T-16362C-16519C-16524G-73G-152C-217C-263G-309.1C-315.1C-340T
TK143	K	16224C-16256T-16311C-16519C-73G-146C-152C-195C-263G-309.1C-315.1C-524.1A-524.2C-524.3A-524.4C
TK144	R1	16287T-16519C-152C-263G-309.1C-309.2C-315.1C
TK136	R0	16104T-16176T-16311C-16519C-263G-315.1C
TK137	U3	16168T-16343G-73G-150T-152C-263G-309.1C-315.1C
TK138	T	16126C-16287T-16294T-16519C-73G-263G-309.1C-309.2C-315.1C
TK139	R0	16325C-16519C-252C-263G-309.1C-309.2C-315.1C
TK140	R0	16209C-16519C-189G-263G-315.1C
TK141	R0	16519C-263G-315.1C
TK142	U2e1	16051G-16129C-16183M-16189C-16193.1C-16256T-16362C-16519C-16524G-73G-152C-217C-263G-309.1C-315.1C-340T
TK143	K	16224C-16256T-16311C-16519C-73G-146C-152C-195C-263G-309.1C-315.1C-524.1A-524.2C-524.3A-524.4C
TK144	R1	16287T-16519C-152C-263G-309.1C-309.2C-315.1C

Table 3.4 Mediterranean Region.

Haplogroup	Number of people	Percentage
R0	4	33%
T2b	1	8%
H5	1	8%
I5a	1	8%
T1a	1	8%
C4a1	1	8%
C	1	8%
T2c	1	8%
K1a	1	8%

3.3 BLACK SEA REGION OF TURKEY

The haplogroups of the samples from Black Sea region can be seen on following table;

Table 3.5 Black Sea Region.

Haplogroup	Number of people	Percentage
R0	8	33%
K1a	3	13%
R0a	2	8%
J2b	1	4%
U3	1	4%
J1b1b1	1	4%
U1b	1	4%
U5a1	1	4%
R6	1	4%
J2a	1	4%
W6	1	4%
H5	1	4%
U7	1	4%
N1c	1	4%

3.4 AEGEAN REGION OF TURKEY

The frequencies of haplogroups in Aegean Region are on table 3.6.

Table 3.6 Aegean Region.

Haplogroup	Number of people	Percentage
R0	4	31%
A4a1	1	8%
G2a	1	8%
X2	1	8%
U3b1a	1	8%
D	1	8%
T2c	1	8%
A4	1	8%
Z1a	1	8%
R	1	8%

3.5 CENTRAL ANATOLIA REGION OF TURKEY

In Central Anatolia Region, the result of the frequencies of haplogroups are on table 3.7.

Table 3.7 Central Anatolia Region.

Haplogroup	Number of people	Percentage
R0	4	21%
T2	3	16%
J2b1a	1	5%
U5a1	1	5%
HV0	1	5%
U3	1	5%
N1c	1	5%
U1a1	1	5%
I1	1	5%
F1b	1	5%
U4	1	5%
C4a2	1	5%
U5b	1	5%
T1b	1	5%

3.6 SOUTHEAST ANATOLIA OF TURKEY

The results of the region are on table 3.8.

Table 3.8 Southeast Anatolia Region.

Haplogroup	Number of people	Percentage
R0	5	50%
J1b	1	10%
J1d	1	10%
D	1	10%
U5b	1	10%
J1d2	1	10%

3.7 EASTERN ANATOLIA REGION OF TURKEY

Haplogroup frequencies are on table 3.9.

Table 3.9 Eastern Anatolia Region.

Haplogroup	Number of people	Percentages
K1a	2	17%
R0	2	17%
U1a1	1	8%
J1d	1	8%
J2a2a	1	8%
J1b1b1	1	8%
T2c1b	1	8%
H5	1	8%
U7	1	8%
N1b	1	8%

3.8 WESTERN THRACE FROM GREECE

The frequencies of haplogroups in samples from Western Thrace are on table 3.10.

Table 3.10 Western Thrace from Greece.

Haplogroup	Number of people	Percentage
R0	12	60%
K	1	5%
K1a	1	5%
U5a1b1	1	5%
H5	1	5%
U3	1	5%
T	1	5%
U2e1	1	5%
HV0	1	5%

3.9 Amova Results

Results of the amova test can be seen on following table;

Table 3.11(a) Amova Results.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	10	63.255	0.04053 Va	0.72
Within populations	215	1199.284	5.57807 Vb	99.28
Total	225	1262.540	5.61860	

Fixation Index FST : 0.00721

Table 3.11(b) Population pairwise FSTs.

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00000											
2	-0.00574	0.00000										
3	0.03530	0.05187	0.00000									
4	0.00908	0.01441	0.06019	0.00000								
5	-0.00499	0.03198	0.06405	0.01246	0.00000							
6	0.00587	-0.00867	0.06578	-0.01567	0.01876	0.00000						
7	-0.02147	0.01159	0.03545	0.01310	0.00258	0.01876	0.00000					
8	0.00505	-0.00457	0.01582	-0.00190	0.02030	-0.00398	0.00751	0.00000				
9	0.04071	0.09804	0.11758	-0.04654	0.04310	-0.05856	0.04587	0.01275	0.00000			
10	-0.00759	0.00441	0.00285	0.02882	0.02350	0.03145	0.00054	0.00058	0.07564	0.00000		
11	-0.01688	-0.00061	0.01372	0.01296	0.00112	0.00662	-0.01182	-0.00614	0.04080	-0.01322	0.00000	
12	-0.01494	-0.01067	**0.01417**	0.00734	0.00098	-0.00660	-0.00795	-0.00469	**0.02813**	-0.01140	-0.00877	0.00000

Numbers for the populations are as follows: 1- Mediterranean region, 2-Arabic(ethnically), 3- Western thrace, 4-East Anatolia region, 5-Aegean Region, 6-Southeast Anatolia region, 7-Central Anatolia region, 8-Black Sea region, 9-Kurdish(ethnically), 10-Marmara region, 11-Turkish(ethnically), 12-All samples

Table 3.11(c) FST P values.

	1	2	3	4	5	6	7	8	9	10	11	12
1	*											
2	0.48649+-0.0667	*										
3	0.00901+-0.0091	0.14414+-0.0278	*									
4	0.35135+-0.0286	0.30631+-0.0243	0.00000+-0.0000	*								
5	0.61261+-0.0618	0.16216+-0.0379	0.00000+-0.0000	0.22523+-0.0365	*							
6	0.27928+-0.0344	0.45045+-0.0650	0.02703+-0.0139	0.58559+-0.0390	0.20721+-0.0451	*						
7	0.92793+-0.0306	0.28829+-0.0466	0.00000+-0.0000	0.24324+-0.0451	0.35135+-0.0417	0.14414+-0.0337	*					
8	0.28829+-0.0485	0.41441+-0.0454	0.07207+-0.0264	0.37838+-0.0264	0.07207+-0.0227	0.53153+-0.0370	0.20721+-0.0305	*				
9	0.06306+-0.0237	0.06306+-0.0194	0.00000+-0.0000	0.88288+-0.0228	0.09009+-0.0303	0.91892+-0.0266	0.03604+-0.0201	0.30631+-0.0528	*			
10	0.60360+-0.0576	0.37838+-0.0354	0.36036+-0.0525	0.04505+-0.0203	0.06306+-0.0237	0.06306+-0.0139	0.45946+-0.0459	0.39640+-0.0562	0.00000+-0.0000	*		
11	0.93694+-0.0244	0.46847+-0.0504	0.04505+-0.0203	0.14414+-0.0309	0.44144+-0.0344	0.21622+-0.0473	0.96396+-0.0196	0.84685+-0.0434	0.02703+-0.0194	0.99099+-0.0030	*	
12	0.92793+-0.0196	0.63063+-0.0333	**0.02703+-0.0139**	0.23423+-0.0473	0.38739+-0.0490	0.65766+-0.0334	0.88288+-0.0327	0.74775+-0.0408	**0.07207+-0.0227**	0.97297+-0.0125	0.99099+-0.0030	*

Numbers for the populations are as follows: 1- Mediterranean region, 2-Arabic(ethnically), 3- Western thrace, 4-East Anatolia region, 5-Aegean Region, 6-Southeast Anatolia region, 7-Central Anatolia region, 8-Black Sea region, 9-Kurdish(ethnically), 10-Marmara region, 11-Turkish(ethnically), 12-All samples

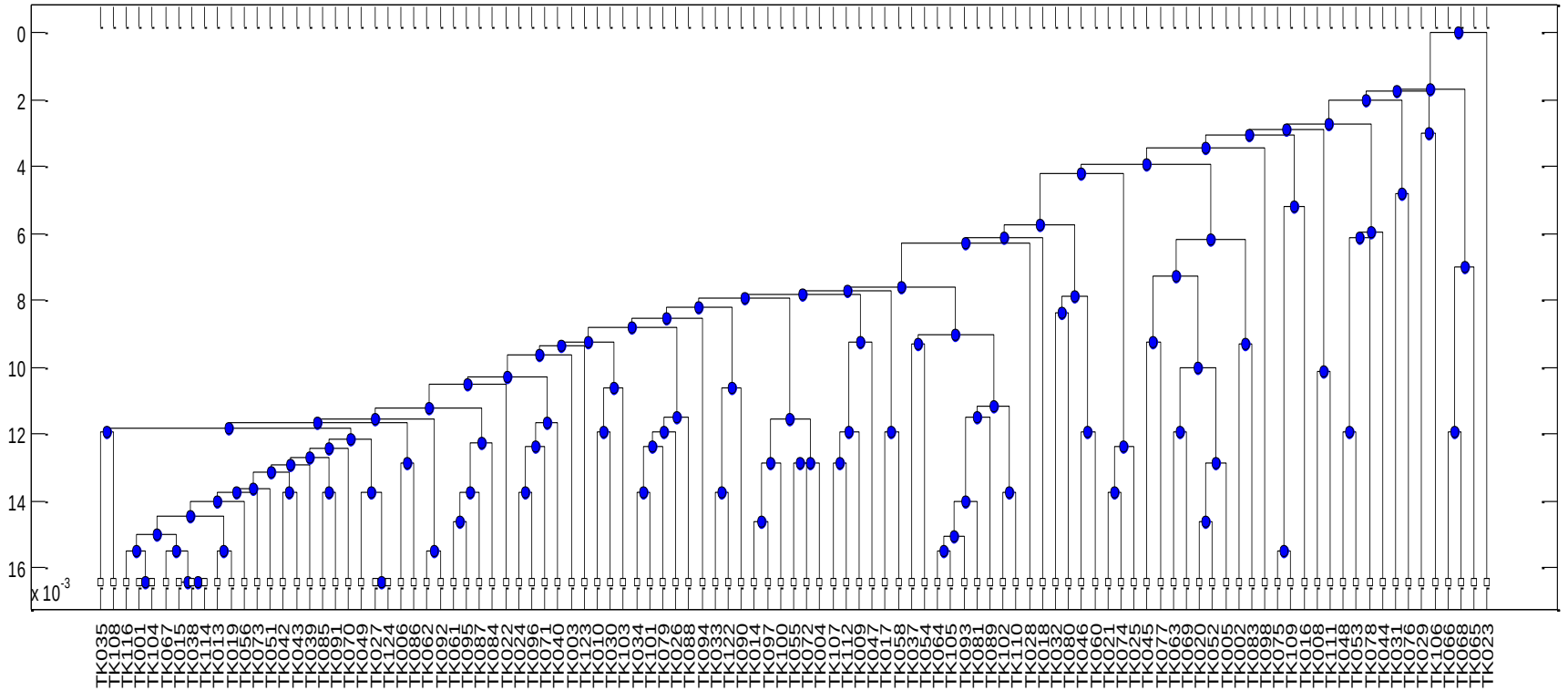

Figure 3.1 Phylogenetic tree for the whole Turkish population.

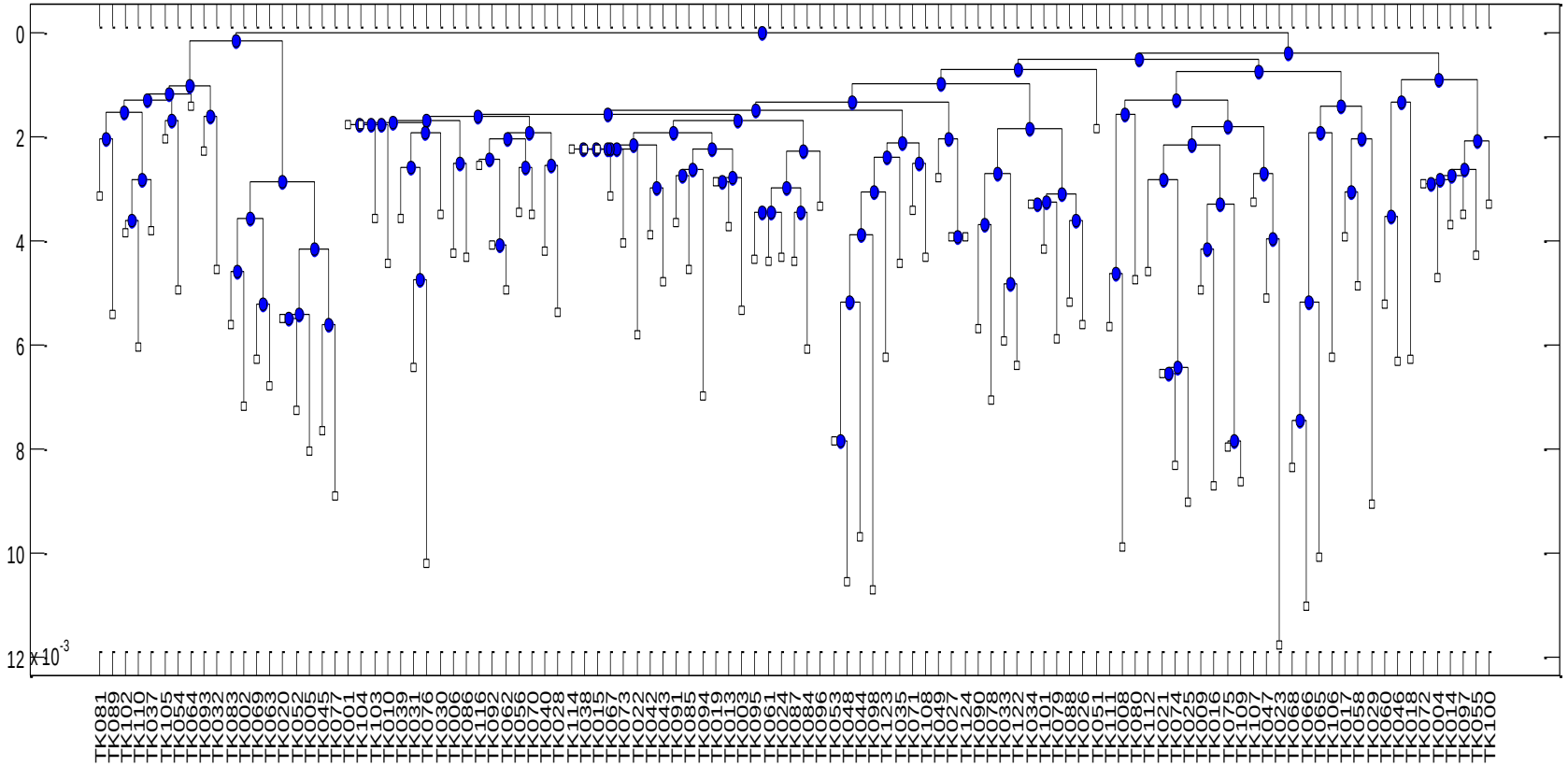

Figure 3.2 Neighbor-joining tree for whole Turkish population.

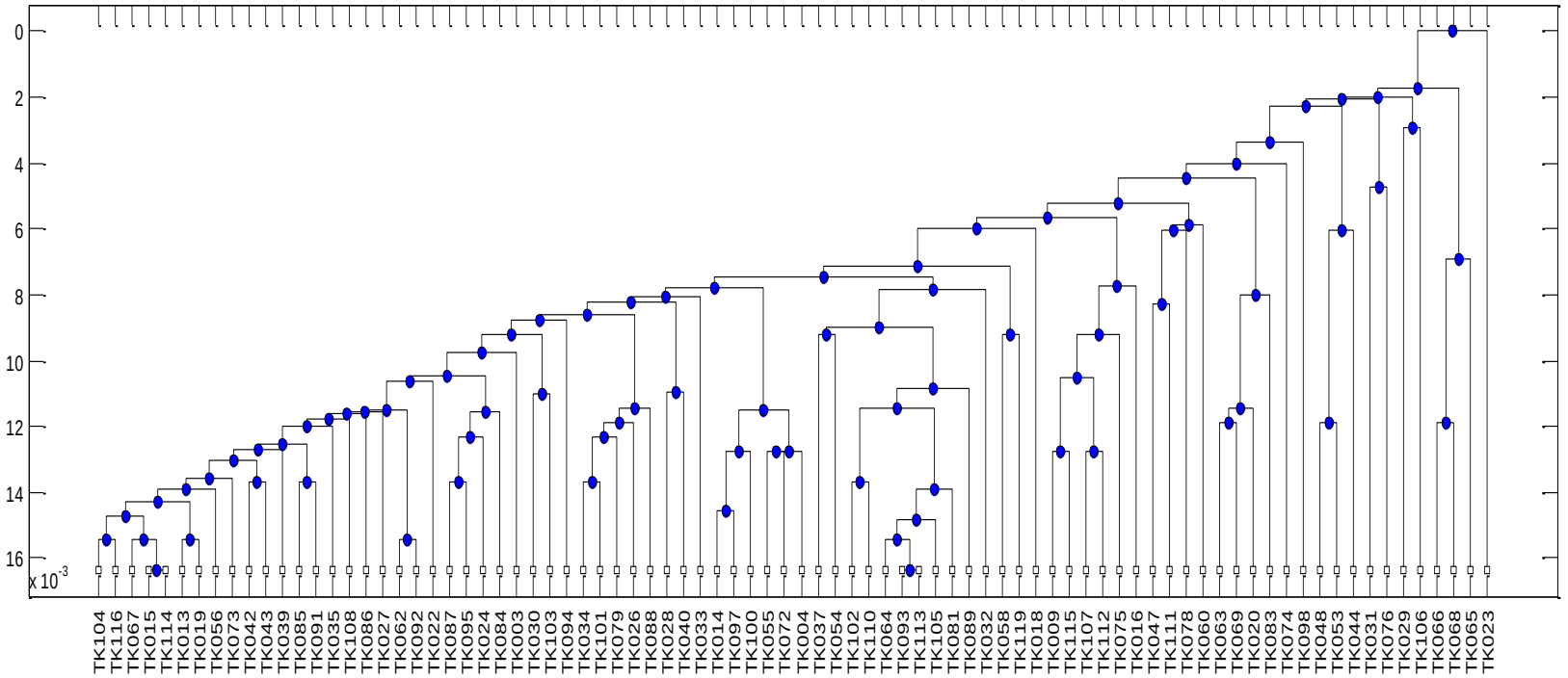

Figure 3.3 Phylogenetic tree for only Turks ethnically.

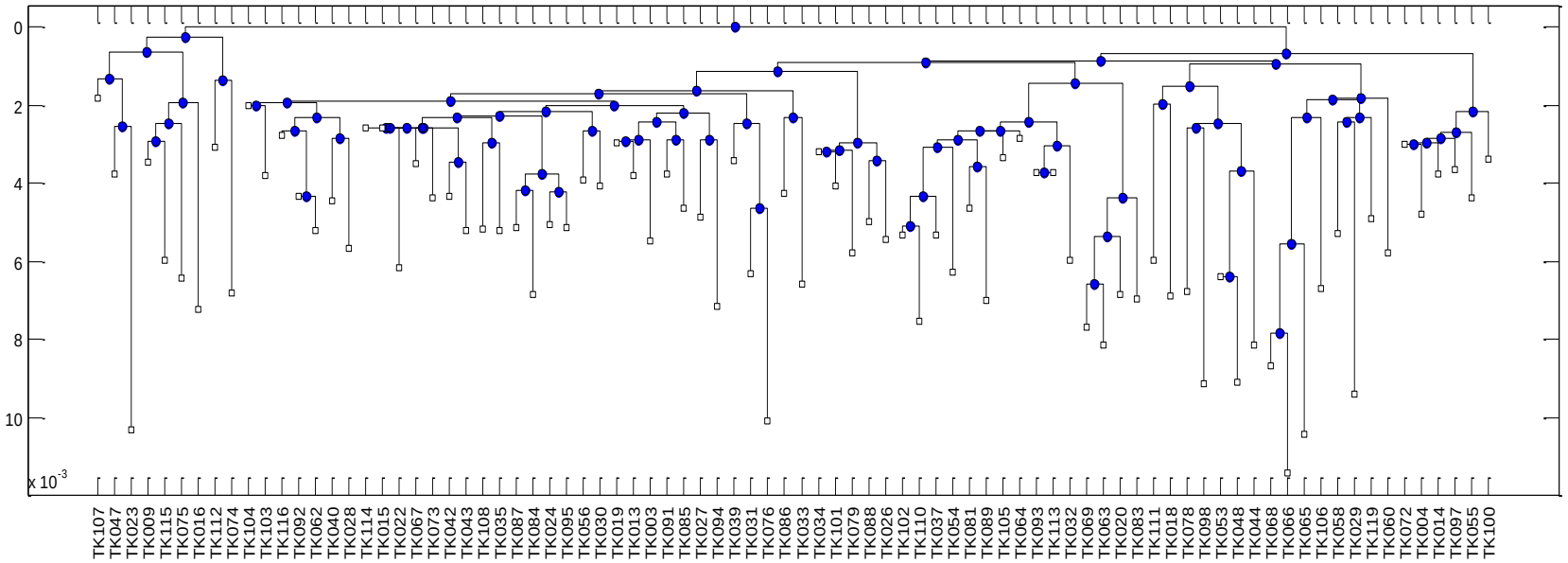

Figure 3.4 Neighbor-joining tree for Turks.

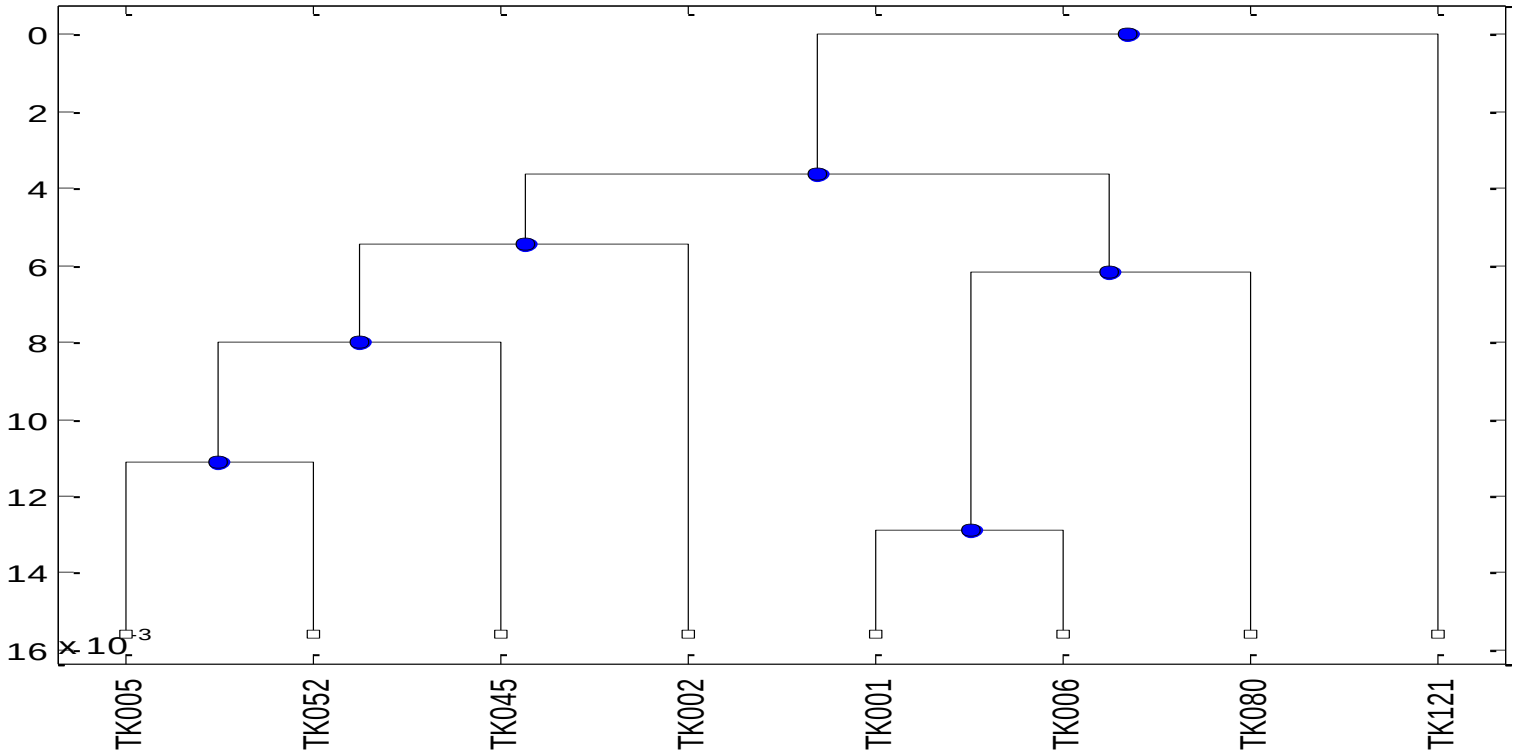

Figure 3.5 Phylogenetic tree for Kurds(ethnically).

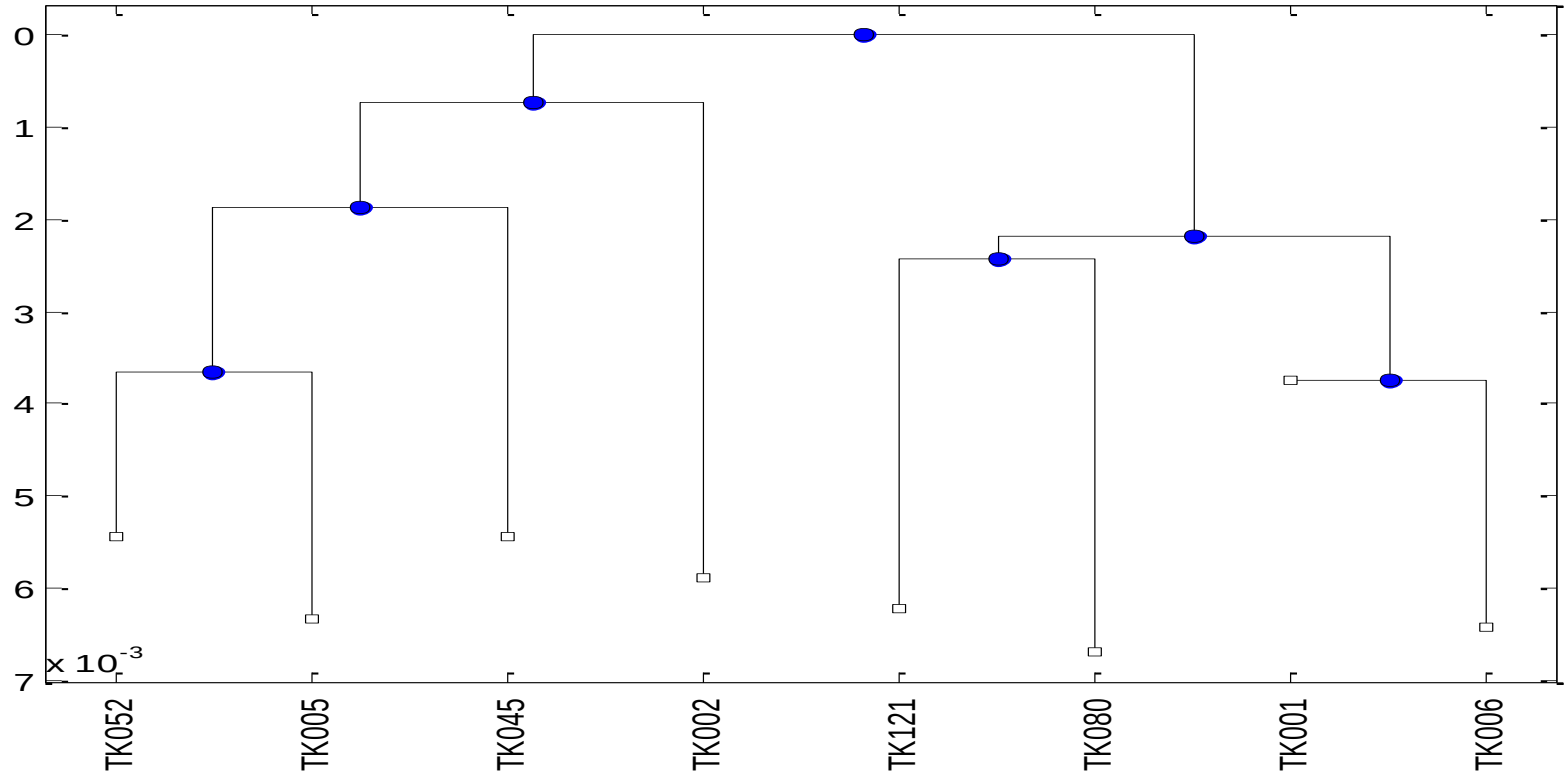

Figure 3.6 Neighbor-joining tree for Kurds.

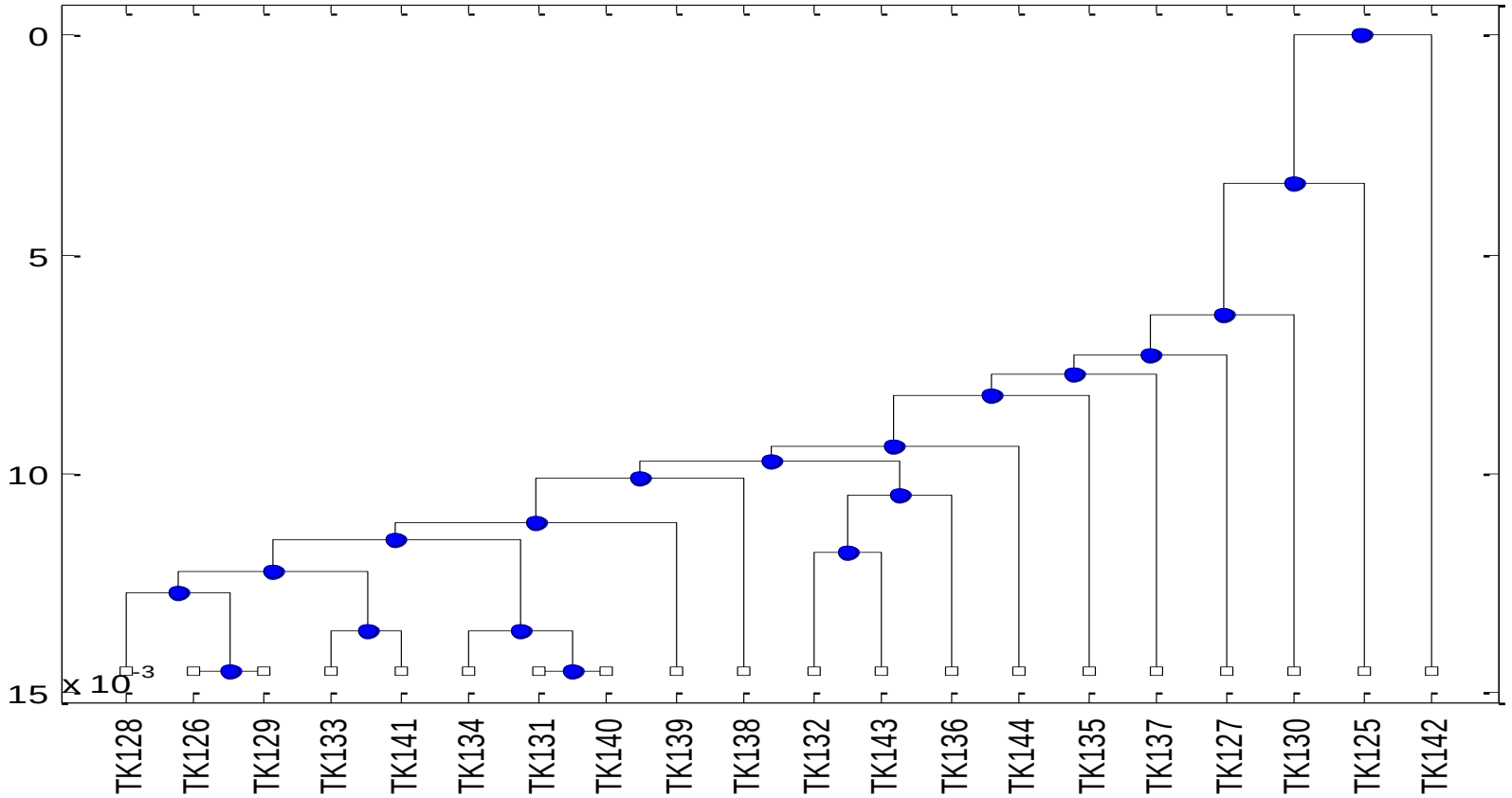

Figure 3.7 Phylogenetic tree for Western Thrace.

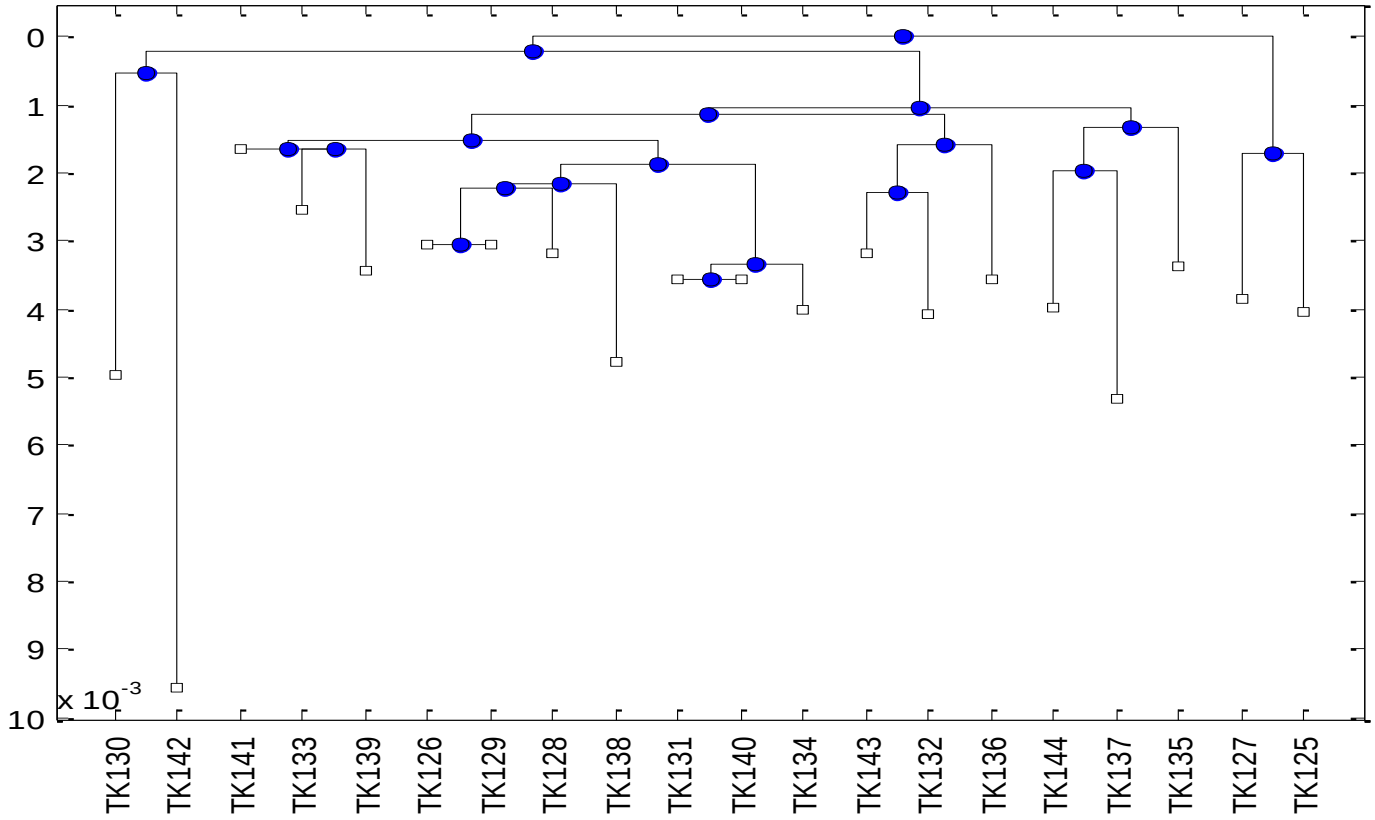

Figure 3.8 Neighbor-joining tree for Western Thrace.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

By using mtDNA analysis, the maternal haplogroup lineages of Turkey were screened in this study. In population genetics, because of sampling is very important to get relevant and more realistic results, extra attention was paid for it. The number and regions of the samples were determined according to population distribution. Limited number of data about mtDNA of Turkey in the literature and the sample collection method of this study makes it more precious and informative for Turkish maternal lineage.

According to frequencies, the most common haplogroup in Turkey is R0 or pre-HV (former name) with the 28,6% frequency. When it is looked in ethnic origin point of view, R0 is again the most common in both Turkish and Kurdish people in Turkey. R0 comes from haplogroup of R which is a sub-haplogroup of macro haplogroup N. Macro-haplogroup N is an Asian mitochondrial haplogroup. Haplogroup R0 is very common in European populations, especially in West Europe countries with 50% frequency in Macedonia [55]. The frequency of R0 declines towards East with 10-30% in the Near East and Central Asia and the Caucasus [56, 57]. The possible origin of R0 is Asia and the time is between 23,600- 54,900 years ago [58]. Most of the Turkish haplogroups are Europian haplogroups and there are also Asian haplogroups in Turkey. So Turkey is a mixture between Europe and Asia in mitochondrial point of view like its geographic location which is a bridge between the two continents.

According to mtDNA haplogroups point of view, Turkey or Anatolia is admixture of populations. There are some migration models which try to explain the occurrence of

this admixture. According to Benedetto et al [59] with studying both mtDNA and Y chromosome data, there have to be continuous migration from Central Asia to Turkey and two dimensional migration between Turkey and Europe in the past (figure 4.1) to make present Turkish genetic structure.

In many researches [50, 51], the most common haplogroup in Turkish people is haplogroup H unlike this research data. Haplogroup H is a sub-haplogroup of R0. To decide whether the haplogroup is H or not, more information is needed. In this study,

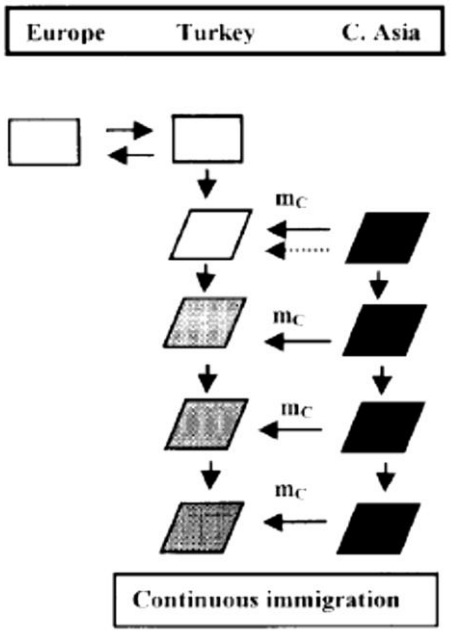

Figure 4.1 Continuous immigration model for Turkey [59].

only control region is sequenced like most of the studies did. Coding region sequence is also needed to go to the tips of the phylogenetic tree. For example, the position of 263 in mtDNA is a defining mutation point for not only H but also R0. In this study, the upper branch chosen to be cautious.

In regional analysis, all of the region's most frequent haplogroup is haplogroup R0 except Eastern Anatolia Region of Turkey. In this region, frequency of haplogroup K1a is as high as frequency of R0. In this region, there are more Kurdish people than Turkish people in ethnic point of view so the difference of frequencies can come from Kurdish people.

The phylogenetic and molecular analyses also support the genetic heterogeneity of Turkish people (figure 3.3, 3.4). Anatolia is a place that has not only mixed cultures as many people say but also mixed genomic material in mitochondrial point of view.

According to amova test results, there is 99,28% variation within the population so it shows that Turkish population has heterogeneous genetic structure in mtDNA (table 3.11(a)).

Population pairwise FSTs and FST P value tables (table 3.11(a,b)) show genetic relations between populations. Both Western Thrace samples and Kurdish samples displayed significant differences from Turkish population. Interestingly, Western Thrace samples close to samples from Marmara region and Kurdish samples close to Southeast Anatolia and East Anatolia regions' samples of the Turkey. On the other hand, Mediterranean region samples and Central Anatolia region samples close to each other.

In addition to samples from Turkey, in this study, 20 samples were studied from Western Thrace of Greece who have Turkish origin. The frequency of R0 is very high in this population which is 60%. They are from Turkish minority. They are closed population in marriage like most of the minorities so it could be the reason for this high number of frequency.

As a conclusion, Turkish population is between Europe and Asia with respect to mitochondrial haplogrouping. Unlike many studies, haplogroup R0 has the highest frequency in Turkish population. And interestingly, it also has highest frequency (60%) in Turkish origin samples from different country. The genetic heterogeneity is very high in Turkish population. According to results, geographical relations effect genetic structure of the populations.

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APPENDIX A

TÜRKİYE POPÜLASYONUNUN MİTOKONDRİAL DNA ANALİZİ

ANKET FORMU

1. Adı Soyadı:

2. Telefon:

3. E-mail:

4. Cinsiyet:

5. Memleket:

6. Doğum yeri:

7. Etnik köken:

8. Doğum yılı:

9. Ailede genetik hastalık var mı?

10. Akraba evliliği durumu?

11. Anne tarafından nineleri

	Adı	Yaşadığı Yer	Yaklaşık Doğumyılı
Anne			
Anneanne			
Nine 1			
Nine 2			
Nine 3			
Nine 4			

Alınan numunenin sadece bu araştırmada kullanılmasına müsaade ediyorum.

İmza Tarih: