ARCHAEOGENOMIC ANALYSIS OF POPULATION GENETIC RELATIONSHIPS AND KINSHIP PATTERNS IN THE SEDENTARY SOCIETIES FROM NEOLITHIC ANATOLIA

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ABSTRACT

ARCHAEOGENOMIC ANALYSIS OF POPULATION GENETIC RELATIONSHIPS AND KINSHIP PATTERNS IN THE SEDENTARY SOCIETIES FROM NEOLITHIC ANATOLIA

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The Neolithic way of life first emerged in the Fertile Crescent (c.10th and early 9th millennium cal BCE) and quickly spread to neighbouring regions such as Central Anatolia and Cyprus, and eventually further westwards. This transition involved to fundamental changes in human lifestyle, with the first emergence of villages during the early Neolithic and the later the growing reliance on farming and herding during the late Neolithic periods. Changes in the social organization of sedentary communities are also hypothesized to have occurred during this period, including changes between early and late Neolithic periods.

Central Anatolia was one of the major regions where these developments took place. Like other regions of early Neolithization, it shows distinctive traditions in the early and late Neolithic settlements in the region. Earlier studies based on small sample sizes from Central Anatolia analyzed genetic relationships among the Neolithic populations in the region. In this study, for the first time, we investigated ancient genomes from Aşıklı Höyük and Çatalhöyük from Central Anatolia, representing early (Aceramic) and late (Ceramic) Neolithic, respectively. We

generated a total of 22 genomes from Aşıklı (n=8) and Çatalhöyük (n=14), and combined these with published genomes from other Anatolian Neolithic sites (Boncuklu, Barcın and Tepecik-Çiftlik).

We first investigated genetic relationships among Anatolian Neolithic groups at both individual- and population-level. We found strong genetic affinity between Aceramic Aşıklı and Boncuklu, supporting the notion that these early Neolithic populations from Central Anatolia may have been part of the same gene pool. Likewise, we observed genetic affinity between Çatalhöyük and other Anatolian Ceramic Neolithic populations (Barcın and Tepecik-Çiftlik). In addition, we identified higher within-population genetic diversity in the Anatolian Ceramic Neolithic populations (Çatalhöyük, Barcın and Tepecik-Çiftlik) compared to those of Aceramic Neolithic (Boncuklu and Aşıklı). Further, our findings based on a larger sample size supported the notion of a possible gene flow from Levant and Iran to Anatolia during the transition from Aceramic to Ceramic Neolithic period, after c.7,500 BCE.

Next, we studied genetic kinship among individuals co-buried within the same structures within Aceramic and Ceramic Neolithic settlements from both Central and Northwest Anatolia, to understand social structures of Neolithic societies in the earlier and later period of Neolithic life in Anatolia. In the two Aceramic Neolithic societies from Central Anatolia, Aşıklı and Boncuklu, we identified close genetic kin-relationships (e.g., first-degree) among co-burials at a high frequency, while the frequency of genetically close relatives was lower among co-buried individuals in Çatalhöyük and Barcın, which represent Ceramic Neolithic societies from Central and Northwest Anatolia, respectively. Our findings supported the notion that genetic kinship patterns among co-buried individuals, who could represent households, might have changed over time during the transition from Aceramic to Ceramic Neolithic in Anatolia.

Keywords: Central Anatolia, Neolithic, Kinship, Genetic Characterization, Ancient Genome

ÖZ

NEOLİTİK ANADOLUDAKİ YERLEŞİK TOPLUMLARDA GENETİK İLİŞKİLERİN VE AKRABALIK ÖRÜNTÜLERİNİN ARKEOGENOMİK ANALİZİ

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İnsanlığın avcı-toplayıcı yaşam tarzından, tarım ve hayvancılığa dayanan yerleşik hayata geçişi Neolitik Dönüşüm olarak adlandırılır ve insanlık tarihindeki önemli değişimlerden biridir. Bu dönüşüm ilk olarak Verimli Hilal bölgesinde ortaya çıkmış (yaklaşık MÖ 10. ve 9. binyıl) ve hızla Orta Anadolu ve Kıbrıs gibi yakın bölgelere, daha sonra da batıya doğru yayılım göstermiştir. Bu noktada, Neolitik geçişin çekirdek bölgelerinden biri olması ve Neolitik yaşam tarzının batıya yayılım rotası üzerinde bulunması sebebiyle Orta Anadolu bölgesi büyük bir öneme sahiptir. Bu çalışmada, Orta Anadolu Neolitiği'nin erken (Çanak-Çömleksiz) ve geç (Çanak-Çömlekli) dönemlerini temsil eden, sırasıyla Aşıklı Höyük ve Çatalhöyük yerleşimlerinden ilk defa antik insan örnekleri çalışıldı. Bu iki yerleşimden toplamda 22 antik genom elde edildi ve karşılaştımalı analizlerde kullanılmak üzere Anadolu Neolitiği'ne ait önceki yıllarda yayınlanmış genomik verilerle birleştirildi. Bu çalışmada Anadolulu Neolitik toplumların genetik yapısı ve akrabalık örüntüleri hem toplum-içi hem de toplumlar-arası düzeyde analiz edildi.

Bu çalışmada ilk olarak Anadolulu Neolitik gruplar arasındaki genetik ilişkileri hem bireyler arası hem de populasyonlar arası karşılaştırmalarla inceledik. Sonuç olarak, Anadolulu erken Neolitik populasyonlarının (Aşıklı ve Boncuklu) genetik olarak birbirine daha yakın olduğunu, Çatalhöyük popülasyonunun ise Anadolulu geç Neolitik dönem gruplarına (Barcın ve Tepecik-Çiftlik) genetik olarak daha benzer olduğunu gözlemledik. Ek olarak, Anadolulu geç Neolitik dönem popülasyonlarının (Çatalhöyük, Barcın ve Tepecik-Çiftlik) grup içi genetik çeşitliliklerinin Anadolulu erken Neolitik popülasyonlara (Aşıklı ve Boncuklu) göre daha yüksek olduğunu bulguladık. Ayrıca, Anadolu'dan daha fazla genetik veri kullanarak ortaya çıkan sonuçlarımız, yaklaşık olarak MÖ 7,500 sonrasında, Levant ve İran Neolitik toplumlarından Anadolu'ya gen akışı olduğu düşüncesini desteklemektedir.

İkinci olarak, Anadolu'da erken ve geç Neolitik dönem yerleşimlerinde, aynı bina içine gömülü olan bireyler arasındaki genetik akrabalık ilişkilerini inceledik. Sonuç olarak, Anadolu'dan her iki erken Neolitik dönem topluluğunda da, birbirine yakın gömülen bireyler arasında yüksek oranda yakın genetik akrabalık ilişkisi (birinci derece) olduğunu; diğer taraftan geç Neolitik dönem topluluklarında ise yakın genetik akrabalık sıklığının azaldığını bulguladık. Sonuçlarımız, en azından Orta Anadolu erken Neolitik topluluklarında, birlikte gömülen ev halkının yakın akraba olabileceğine işaret etmektedir. Ayrıca, bulgularımız Anadolu'da Çanak-Çömleksiz (erken) Neolitik dönemden Çanak-Çömlekli (geç) Neolitik döneme geçiş sırasında hanehalkını temsil ettiği düşünülen birlikte gömülü bireyler arasındaki genetik akrabalık örüntülerinin zamanla değişmiş olabileceği görüşünü desteklemektedir.

Anahtar Kelimeler: Orta Anadolu, Neolitik, Akrabalık, Genetik Karakterizasyon, Antik Genom

To My Family and Health

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ABBREVIATIONS

aDNA: ancient DNA

BCE: Before Common Era

bp: base pair

CRS: Cambridge Reference Sequences

DNA: deoxyribonucleic acid

dNTP: deoxyribonucleotide triphosphates

UV: Ultraviolet

RNAse: Ribonuclease

dH2O: distilled water

nm: nanometer

HYA: Synthetic Hydroxyapatite powder

mg: miligram

EDTA: Ethylenediaminetetraacetic acid

M: Molar

pH: Power of Hydrogen

EB: Elution Buffer

HPG: haplogroup

PCA: Principal Component Analysis

min: minute

ml: milliliter

mm: millimeter

mtDNA: mitochondrial DNA

PCR: Polymerase Chain Reaction

rpm: revolutions per minute

s: second

μ l: microliter

cal: calibration

PCA: principal component analysis

SNP: single nucleotide polymorphism

PMD: post mortem damage

ROH: Runs of homozygosity

θ: Kinship coefficient

CHAPTER 1

GENERAL INTRODUCTION

1.1 Ancient DNA: an overview

Ancient DNA (aDNA) is a type of DNA obtained from archaeological remains and museum samples (e.g., tooth, bone, hair, tissue and seed) (Pääbo et al., 2004). In the last four decades, aDNA techniques were developed and used to address various questions about phylogeny, genetic history of populations, origins of domestication, diet and behaviour (Gilbert et al., 2005; Pääbo, 2014; Pääbo et al., 2004).

The first aDNA analysis was on a sample from Quagga (an extinct subspecies of zebra) (Higuchi et al., 1984). Svante Pääbo's 1985 study, where he examined a sample from 2400-year-old mummy, was the first aDNA study on humans (Pääbo, 2005). However, it was later understood that the aDNA sequence obtained from the sample was caused by modern human DNA contamination (Knapp et al., 2015; Del Pozzo et al., 1989). Results of these earlier studies highlighted the fact that contamination of aDNA samples by environmental and modern human DNA is highly likely. Thus, since that time, extreme wet-lab precautions for aDNA analysis have been taken to avoid possible contaminations (Hofreiter et al., 2001; Gilbert et al., 2005; Pääbo et al., 2004; Ottoni et al., 2011). Later, a number of computational methods have also been developed to confirm authenticity of the aDNA sequences (Skoglund et al., 2012; Knapp et al., 2015 (see Materials and Methods for details).

Besides modern human DNA contamination, there are other technical challenges to study aDNA due to the nature of aDNA molecule. First, because aDNA is mixed with large amounts of soil bacteria DNA, it can be obtained in low quantity. Therefore, this property of aDNA makes it highly prone to environmental

contamination. Second, aDNA is degraded into fragments and that size of the fragments decreases over time. Thus, aDNA sequences are relatively shorter compared to those of modern DNA (Knapp et al., 2015; Sawyer et al., 2012; Pääbo et al., 2004; Hofreiter et al., 2001; Pääbo, 1989). The third challenge is the molecular damages in the aDNA, which occur shortly after the death and are called postmortem damage (PMD). The most common type is deamination of cytosine at 5'-ends of the aDNA fragments, which generates uracil and is read as thymine when sequenced. Thus, postmortem damages lead to transitions from C to T at 5' end and G to A at 3' end (on the opposite strand) of the reads. These patterns can be used as an indicator of the authenticity of aDNA sequences (Skoglund et al., 2012; Briggs et al., 2007; Olivieri et al., 2010; Helgason et al., 2007; Paabo et al., 2004).

Due to these properties (DNA degradation into short fragments, low DNA quantity and also difficulties in confirming authenticity of the obtained sequences) Sanger sequencing-based methods of targeted aDNA fragment amplification is always challenging. However, in the last decade, these difficulties have been partly overcome with the technical innovations such as high-throughput sequencing techniques (i.e., shotgun sequencing). These sequencing technologies, which can generate hundreds of millions of short reads (i.e., Illumina HiSeq platforms) per run, have transformed aDNA analysis. In addition, a number of methods based on optimization of DNA extraction and library preparation have been also developed to obtain sufficient authentic aDNA from ancient samples. For example, hybridization capture-based approaches (e.g., whole-genome capture, targeted SNP-capture) appear as a promising way forward (Carpenter et al., 2013).

1.1.1 Ancient DNA as a tool for archaeogenomic studies

The field of ancient DNA, especially studies on human remains is a rapidly growing research area thanks to the technical developments mentioned above. Ancient genome analysis is today a highly efficient and direct method to study

demographic history and genetic characterization of past populations including determination of maternal and paternal lineages, direction and source of human migrations, admixture among ancient populations, inferring familial relationships within a population and also estimating phenotypic traits (e.g., hair color and blood type) of ancient individuals (Hofreiter et al., 2001; Slatkin and Racimo, 2016). In addition, aDNA data obtained from archaeological human samples allows to resolve important questions about anthropology and evolutionary biology, including natural selection (i.e., variants of the *LCT* gene associated with lactose tolerance) (Allentoft et al., 2015) and social structures within the past communities, e.g., to understand genetic kinship structure among the individuals from a mass grave (Schroeder et al., 2019). aDNA is also used to understand animal and plant domestication that was one of the major steps in human history, allowing food production and causing significant changes in lifestyle (Hofreiter et al., 2001; Zeder et al., 2006).

aDNA techniques have thus been recently used to better understand prehistoric demographic events that accompanied the Neolithic Transition, which was one of the fundamental changes in human evolutionary history. In this respect, Anatolia becomes quite important with its geographical location and valuable collections of archaeological remains from different prehistoric sites and periods to address the questions about genetic history of past populations and as well as major demographic events in the region.

1.2 The Neolithic Transition

One of the major changes in human history was the Neolithic Transition from hunting-gathering to sedentary lifestyle with practicing farming and herding. The Neolithic way of life first appeared in the Fertile Crescent, in the Near East (Düring, 2010; Price and Bar-Yosef, 2011). The Fertile Crescent, including Southern Levant, Upper Mesopotamia and Zagros, was the primary region of Neolithic Transition in the Near East and this transition occurred roughly between

10th and early 9th millennium cal BCE (Baird, 2012; Hillman, 1996). Later, with the expansion of Neolithic lifeway among communities living in the Fertile Crescent, this sedentary lifestyle spread out of the region, for example, to Central Anatolia roughly later 9th millennium cal BCE (Baird, 2012; Özbaşaran, 2011), and then through the West Anatolia into Europe after ~7,000 cal BCE (Özdoğan, 2011; Düring, 2010).

The Neolithic Transition consists of a number of development stages and these gradual changes in sedentary lifestyle occurred over time. It is suggested that the initial phase emerged in the southern Levant region. Communities living in the late Epipalaeolithic period of the southern Levant, who are known as Natufians, showed the earliest evidence of initial sedentism c.12,500-9,500 cal BCE. After the Younger Dryas (c. 10,800-9,500 BC) which was a cold and dry period for those regions, people started to practise sedentary lifestyle and agriculture (Byrd, 2005; Düring, 2010). This led to the emergence of sedentary hunter-gatherer groups which are known as the Pre-Pottery Neolithic (PPN) and shows gradual changes through farming and domestication processes. The Pre-Pottery Neolithic (PPN) period consists of two sub-periods: (i) PPNA (Pre-Pottery-Neolithic-A) is between c.9,500-8,500 BCE and known as the initial period of sedentary lifestyle; (ii) PPNB (Pre-Pottery-Neolithic-B) is between c.8,500-7,000 BCE, and shows initial domestication and development of farming practices. The PPNB period is known as Aceramic Neolithic in Anatolia. The later period of Neolithic is named as the Pottery Neolithic (PN) (Ceramic Neolithic in Anatolia) (c.7,000-5,500); during this period farming societies have emerged who were practising full-scale farming and food production (Özdoğan, 2011; Düring, 2010). These gradual changes on human lifestyle during the Neolithic Transition also allowed a number of shifts in social organizations of communities such as emergence of villages, increase in population sizes and in technological innovations, appearance of complex social institutions and major changes in nutrition and health (Larsen, 1995; Düring, 2010). For instance, with the advance of Neolithic Transition, people started to produce their own food by domesticating plants and animals as mentioned above, and later lived

with intentionally domesticated animals (e.g., sheep, goat, cattle, horse) in settlements, including other animals (i.e., mice and dog) which might have facilitated the spread of diseases (Düring, 2010). Another effect of the Neolithic Transition was about the changes on the social networks such that human groups began to live together as larger societies and these societies were in interaction with each other (Byrd, 1994, 2005; Düring, 2010). The gradual shifts from the initial to later phases of Neolithic lifestyle were also observed in the social organization of communities and the arrangements of settlements (Byrd, 1994, 2005; Baird et al., 2017; Özbaşaran et al., 2018).

In addition, archaeological evidences suggest a number of major regions of Neolithic Transition (e.g., southern Levant, Upper Mesopotamia and Central Anatolia) and each of those represents its own cultural and historical developments during the phases of Neolithic life. Central Anatolia is one of these core regions and shows its distinctive traditions during the Neolithic Transition with gradual changes from early to later period (Düring, 2010; Özbaşaran, 2011; Baird, 2012).

1.2.1 The case of Central Anatolia

Central Anatolia is a quite important region due to being one of the core regions of Neolithic Transition and being on the route of Neolithic spread into westwards and Europe. Central Anatolia is known with its distinctive features observed during a sedentary lifestyle (Baird, 2012). The earliest documented example of sedentism in Central Anatolia is observed in Pinarbaşı between c.9,000-7,800 BCE, which is a rock shelter and dated to the late Epipaleolithic period (occupation date c.14,000-6,000 BCE) (Baird, 2012). Later, an early evidence of domestication (i.e., domestic cereals) was found in Boncuklu Höyük (Konya plain) and Aşıklı Höyük (Cappadocia region), which represent the early (Aceramic) Neolithic in Central Anatolia (Baird, 2012; Özdoğan, 2011; Düring, 2010). The later period of Neolithic in Central Anatolia (Ceramic Neolithic), after c.7,500 BCE, was represented by fully practiced farming and sedentism including plant and animal domestication

that led to emergence of farming societies (e.g., Çatalhöyük, Tepecik-Çiftlik Höyük) (Özdoğan, 2011; Düring, 2010). Çatalhöyük is a larger size Ceramic Neolithic site located in Central Anatolia. Central Anatolian Aceramic Neolithic, Aşıklı (later levels of the site), and Ceramic Neolithic, Çatalhöyük, both represent dense-clustered settlement structure (Özbaşaran, 2011). In addition, Central Anatolian Neolithic settlements show a house tradition by representing building continuity where a new house was built on top of a previous one using the same wall structure (Düring, 2010; Özbaşaran, 2011; Baird, 2012). Although, this building pattern was observed in Neolithic settlements from neighbouring regions, it has been maintained for a long time in Central Anatolia. In addition, these aforementioned Aceramic (Boncuklu ve Aşıklı Höyük) and Ceramic (Çatalhöyük) Neolithic sites represent such examples of the Central Anatolian model (Özbaşaran, 2011). Therefore, these characteristics of Central Anatolian Neolithic settlements make the region quite important to study Neolithic Transition.

There are two hypotheses about the Neolithic Transition in Central Anatolia; the first is the colonization of the region by farmers from the Fertile Crescent, and the second suggests that Central Anatolian farmers were the continuation of the local late Epipalaeolithic groups living in the region who adopted sedentary lifeway and farming. The second hypothesis is well-accepted based on the archaeological observations such as: (i) cultural features of the Central Anatolian Neolithic differs from the those of Fertile Crescent Neolithic, (ii) the cultural continuity was found between the Central Anatolian early Neolithic and the local late Epipalaeolithic groups in the region (Baird, 2012). For instance, microliths that are common in the chipped stone traditions in the late Epipalaeolithic period, were also found in Boncuklu Höyük and Aşıklı Höyük which represent the early Neolithic in Central Anatolia and are dated to approximately 8,500-7,500 BCE (Düring, 2010; Özbaşaran, 2011; Baird, 2012). These microliths were already out of use at 8,500 BCE in the Fertile Crescent and were not found after 7,500 BCE in the Central Anatolia (e.g., at Canhasan and Musular) (Düring, 2010). Thus, this observation supports the notion that the microlith tradition in the Central Anatolian Aceramic

Neolithic sites was derived from those of local late Epipalaeolithic groups in the region (Düring, 2010; Baird, 2012). These lines of archaeological evidence suggest that the local sedentary foragers from c.9th millennium BCE adopted sedentary lifeways and played a role as a part of the earliest farming communities in the Central Anatolia region (Baird, 2012). Another interesting point of Central Anatolian early Neolithic is that the sub-oval building pattern which was observed in both Boncuklu Höyük and earliest levels of Aşıklı Höyük, are different than those in the contemporaneus PPNB sites from the southeast Anatolia, (i.e., Cafer on the Upper Euphrates) (Baird, 2012; Düring, 2010; Cauvin et al., 1999).

At this point, ancient DNA analysis becomes a valuable approach to test the hypotheses about spread of farming into Anatolia and westwards as well as about genetic relationships among the early Holocene populations from Anatolia (e.g., Pınarbaşı, Boncuklu, Aşıklı and Çatalhöyük).

1.2.2 Ancient DNA studies about the Neolithic Transition

One of the major questions about the Neolithic Transition addressed by aDNA studies has been whether farming arrived in Europe by cultural transmission or migration; in other words, by cultural diffusion (spread of ideas) or demic diffusion (spread of people) (Cavalli-Sforza, 1997; Özdoğan, 2011).

This was first addressed about a decade ago by ancient mitochondrial DNA (mtDNA) analyses, which investigated questions about demographic and genetic history of past populations during the Neolithic Transition in Europe. MtDNA is a molecular marker to study genetic structure, admixture and demographic patterns of the past populations. In a mtDNA study, it was demonstrated that the Skandinavian farmers were genetically distinct from the Mesolithic groups in the same region (Malmström et al., 2009). Another study on ancient mtDNA showed that the first farmers of Europe had genetic affinity to modern-day Near Eastern

populations (Haak et al., 2010). Results of these studies have supported the idea of farming reached Europe via migration.

However, because mtDNA analysis only traces the maternal lineage, these studies might lead to different demographic inference about the history of populations compared to inferences from genomic data (Reich et al., 2010). Thus, after the innovation of next generation sequencing technologies, aDNA studies reanalysed the same question using ancient genome-wide data. Archaeogenomic analyses of the last decade confirmed that the first farmers of Europe were genetically distinct from European Mesolithic groups, while these European farming groups were similar to each other (Lazaridis et al., 2014; Skoglund et al., 2012, 2014). These studies also revealed that the gene pool of modern-day Europeans was built up by the genetic admixture of European Neolithic and Mesolithic groups. In addition, European farmers had genetic affinity to modern-day populations from southern Europe (e.g., Italy and Spain) especially to Sardinians (Skoglund et al., 2014). The overall results supported the idea of spread of farming to Europe by a migration wave probably routed in the Near East.

Recently, aDNA studies on Anatolian human remains published genetic profiles of the late Neolithic and Chalcolithic individuals from Northwest Anatolia (from Barcın Höyük and Kumtepe, respectively) (Mathieson et al., 2015; Omrak et al., 2016). These studies from Neolithic Anatolia mainly focused on one important question in archaeology, whether farming reached Europe by migration or cultural transmission from the Near Eastern regions, including Anatolian plateau. Findings of these studies showed genetic similarity between the Northwestern Neolithic Anatolians and the European farmers (Mathieson et al., 2015; Hofmanová et al., 2016; Omrak et al., 2016). In addition, Hofmanová and colleagues (2016) suggested that Central European early farmers were mixture of the local hunter-gatherers and the Neolithic groups who migrated from Southwest Asia including Northwest Anatolia and the West Aegean coast, but genetically more similar to the latter (Hofmanová et al., 2016).

In another study from Anatolia, ancient genomes from Boncuklu Höyük and Tepecik-Çiftlik Höyük, representing early and late Neolithic periods in Central Anatolia, respectively, were analysed (Kılınç et al., 2016). Kılınç et al.'s (2016) study also indicated a genetic affinity between Central Anatolian Neolithic groups and European farmers using archaeogenomic analysis (Kılınç et al., 2016). Moreover, another recent study, combining Anatolian data with published genomic data of Neolithic groups from Levant and Iran, showed more genetic affinity between European farmers and Anatolian Neolithic individuals compared to those of Levantine and Iranian Neolithic (Kılınç et al., 2017).

At the same time, recent aDNA studies from Anatolia have revealed a number of genetic characteristics of Anatolian Neolithic populations, especially in Central Anatolia. For example, Kılınç et al. (2016) showed a low genetic diversity in the early Neolithic population from Central Anatolia (Boncuklu), while the genetic diversity is higher in the population from later Neolithic period in the region (Tepecik-Çiftlik). Another recent study examining one ancient individual from Pınarbaşı, representing late Epipaleolithic (occupation date c.14,000-6,000 BCE) in Central Anatolia, and five individuals from Boncuklu, representing early Neolithic in Central Anatolia, found genetic continuity between early Neolithic and local late Epipaleolithic groups in the region (Feldman et al. 2019). Overall these results suggest that the Central Anatolian Neolithic groups are genetically distinct from Near Eastern Neolithic individuals based on population genetic analysis using ancient genomic data (Kılınç et al., 2016, 2017; Feldman et al., 2019).

To date, various questions about the Neolithic Transition in SW Asia have been examined using aDNA analysis, but there are still a number of questions that have remained unanswered: whether there was genetic continuity among Aceramic and Ceramic Neolithic settlements in Central Anatolia, Aşıklı, Boncuklu and Çatalhöyük, which represent cultural and material similarities; how were the genetic characteristics and social structures of the Anatolian Neolithic populations, especially in Central Anatolia, and which changed over time, including genetic kinship structures within Neolithic societies. In this thesis, questions about genetic

relationships among and within Anatolian Neolithic populations have been studied using ancient genomes obtained from a number of Anatolian Neolithic sites. These sites are briefly described in the following section.

1.3 Anatolian Neolithic sites studied in this thesis

1.3.1 Aşıklı Höyük

Aşıklı Höyük is one of the most important early (Aceramic) Neolithic settlements in Central Anatolia, located in the Aksaray province within the Volcanic Cappadocia region (Figure 1.1). The occupation of the site is dated to 9,000-7,400 BCE and shows evidence of the early emergence of fully sedentary communities between 8,400-7,400 BCE (Özbaşaran, 2011; Özbaşaran, 2012; Özbaşaran, 2013; Stiner et al., 2014). Archaeological evidence indicates that Aşıklı people lived continually for approximately 1000 years in the settlement. This uninterrupted occupation could be observed by gradual changes on building shapes and architecture as well as in social and technological lifeways. The oldest levels (Levels 4 and 5) of the site, dated approximately 9,000-8,200 cal BCE, are associated with the sub-oval building structure; this period extending from Level 5 to 4 represents the emergence of Pre-Pottery (Aceramic) Neolithic (PPN) in Central Anatolia (Özbaşaran, 2011). There is continuity in the use of space in Levels 3-4, but the structure of the buildings are different with respect to plan and layout from those in the later layer (Level 2). Specifically, the sub-oval structures exist together with rectangular buildings in the early levels, while densely built rectangular buildings are common in Level 2 (Özbaşaran, 2011). These gradual changes in building style were also observed in other Near East Neolithic sites (Stiner et al., 2014). In the Level 2 buildings were reconstructed on top of the old house structures. This continuity on the building is named as *"house series"* and this culture was also observed particularly in another Aceramic Neolithic settlement in Central Anatolia, Boncuklu Höyük (Konya plain). In addition, this type of house

building tradition was most common in Çatalhöyük (Konya plain), which is a Ceramic Neolithic site in Central Anatolia and is dated to a later period than Aşıklı Höyük (Düring, 2010). Moreover, Aşıklı Höyük shows a "clustered neighbourhood settlement" structure, which is also observed in Çatalhöyük. The clustered neighbourhood structure consists of three characteristics; lack of streets, absence of doors to enter houses and aforementioned the "*house series"* pattern (Özbaşaran, 2011; Düring, 2010).

Although Aşıklı Höyük is one of the earliest Neolithic settlements in Central Anatolia, it is dated to a later period compared to early Neolithic settlements from Levant and Mesopotamia (c.9,500-9,000 BCE). Aşıklı people were cultivating domestic crops, in addition to large scale hunting-gathering, and it is known that a group of the domesticated plants found in Aşıklı Höyük appear to have been derived from the Fertile Crescent (Düring, 2010). Therefore, it has been questioned in archaeology whether Aşıklı population migrated from the Fertile Crescent or was one of the hunther-gatherer groups that adopted sedentary lifestyle and farming (Düring, 2010). Interaction between those two regions was also observed from obsidian evidence (Düring, 2010). On the other hand, Aşıklı Höyük represents distinct cultural patterns from Neolithic sites of the Fertile Crescent. These include the common use of microlith industries, settlement structures and presence of public buildings. Thus, distinct characteristics of Aşıklı Höyük appear inconsistent with the colonisation model, which assumes that the Aşıklı people were immigrants from the Fertile Crescent and did not change culturally (Düring, 2010).

1.3.2 Boncuklu Höyük

Boncuklu Höyük is another important Aceramic (early) Neolithic site from Central Anatolia, located in the Konya plain between Pınarbaşı and Çatalhöyük sites (Figure 1.1). The occupation of the site is dated to 8,300-7,600 cal BCE based on radiocarbon dating (Baird, 2012; Baird et al., 2012, 2018). At Boncuklu widespread evidence of use of wild resources (e.g. wild cattle, boar, fish and

wetland birds, also nuts and fruits) was found along with small-scale cultivation of wheat, lentils and peas (Baird, 2012; Baird et al., 2018). The microlithic industry at Boncuklu was similar to local late Epipalaeolithic (Pınarbaşı) industries. This suggests a continuity between earlier local Epipalaeolithic and early Neolithics in the region (Baird, 2012; Baird et al., 2018), which was supported by a recent aDNA study (Feldman et al., 2019).

The Boncuklu Höyük site consists of a series of sub-oval buildings with mudbrick walls and these buildings show domestic use. These houses were typically reconstructed over the same location for multiple generations and this structure was also characteristic of neighbouring sites at 10th-7th millennia cal BCE both in Central Anatolia, such as Aşıklı in the Aceramic Neolithic period (Özbaşaran, 2012) and Çatalhöyük in the Ceramic Neolithic period (Hodder, 2006), and in the Levant, such as the PPNA Jericho (Byrd, 2005) and PPNB Tell Halula (Kuijt et al., 2011). This is suggested as a symbol of continuity among small tight-knit households related to these building structures, and that perhaps the households living in those building sequences were genetically and/or socially related (Baird et al., 2017).

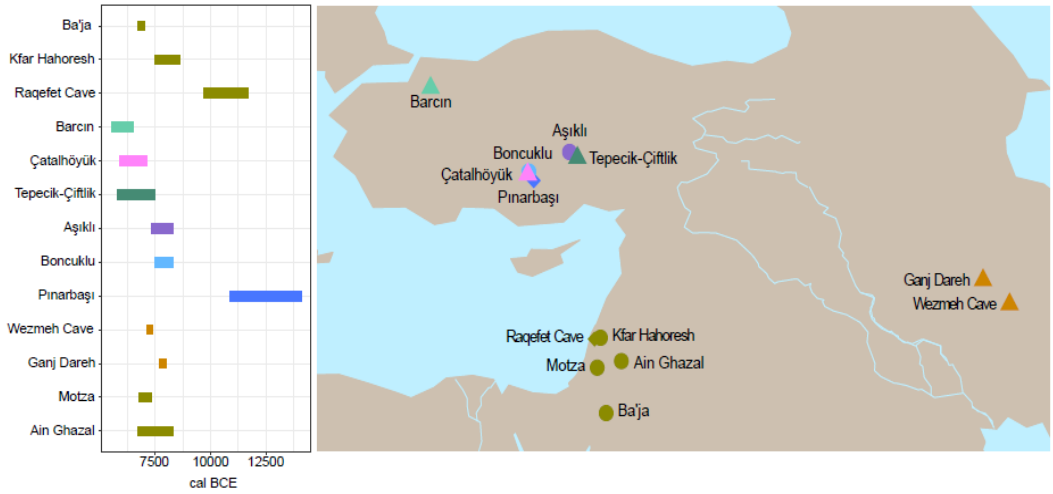


Figure 1.1 Geographic map of the early Holocene sites from Anatolia, Levant and Iran with the occupation dates of the sites.

1.3.3 Çatalhöyük

Çatalhöyük is located on the Konya Plain, 9 km south of the Boncuklu Höyük, in Central Anatolia (Figure 1.1). There are two separate mounds in the site; the larger East Mound and the smaller West Mound. The East Mound contains the Ceramic Neolithic period settlement and is dated to c.7,100-5,950 cal BCE (Bayliss et al., 2015). The West Mound includes the Early Chalcolithic period settlement and shows occupation evidence until the mid-6th millennium BCE (Orton et al., 2018). The Neolithic East Mound consists of mudbrick domestic building structures with external spaces and these spaces were used for daily activities (e.g., domestic refuse, penning deposit) (Hodder and Cessford, 2004). The buildings in the site were mostly located next to each other and many structures were bound to each other by adjacent roofs (Düring, 2010). At Çatalhöyük public structures were not found; instead, it is thought that individual houses were not only used for domestic activities, but also used as socio-cultural spaces (Hodder and Cessford, 2004). Çatalhöyük is a larger size Ceramic Neolithic site and the population size range is estimated as 3,000-8,000 (Hodder and Cessford, 2004; Hodder, 2006; Düring, 2010). The evidence for domesticated cereals as well as the domesticated cattle, sheep and goats were widely found at the site (Bogaard et al., 2013; Russell et al., 2013).

1.3.4 Barcın Höyük

Barcın Höyük is located in the centre of the Yenişehir Plain, Bursa, in northwestern Turkey (Figure 1.1). The Neolithic levels of the site showed an uninterrupted occupation from 6,600 to 6,000 cal BCE (Gerritsen and Özbal, 2019). The Neolithic period at Barcın Höyük consists of seven subphases; from early to late, from VIe (the earliest level) to VIa, respectively, with Level VIe is known to represent one of the earliest farming communities in the Marmara Region (Gerritsen and Özbal, 2019). The architecture of the Neolithic period shows

continuous occupation and rectangular buildings that were made of wood and mud walls (Gerritsen and Özbal, 2019). At Barcın Höyük, houses were in rows and were surrounded by open courtyard areas in which people carried out outdoor activities. The main components of subsistence economy were agriculture with domesticated plants and animal husbandry with sheep and cattle as herding animals (Balcı et al., 2019). It is suggested that the first settlers arrived around 6,600 cal BCE, were farmers and agriculture and animal herding were brought by them (Gerritsen and Özbal, 2013; Gerritsen et al., 2013). Furthermore, evidence of dairy products was found based on organic remains analyses on pottery in Barcın Höyük (Özbal et al., 2013).

1.3.5 Tepecik-Çiftlik Höyük

Tepecik-Çiftlik Höyük is located in the Melendiz/Çiftlik Plain on the southwest of the Volcanic Cappadocia region, in Central Anatolia (Figure 1.1). The site shows an uninterrupted occupation from the Aceramic Neolithic Period to the early Chalcolithic Period, approximately between 7,500-5,800 cal BCE (Bıçakçı et al., 2012). Architectural remains were not found in the the Aceramic Neolithic; while the Ceramic Neolithic levels contain single large structures and wide open areas. But in the later level, which is the final level of Neolithic, different structures emerged in the open areas of the site. In the Aceramic Neolithic levels, evidence of agriculture and animal breeding was found, while hunting and gathering still continued (Bıçakçı et al., 2012).

1.4 Aims of This Thesis

This thesis uses ancient genome analysis to address the following goals:

1. To determine whether the early Neolithic populations in Central Anatolia, Boncuklu Höyük from the Konya Plain and Aşıklı Höyük from the Cappadocia region, had a strong genetic affinity to each other, given that

archaeological material culture evidence indicates similarities between two settlements. In addition, I wish to test the hypothesis that Central Anatolian early Neolithic populations were descendants of local late Epipaleolithic groups in the region (Chapter 2).

2. To investigate the genetic relationship between the populations from Aceramic and Ceramic Neolithic periods in Anatolia, especially in Central Anatolia among Boncuklu, Aşıklı and Çatalhöyük (Chapter 2).
3. To infer regional migration dynamics during the transition from the Aceramic to Ceramic Neolithic periods in Anatolia. Here I will specifically study the genetic affinities among early and late Neolithic communities from Central Anatolia and other groups from the Near East (e.g., Levant and Iran) (Chapter 6).
4. To understand whether genetic kin-relationships among potential household members may have been an important factor in the social organization of early and/or late Neolithic societies in Anatolia, especially in Central Anatolia, by studying genetic kinship levels and pedigree relationships among individuals buried within buildings (Chapter 6).
5. To test the hypothesis that genetic kinship patterns among household members have changed through time, especially during the transition from the Aceramic to Ceramic Neolithic periods in Anatolia (Chapter 6).

POPULATION GENETIC CHARACTERIZATION OF CENTRAL ANATOLIAN NEOLITHIC COMMUNITIES: FIRST INSIGHTS INTO AŞIKLI HÖYÜK AND ÇATALHÖYÜK

CHAPTER 2

INTRODUCTION

The adoption of sedentism, farming, stock breeding, and herding by previously hunter-gatherer groups, termed the Neolithic Transition, was one of the main social changes in human history (Price and Bar-Yosef, 2011; Düring, 2010). This transition witnessed many first-time events such as emergence of villages and increase in population sizes, technological developments, appearance of complex social institutions and changes in nutrition and health. All of these major changes inevitably affected humans' lives and social organizations (Price and Bar-Yosef, 2011; Düring, 2010).

The Neolithic Transition first started in the Fertile Cresent, including southern Levant, Upper Mesopotamia and Zagros around the 10th and early 9th millennium cal BCE (Baird, 2012; Price and Bar-Yosef, 2011). This new lifeway spread into neighbouring regions, including Central Anatolia (c.9th millennium cal BCE) (Baird 2012; Özbaşaran 2011), and later into westward and Europe (c.7,000 BCE) (Özdoğan, 2011; Düring, 2010). Herein, the Anatolian Plateau, especially Central Anatolia, plays a key role as a part of the core regions of Neolithic Transition and also due to being partly on the route of the Neolithic spread westward. The Central Anatolia region is important to study Neolithic Transition because of its long-standing traditions and thus, the region becomes unique. For example, (i) house sequence tradition which was observed as the continuity between the old and new buildings, (ii) clustered neighbourhood settlement patterns (e.g., in Çatalhöyük and

at the later levels of Aşıklı Höyük), and (iii) continuity in the microlith tradition between the local late Epipaleolithic and early Neolithic in the region (Baird, 2012; Özbaşaran, 2011) (see General Introduction for details). In this respect, Central Anatolia hosted important Neolithic sites, including two Aceramic (early) Neolithic sites, Boncuklu Höyük in Konya Plain (8,300-7,600 cal BCE) and Aşıklı Höyük in Cappadocia region (8,400-7,400 cal BCE), and two Ceramic Neolithic sites, Çatalhöyük in Konya Plain (c.7,100-5,950 cal BCE) and Tepecik-Çiftlik Höyük in Cappadocia region (c.7,500-5,800 cal BCE) (see General Introduction for details of the sites). Thus, characteristics of the Central Anatolian Neolithic settlements make the region quite important to study questions about the transition from hunting-gathering to sedentary lifestyle within Anatolia.

To date, a number of archaeogenomic studies from Anatolia have studied questions about the Neolithization of Central Anatolia and spread of Neolithic lifeway westward, eventually into Europe (see General Introduction for details), using ancient genomes (Kılınç et al., 2016, 2017; Feldman et al., 2019). In a recent study about Central Anatolian Neolithic, for the first time, human remains from Aceramic Neolithic, Boncuklu (c. 8,300-7,500 cal BCE, in Konya plain), and Ceramic Neolithic, Tepecik-Çiftlik (c. 7,500-5,800 cal BCE, in Cappadocia region), were examined using ancient DNA and genome analyses (Kılınç et al., 2016). Kılınç et al.'s (2016) study showed a genetic affinity between Anatolian and European Neolithic populations relative to European hunter-gatherers. Later, a number of studies also revealed genetic affinity between European farming groups and Neolithic populations from Anatolia compared to Neolithic groups from Levant and Iran (Kılınç et al., 2017; Feldman et al., 2019).

At the same time, genetic characteristics of the Anatolian Neolithic populations, especially in Central Anatolia, were also investigated in these studies using ancient genome analysis (Kılınç et al., 2016, 2017; Feldman et al., 2019). Kılınç et al.'s (2016) study indicated that within-population genetic diversity was lower in Boncuklu, representing early Neolithic from Central Anatolia, relative to those of Tepecik-Çiftlik and Barcın populations (later Neolithic period) from Central and

Northwest Anatolia, respectively, as well as to European farmer groups. In addition, this study suggested small effective population size in Boncuklu using runs of homozygosity (ROH) analysis, resembling to European hunter-gatherers (Kılınç et al., 2016). These results of Kılınç et al.'s (2016) study supported the notion of small sedentary villages in Central Anatolian early Neolithic which was suggested by archaeological evidence (Baird, 2012).

In addition, the question of genetic continuity between local late Epipaleolithic and early Neolithic populations in Central Anatolia was also addressed in the aforementioned studies. Feldman et al.'s (2019) study showed that early Neolithic populations in Central Anatolia (Boncuklu) might have descended from the local late Epipaleolithic groups (Pınarbaşı) in the region, which was earlier suggested in Kılınç et al. (2017). Interestingly, Hofmanová et al.'s (2016) study addressed a similar question with Feldman et al.'s (2019) study for Central Europe, whether early European farmers descended from local hunter-gatherers or migrated from Southwest Asia into Europe, using ancient genomic data from Northwest Anatolian Neolithic and Greek early Neolithic. In contrast to the case of genetic continuity in Central Anatolia, Hofmanová et al. (2016) showed that early farmers from Europe had a strong genetic affinity to Neolithic populations from Anatolia and Greece rather than local hunter-gatherer groups, suggesting the migration of farmers from Southwest Asia to Europe.

Furthermore, to understand genetic relationships between Neolithic populations from Central Anatolia and Near East (Levant and Iran), archaeogenomic studies from Anatolia used a larger dataset combining published ancient genomes from those two regions (Kılınç et al., 2017; Feldman et al., 2019). These recent studies suggested lack of close genetic affinity between early Neolithic population (Boncuklu) from Central Anatolia and Neolithic groups from Levant and Iran. On the other hand, Kılınç et al.'s (2017) study showed a genetic affinity between the Levantine/Iranian farmers and the Anatolian late Neolithic populations (Barcın and Tepecik-Çiftlik) compared to those of Anatolian early Neolithic, suggesting a gene

flow from Levant and Iran to Anatolia during the transition from Aceramic to Ceramic Neolithic period in Anatolia (after c.mid-8th millennium BCE).

However, in these previous studies data was limited due to the small number of samples such that they used ancient genomic data obtained from only one Aceramic (Boncuklu) and one Ceramic (Tepecik-Çiftlik) Neolithic site from Central Anatolia. Although those two settlements represent Central Anatolian Neolithic, there is a spatial distance between Boncuklu and Tepecik-Çiftlik, that are located in Konya Plain and Cappodocia region, respectively. Thus, by using a limited dataset it is difficult to understand whether the genetic difference between the two Neolithic periods of the region is temporal or spatial. Here, we address the same questions about (i) the genetic relationships between Neolithic groups from Central Anatolia, and from the Levant and Iran, (ii) the genetic continuity between local late Epipaleolithic and early Neolithic populations in Central Anatolia. For this, we used newly generated genomes from two sites, Aşıklı Höyük and Çatalhöyük, representing Aceramic and Ceramic Neolithic in Central Anatolia, respectively, and combined these with the published genomic data from Boncuklu Höyük, Tepecik-Çiftlik Höyük and Barcın Höyük. In this study, we also aimed to understand genetic relationships among Anatolian Neolithic populations, especially in the Central Anatolia region, including genomic data from Aşıklı Höyük and Çatalhöyük.

In addition, it is known from the archaeological evidences that there are similarities and also differences among these three Central Anatolian Neolithic settlements, Aşıklı Höyük, Boncuklu Höyük and Çatalhöyük, with respect to cultural traditions and building structures. For instance, all three settlements present the building sequences structure named as "*house series*", which is known as a characteristic of Central Anatolian Neolithic. Another interesting point is that Boncuklu and Çatalhöyük are spatially closer to each other and also show cultural similarity such as lack of public buildings and division of house floors as "*dirty*" and "*clean*" areas, while public buildings were observed in Aşıklı Höyük (Baird, 2012; Özbaşaran, 2011; Hodder, 2007). On the other hand, Çatalhöyük and the later

levels of Aşıklı Höyük show clustered building patterns (Düring, 2005; Baird, 2012; Özbaşaran, 2011; Hodder, 2007). Meanwhile, Boncuklu and the earliest levels of Aşıklı share a similar building tradition which is the sub-oval building pattern (Özbaşaran, 2011; Baird, 2012). Because of these similarities and dissimilarities, archaeologists working in the region have been questioning whether (i) there was any genetic relationship among Neolithic populations from these three settlements (Boncuklu, Aşıklı and Çatalhöyük); and (ii) Çatalhöyük is genetically continuation of one of those Aceramic Neolithic sites (Boncuklu and/or Aşıklı). Thus, in this study we also tested genetic continuity among Neolithic populations from Boncuklu Höyük, Aşıklı Höyük and Çatalhöyük using ancient genomic data, specifically asking whether Çatalhöyük may show more genetic similarity to either Boncuklu Höyük or Aşıklı Höyük.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of samples

In this study, bone and teeth samples from 30 Aşıklı and six Çatalhöyük individuals were studied using aDNA analysis. Aşıklı samples were obtained from anthropologists of the excavation site (Ömür Dilek Erdal and Yılmaz Selim Erdal) including different type of bone samples (e.g., petrous bones) from 30 individuals. Six Çatalhöyük samples were selected based on their physical preservation levels among more than 400 Çatalhöyük samples collected by archaeological team of the excavation site.

After prescreening at low depth sequencing, eight Aşıklı and six Çatalhöyük individuals were selected for deep sequencing and used for further analysis. In addition, eight individuals from Çatalhöyük that were initially sequenced as part of wider study (Yaka et al., under review in Curr.Biol.), were also added to the dataset and combined with the genomic data generated in this study. DNA extraction, library preparation and sequencing of the aformentioned additional eight genomes from Çatalhöyük were performed by Ayça Doğu and Damla Kaptan (n=7) from our group (METU), and by Maciej Chyleński (n=1) at Adam Mickiewicz University (Poznan, Poland). Detailed archaeological information about the studied individuals is given in the Appendix Table B.2 and in the aforementioned manuscript under review.

3.2 Sample preparation and DNA extraction

Sample preparation, DNA extraction and library preparation from Aşıklı Höyük (n=30) and Çatalhöyük (n=6) samples were performed in a dedicated ancient DNA

facility at Middle East Technical University (Ankara, Turkey). All the extensive precautions to avoid contamination were taken during the grinding, extraction and library preparation processes. Tools and surfaces were regularly cleaned with bleach, RNAse AWAY and long exposures (>30 min) with UV light in the aDNA laboratory. Samples were decontaminated and prepared following the protocols described in Yaka et al. (2018): The outer surface of the samples were carefully removed and discarded using either a single use blade or Dremel drill with a single use cutting disc. Each sample was irradiated with UV-light for 30 minutes from two sides in a cross-linker. The samples were ground into fine powder using freezer mill (no. 6750). DNA was extracted using 120-200 mg bone powder from each sample following silica spin column method with slight modifications combining two protocols, Ottoni et al. (2011) and Dabney et al. (2013). Two negative extraction controls were used for every 8-10 samples.

3.3 Library preparation and initial sequencing

DNA extracts were used to build shotgun sequencing libraries. We prepared double stranded DNA libraries using 20 µl of extract with blunt-end ligation method following the protocol described in Meyer et al. (2010) and Günther et al. (2015). Library amplification of each sample was carried out in six replicates using specific single-indexing primers. The total volume of each reaction was 25 µl and it contained the following mixture in final concentrations: 1X AmpliTaq Gold Buffer, 250 nM of each dNTP, 2.5 mM MgCl2, 200 nM each of the IS4 primers and an indexed-P7 primer, 2.5U AmpliTaq Gold (Life Technologies) and 3 µl DNA library. The PCR cycle conditions were 94 C° for 10 min followed by 10-14 cycles of 94 C° for 30 sec, 60 C° for 30 sec, 72 C° for 45 sec, and a final extension at 72 C° for 10 min. After the amplification of six library replicates seperately, they were pooled and purified with AMPure XP beads (Agencourt). Then, purified libraries were profiled on a 2100 Bioanalyzer using the High Sensitivity DNA Kit (Agilent Technologies) for the DNA quantification and quality control. Negative controls

prepared at each step were also included during the library preparation and amplification steps, and these negative controls were inspected by Real Time PCR (BioRad) and did not show sign of contamination. Libraries were pooled in equimolar concentrations (final vol of 10 nM total pool) for the initial sequencing (prescreening) on Illumina HiSeq X and HiSeq 2500 platforms at SciLife, Stockholm, with 150 and 100 bp paired-end reads on single or several lanes. In order to obtain high-coverage genomic data, libraries that yielded sufficient reads from the initial screening process were then sequenced deeper in pools of three to six libraries per lane.

3.4 Whole genome in-solution capture and resequencing

To increase the depth of coverage, we enriched the best libraries of 11 individuals (Appendix Table B.1) for human genomic DNA using the MYbaits Human Whole Genome Capture Kit (African baits) from Arbor Biosciences (Ann Arbor, MI) following the manufacturer's instructions (http://www.mycroarray.com/pdf/MYbaits-manual-v4.pdf). Here, African baits were chosen to avoid biasing for European alleles. The captured libraries were amplified for 16–19 cycles with primers IS5 and IS6 (Kılınç et al. 2016), using either Herculase II Fusion DNA Polymerase (Agilent Technologies) or KAPA HiFi HotStart Polymerase (Kapa Biosystems). Enriched libraries were purified with AMPure XP beads and profiled on the Bioanalyzer 2100 using the High Sensitivity DNA kit (Agilent Technologies). Purified libraries were pooled in equimolar concentrations for prescreening on the Illumina HiseqX and Hiseq 2500 platforms at SciLife, Stockholm, with 150 and 100 bp paired-end reads on single or multiple lanes. Human DNA endogenous proportions increased between 1.2- and 27-fold (median 8×) after the enrichment (Appendix Table B.1).

3.5 Sequence read processing and alignment

We processed sequencing data from each library following the methods described in (Kılınç et al., 2016). First, the residual adapter sequences in *FASTQ* files were trimmed and the paired-end sequencing reads were merged using *MergeReadsFastQ_cc.py* (Kircher, 2012), with an overlap of at least 11 bp between the pairs. Then, we mapped the merged reads with single-ended mode to the human reference genome (version hs37d5) (Kent et al., 2002) using *BWA aln* (v0.7.12) (Li and Durbin, 2009) and the following parameters; "*-n 0.01 -o 2"* and "*-l 16500"* (Skoglund et al., 2014; Lazaridis et al., 2014). We merged different libraries of the same individual and removed PCR duplicates collapsing the reads with identical start-end positions into consensus sequences using *FilterUniqueSAMCons.py* (Kircher, 2012). Finally, we filtered the reads with more than 10% mismatches to the human reference genome and the reads less than 35 bp length (Kılınç et al., 2016). We processed the published ancient DNA sequence data from Appendix Table B.3 using the same procedures for comparative analysis. (The mapping process was performed by bioinformaticians; Kıvılcım Vural from our group, and Arielle R. Munters from Uppsala University, Sweden).

3.6 Authentication of data, contamination estimates and molecular sex determination

The authenticity and level of contamination in the generated ancient genomes were estimated using three approaches: (i) the characteristic damage patterns of ancient DNA, (ii) mtDNA-based estimation of contamination, and (iii) X chromosome-based estimation of contamination in male individuals.

3.6.1 Postmortem damage

We assessed the authenticity of our ancient genomes from Aşıklı (n=8) and Çatalhöyük (n=14) by checking the characteristic damage patterns at the first 30 positions at the 5'- and 3'-ends of the reads of aDNA using *PMDtools* (Skoglund et al., 2014). Authentic aDNA molecules contain a high frequency of post-mortem damage patterns; cytosine to thymine (C to T) transitions at 5'-ends of reads, and guanine to adenine (G to A) transitions at the 3'-ends. C to T transitions occur due to deamination of cytosine at 5'-ends of the broken aDNA fragments and observed as a complementary G to A transitions at the 3'-ends. These post-mortem damage patterns increase from the middle to the ends of the sequence reads (Briggs et al., 2007; Skoglund et al., 2014).

3.6.2 Mitochondrial contamination

We tested all samples from Aşıklı (n=8) and Çatalhöyük (n=14) for mtDNA contamination using *contamMix* software (Fu et al., 2013), which calculates the posterior probability of mtDNA contamination using a likelihood method (Bayesian approach) (Green et al., 2008). This method estimates contamination rates by comparing the mtDNA consensus sequence of each sample with all mitochondrial reads obtained from that sample and also with the mtDNA sequences from modern-day populations (Fu et al., 2013; Green et al., 2008). For this, consensus mtDNA sequence of each individual was called from *BAM* files using *samtools* (v.1.9) *mpileup* and *vcfutils* modules (Li et al., 2009). These consensus sequences were combined with a set of 311 modern-day human mtDNA sequences from worldwide populations (provided by *contamMix*) and contamination rates were estimated with and without transitions using *contamMix* software (Appendix Table B.1).

3.6.3 X chromosome contamination

We estimated levels of contamination for male individuals from Aşıklı (n=1) and Çatalhöyük (n=4) by examining heterozygosity of the X-chromosome, since males have only one X-chromosome. For this, we used a maximum likelihood method implemented in the ANGSD software (Rasmussen et al., 2011), with the following parameters described in the software manual; *"-r X:5000000-154900000 -doCounts 1 -iCounts 1 -minMapQ 30 -minQ 30"* for X chromosome positions. Next, the *contamination.R* script, implemented in the ANGSD, was run to estimate X-chromosome-based contamination ratio using Fisher's exact test and the jackknife procedure. The results are given in the Appendix Table B.1.

After applying these three methods, we evaluated the results in combination, considering different levels of specificity and sensitivity of each method. All individuals' data (n=22) examined here, passed at least two of the contamination estimation approaches (Appendix Table B.1). Given these results, we included all samples in further analyses.

3.6.4 Molecular sex determination

To assess the molecular sex of all individuals, we calculated the ratio of reads mapping to the Y chromosome to mapping to both X and Y chromosomes using the Ry method as described in (Skoglund et al., 2012, 2013), and including reads with mapping quality of at least 30. One individual from Aşıklı and four individuals from Çatalhöyük were assigned as biologically males. Seven individuals from Aşıklı and ten from Çatalhöyük were assigned as biologically females (Appendix Table B.1). These results are consistent with the osteological sex estimation of individuals (personal communication with anthropologists Ömür Dilek Erdal and Yılmaz Selim Erdal).

3.7 Mitochondrial DNA and Y chromosome haplogroup analyses

3.7.1 Mitochondrial DNA

We obtained mitochondrial DNA (mtDNA) sequences with mean coverage per individual ranging between 3-258x (Appendix Table B.1). In order to assign mtDNA haplogroups, consensus mitochondrial sequence of each individual was called using *samtools* (v1.9) *mpileup* and *variant caller* (Li et al., 2009) following the parameters specific to aDNA; filtering the sites with a depth <3x and a mapping quality <30 (Kılınç et al., 2018). We assigned mtDNA haplogroups to each individual using the SNPs at informative nucleotide positions of the consensus sequences and analysed in HaploGrep2 (v2.1.1) (https://haplogrep.uibk.ac.at/) (Weissensteiner et al., 2016). The mitochondrial coverage and haplogroups are given in Appendix Table B.1 and B.2.

3.7.2 Y chromosome

To assign Y chromosome haplogroups to one Aşıklı and four Çatalhöyük male individuals, we used the *yHaplo* program (Poznik et al., 2016). We genotyped each male individual based on 13,508 ISOGG (International Society of Genetic Genealogy, http://isogg.org) consortium SNPs following the method described in van de Loosdrecht et al. (2018). We called all single base substitutions using *BAM* files mapped to human reference genome (version hs37d5) with *samtools* (v1.9) *mpileup* (Li et al., 2009). We filtered the sites with a mapping quality and a base quality less than 30, and also excluded insertions/deletions and sites with multiple alleles. The haplogroups are given in Table 4.1.

3.8 SNP-calling and dataset preparation

We compiled three different datasets by merging the genomic data generated from ancient individuals in this study (n=22) and published ancient genomes from previous studies (n=191, Appendix Table B.3) with three datasets from modern-day populations:

Dataset1: The Human Origins SNP Array dataset (Lazaridis et al., 2014; Patterson et al., 2012) consists of 594,924 autosomal SNPs, including both transitions and transversions, genotyped in 2,730 modern-day individuals from 203 populations. Dataset1 was used for *PCA* and *ADMIXTURE* analyses.

Dataset2: The 1000 Genomes whole genome sequencing dataset (phase 3) (Auton et al., 2015) comprised of 1,938,919 biallelic transversion SNPs genotyped in African Yoruba individuals (YRI) (n=108) was used for Dataset2. This SNP dataset was preferred to reduce the potential effect of post mortem damage (by only including transversions), to maximize the overlaps between the genomic data of ancient individuals and also to avoid ascertainment bias. Dataset2 was prepared by extracting all transversion SNPs and applying a minor allele frequency ≥10% in Yoruba population using *vcftools* (Danecek et al., 2011) as described in (Kılınç et al., 2016; Günther et al., 2015), to avoid the effect of Eurasian admixture into Yoruba individuals (Dataset2 was prepared by our aDNA group (METU) together with Uppsala aDNA group). Dataset2 was used for f_3- and *D*-statistic and also for genetic relatedness estimations.

Dataset3: X chromosome SNPs from 1000 Genomes Project phase 3 (Auton et al., 2015) dataset that were genotyped in west African Yoruba individuals, were extracted and filtered with a minor allele frequency of 10% using "*transversion"* positions (across n=56 female and n=52 male Yoruba individuals). The pseudoautosomal regions from X chromosomes as identified in the human reference genome (hs37d5) were also removed (Dataset3 was prepared together

with Dilek Koptekin). The remaining 73,799 transversion SNPs were used for X chromosome-based kinship estimation.

We merged genomes of ancient individuals with Dataset1 and with Dataset2 as follows: we extracted reads with mapping quality of at least 30, and used the genotype information at each position that overlapped with a variant in these datasets, using only reads where the position had base quality of at least 30 using *samtools mpileup*. We haploidized our data as in (Günther et al., 2015), such that when multiple reads overlapped with the same position we randomly selected one read and this position was assumed as homozygous in the ancient individuals using an in-house python script (prepared by Torsten Günther from Uppsala University). The reason for pseudo-haploidization was to avoid bias during the SNP-calling from heterozygous sites in the low-coverage ancient genomes. Because of the low-coverage SNPs cannot be retrieved accurately and thus one read is randomly chosen per site to generate haploid genotype of ancient individual (Günther and Nettelblad, 2019). We removed any non-biallelic SNPs and the transitions and indels that are found in an ancient individual. We also applied the same process to published genomes (n=114) of ancient individuals from Anatolia and West Eurasia (Appendix Table B.3) as described in the previous sections, to homogenize the data treatment.

3.9 Principal component analysis

Principal component analysis (PCA) is a cluster-based method that reduces the dimensionality of the dataset, while increasing its interpretability and preserving the information from the data (Jolliffe and Cadima, 2016; Patterson et al., 2006). Herein, we first carried out PCA to investigate genetic affinities among studied Aşıklı and Çatalhöyük individuals using Dataset1 and following the method described in Kılınç et al. (2016). We conducted PCA using a total of 49 modern-day West Eurasian populations from the Human Origins SNP Array dataset (Lazaridis et al., 2014; Patterson et al., 2012) and projected 136 ancient individuals

(114 previously published and 22 reported here) onto the first two principal components inferred from modern-day individuals. Here, the projection method was used to avoid the effect of excessive amounts of missing data in the ancient genomes. To perform PCA, *smartpca* program of the *EIGENSOFT* software (Patterson et al., 2006) was used with the parameters; numoutlieriter:0 and lsqproject:YES. We visualized the results in R (v3.2.4) (http://www.r-project.org/). The modern-day populations are listed in the Appendix Table B.3.

3.10 f_3- and *D*-statistics

To investigate the genetic relationships between populations, we first computed outgroup f_3-statistics using Dataset2 with *qp3Pop* program in the *ADMIXTOOLS* package (Patterson et al., 2012), either at the individual- or population-level. The outgroup f_3-statistic quantifies shared drift between individuals/populations as their divergence from an outgroup population; i.e. it is a measure of genome-wide genetic similarity. We used the Yoruba population from 1000 Genomes Project phase 3 (Auton et al., 2015) as outgroup. Next, we converted population-level f_3-statistics into a pairwise distance matrix by subtracting all values from 1, we then summarized this distance matrix on two-dimensions using multidimensional scaling (MDS) analysis with the *"cmdscale"* function in R (v3.2.4) (http://www.r-project.org/). The goodness of fit for MDS was computed calculating a new distance matrix from the MDS output and calculating its Pearson correlation coefficient with the original distance matrix (http://cognitionandreality.blogspot.com/2015/04/computing-fit-of-mds-solution-using-r.html). We calculated individual-level outgroup f_3-statistics within each population to compare genetic diversity levels of populations.

We further carried out *D*-statistics to evaluate the genetic affinities of Aşıklı and Çatalhöyük either at individual- or population-level using Dataset2 with *qpDstat* program in the *ADMIXTOOLS* package (Patterson et al., 2012). *D*-statistics is a useful method to estimate the direction of possible gene flow among tested

individuals/populations using an outgroup (Patterson et al., 2012). We used the Yoruba population from 1000 Genomes Project phase 3 (Auton et al., 2015) as outgroup to avoid ascertainment bias. Statistical significance and the standard error bars were calculated using block-jackknife estimation by *ADMIXTOOLS*. In the plots, confidence intervals indicate ± 2 standard errors from the mean and a cutoff of $|Z| \geq 3$ was used for nominal statistical significance. Significant deviations (Z-score; Z = D/standard error) from zero indicates deviation from the proposed tree with topology (W,X;Y,Z). Here, W, X, Y, Z are representing any four populations. When W is the outgroup, if the Z-score is significantly positive or negative, then we can reject the topology. If the Z-score is significantly positive, this is compatible with gene flow that occured either between W and Y or X and Z; while negative values are compatible with gene flow that occured either between W and Z or X and Y.

3.11 *ADMIXTURE* analysis

We performed unsupervized genetic clustering using the *ADMIXTURE* software (Alexander et al. 2009), to estimate ancestry components in our studied ancient genomes. For this, we used the genomic data of modern-day Eurasian, African, Asian, and American populations from the Human Origins dataset (n=231) (Lazaridis et al., 2014, Patterson et al., 2012) and merged these with the genomes of ancient individuals from Anatolia and west Eurasia (Appendix Table B.3). We filtered the dataset by pruning for linkage disequilibrium (LD) following the parameters "*--indep-pairwise 200 25 0.4*" and for missing genotype with "*–geno 0.99*" using *PLINK* (Purcell et al., 2007), such that 518,401 SNPs remained for the analysis. The pruning is performed to remove the SNPs in high LD. We conducted *ADMIXTURE* analysis in ten replicates with different random seeds for each value of K ranging from 2 to 15, and determined the clusters of each ancient individual using the "*projection*" function of *ADMIXTURE*. We then visualized the results using *PONG* program (Behr et al., 2016).

3.12 Runs of homozygosity

We analyzed runs of homozygosity (*ROH*) in our high-coverage ancient genome (Aşıklı 128) and published high-coverage ancient genomes (n=6) from Anatolia and west Eurasia (Bon002, Bar8, Loschbour, Stuttgart, NE1 and WC1), following the methods described in Kılınç et al. (2016). We performed diploid genotype calling with autosomal transversions in Yoruba population from 1000 Genomes Project phase 3 (Auton et al., 2015) using *samtools (v1.9) mpileup* (Li et al., 2009), which generated between 1,798,444 and 1,893,648 transversion SNPs for these seven individuals. We estimated the distribution of *ROH* using *PLINK* (v1.9) (Purcell et al. 2007) with the parameters *"--homozyg , --homozyg-window-snp 50, --homozyg-window-het 1, --homozyg-windowthreshold 0.05, --homozyg-snp 50, --homozyg-kb 500, --homozyg-density 50, --homozyg-gap 100*".

We next calculated the genomic inbreeding coefficient using the F_{ROH} to estimate the level of inbreeding. F_{ROH} measures individual homozygosity, which is the proportion of the genome covered by *ROH* (McQuillan et al., 2008; Keller et al., 2011). We calculated F_{ROH} by dividing the summed length of *ROH* (> 1.5 Mb) for each individual by the total length of autosomal chromosomes covered by SNPs in megabases (McQuillan et al., 2008; Keller et al., 2011).

3.13 Phenotypic SNPs analysis

A number of functional SNPs (e.g lactose tolerance, skin pigmentation, eye color) in one high-coverage Aşıklı genome (Aşıklı 128) were analyzed. This analysis was restricted to one individual with the highest coverage (5x) to avoid excessive amounts of missing data and to be able to call diploid genotypes with at least modest confidence. SNPs associated with phenotypes of interest were called from the *BAM* file using *samtools* (v1.9) *mpileup* (Li et al., 2009) following the method described in van de Loosdrecht et al. (2018). We filtered out reads with mapping quality score <30 and positions with base quality score <30. Alleles predicting

skin, eye and hair color were retrieved and analyzed using the HIrisPlex tool (http://hirisplex.erasmusmc.nl/), to compute the probability of skin, eye and hair shade for Aşıklı 128 individual.

In addition, derived allele variants in the *MCM6* gene associated with lactose tolerance in Europeans (rs4988235) (Enattah et al., 2002), Africans (rs41456145, rs145946881) (Ranciaro et al., 2014; Jones et al., 2013) and Middle Easterners (rs41380347) (Ranciaro et al., 2014; Jones et al., 2013) were also analyzed for the Aşıklı individual (Aşıklı 128).

CHAPTER 4

RESULTS

In the present study, I report new archaeogenomic data from Aşıklı Höyük (n=8) and Çatalhöyük (n=6) that I produced as part of this thesis work, representing Aceramic and Ceramic Neolithic populations from Central Anatolia, respectively. This data was supplemented by additional genomes produced from Çatalhöyük (n=8) and published in Yaka et al. (under review in Curr.Biol.) (Table II.1).

The authenticity of the data was confirmed using three methods as described in the previous section (Materials and Methods). First, postmortem damage profiles of samples were assessed and all samples showed >25% PMD at 5'- and 3'-ends (Appendix Figure A.1; Appendix Table B.1). Next, contamination estimate analysis was performed using two different approaches; mitochondrial-based contamination estimates for all samples and X-chromosome-based contamination in males. All individuals passed the authenticity test based on mtDNA contamination estimates with the overall authenticity levels ≥93% (Appendix Table B.1). Ancient genomes examined here (n=22), passed at least two of the contamination estimation approaches (Appendix Table B.1). Given these results, we included all samples into further analyses.

Next, we analysed newly generated genomic data comparatively with published ancient genomes from Neolithic and early Holocene populations from Anatolia (Pınarbaşı, Boncuklu, Barcın and Tepecik-Çiftlik Höyük) and West Eurasia using population genetic analysis (Appendix Table B.3). Details about the generated data and sequencing statistics are given in Table 4.1 and Appendix Table B.1. Here, genetic relationships among individuals and populations from Neolithic Anatolia were analyzed. Note that, using information about kinship relationships identified among individuals from each of the Anatolian Neolithic settlements (see the

Chapter 8), for population genetic analyses only one individual for each group of related individuals was chosen, to avoid bias (i.e. non-independent data points) and clustering of subgroups within populations in the analyses.

Table 4.1 Archaeological, anthropometric and genetic characteristics of sequenced individuals (old adult: 50+ years, middle adult: 35-50 years, young adult: 20-35 years, adolescent: 12-20 years, child: 3-12 years, infant: 2 months-3 years). The table is adopted from Yaka et al. (under review in Curr.Biol.).

Individual ID	**Site**	**Stratigrahic level**	**Building**	**C14 date (cal. BCE)**	**Age class**	**Molecular sex**	**Genome coverage**	**Mitochondrial DNA haplogroup**	**Y chromosome haplogroup**
128	Aşıklı Höyük	4	B3	8225-7955	Child	XX	5.0	K1a4	-
129	Aşıklı Höyük	4	B3	8170-7735	Young adult	XX	0.79	K1a4	-
133	Aşıklı Höyük	4	B1	8170-7735	Old adult	XX	1.16	K1a4	-
131	Aşıklı Höyük	4	B1	8200-7740	Child	XX	0.09	T2c1a	-
136	Aşıklı Höyük	4	B1	8175-7655	Adult	XX	0.15	T2c1a	-
2	Aşıklı Höyük	2A	AB	7585-7475	Young adult	XX	0.02	H2a2a	-
33	Aşıklı Höyük	2C	C	7945-7595	Child	XY	0.07	U3a	G2a2b
40	Aşıklı Höyük	2B	BH	7935-7590	Old adult	XX	0.03	N1a1a1	-
30006 F.7615	Çatalhöyük	North G	114	6645-6480	Infant	XX	0.07	K1a4	-
8587 F.1013	Çatalhöyük	North G	114	-	Neonate	XX	0.14	T2e	-

Table 4.1 (continued)

2728 F.258	Çatalhöyük	South M	50	6695-6506	Infant	XX	0.08	K1a	-
2842 F.274	Çatalhöyük	South M	50	6690-6506	Child	XX	0.09	K1a	-
2017 F.96	Çatalhöyük	South M	50	6815-6595	Neonate	XX	0.03	T2	-
1885 F.84	Çatalhöyük	South M	50	6905-6600	Child	XY	0.07	K1a	G2a2a1
2033 F.84/86	Çatalhöyük	South M	50	6690-6590	Child	XY	0.01	H2a2a1d	H3a1
2779 F.265	Çatalhöyük	South M	50	-	Infant	XY	0.27	H2a2a	C1a2
5357 F.576	Çatalhöyük	South K	17	7035-6650	Infant	XY	0.06	N1a1a1	C1a2
21855 F.8214	Çatalhöyük	South K	17	-	Child	XX	0.07	H2a2a1	-
21981 F.8153	Çatalhöyük	South N	89	-	Infant	XX	0.09	K1a17	-
5747 F.1064	Çatalhöyük	South M	91	6640-6490	Infant	XX	0.12	T2c1	-
11739 F.1912	Çatalhöyük	TP Late Neolithic	NA	6235-6075	Middle adult	XX	0.2	K1b1	-
20217 F.3931	Çatalhöyük	TPC Late Neolithic	NA	6415-6240	Child	XX	0.06	K1a4b	-

4.1 Uniparental marker analysis

Mitochondrial DNA haplogroups of Aşıklı and Çatalhöyük individuals were assigned as described in the previous section (Materials and Methods). We observed haplogroup K1a4, one of the common haplogroups observed in Neolithic populations from Southwest Asia (Brandt et al., 2013), in three individuals from

Aşıklı (Aşıklı 128, 129, 133). Two Aşıklı individuals (Aşıklı 131, 136) belonged to T2c1a and the remaining three individuals from Aşıklı (Aşıklı 2, 33, 40) belonged to haplogroups H2a2a, U3a and N1a1a1, respectively. We also found haplogroup K1a4 in one Çatalhöyük individual (30006) and a subtype of haplogroup K1 (K1a) in three individuals (2728, 2842, 1885). Three Çatalhöyük individuals (21981, 11739, 20217) belonged to three subtypes of K1 (K1a17, K1b1, K1a4b, respectively). Three individuals from Çatalhöyük (8587, 2017, 5747) belonged to subtypes of T2 (T2e, T2, T2c1) and one (5357) belonged to N1a1a1, one of the most abundant haplogroups in Near Eastern and European farmer populations (Fernandez et al. 2014, Brandt et al. 2013). The remaining Çatalhöyük individuals (2033, 2779, 21855) belonged to subtypes of H2a2a (H2a2a1d, H2a2a, H2a2a1), respectively.

We further determined Y chromosome haplogroups of male individuals from Aşıklı and Çatalhöyük as described in the previous section (Materials and Methods). We observed haplogroup G2a2b in the single Aşıklı male individual. Two male individuals from Çatalhöyük were assigned to haplogroup C1a2 and remaining two Çatalhöyük individuals belonged to two other haplogroups, G2a2a1 and H3a1 (Table 4.1).

The mitochondrial DNA and Y chromosome haplogroups found in both Aşıklı and Çatalhöyük populations were common in the early farmer groups reported earlier from Europe and Levant (Brandt et al., 2013; Fernandez et al., 2014; Haak et al., 2015). Overall these results suggested that Aşıklı and Çatalhöyük maternal and paternal lineages belonged to the Neolithic substrate.

4.2 Inter- and intra-population genetic affinities of Anatolian Neolithic populations

To summarise the genetic relationships between the studied Aşıklı and Çatalhöyük individuals, and other Neolithic individuals from Anatolia as well as early

Holocene individuals from Iran, Levant, Caucasus and Europe, we first performed principal component analysis (PCA). PCA was conducted using 49 modern-day West Eurasian populations from the Human Origins SNP Array dataset (Patterson et al., 2012; Lazaridis et al., 2014) and the 136 ancient individuals (114 earlier published and 22 generated in this study) including Aşıklı and Çatalhöyük individuals were projected onto the first two principal components calculated from modern-day populations (Figure 4.1B).

On the PC1 and PC2 space, Aşıklı and Çatalhöyük individuals were grouped together with individuals from Boncuklu (Aceramic Neolithic), Barcın and Tepecik-Çiftlik (Ceramic Neolithic) and Pınarbaşı (late Epipaleolithic) from Anatolia, and formed an Anatolian cluster (Figure 4.1B), as previously reported in Kılınç et al. (2016, 2017). Our PCA results revealed genetic affinity between Aşıklı and Boncuklu individuals, representing Central Anatolian early Neolithic, while Çatalhöyük individuals were located between other Anatolian and Levantine Neolithic groups in the PC1 space. Despite the geographical proximity of the Anatolian Neolithic populations from different periods (Aşıklı, Boncuklu, Çatalhöyük, Barcın and Tepecik-Çiftlik) (Figure 4.1A), each of these groups formed their own clusters, albeit close to and partly overlapping with each other in the PCA (Figure 4.1B). Overall, the PCA shows a Neolithic Anatolian cluster distant from European and Caucasus hunter-gatherers (WHG, EHG, Iron Gates, CHG) as well as from Levantine and Iranian farmers (Figure 4.1 and Appendix Table B.3).

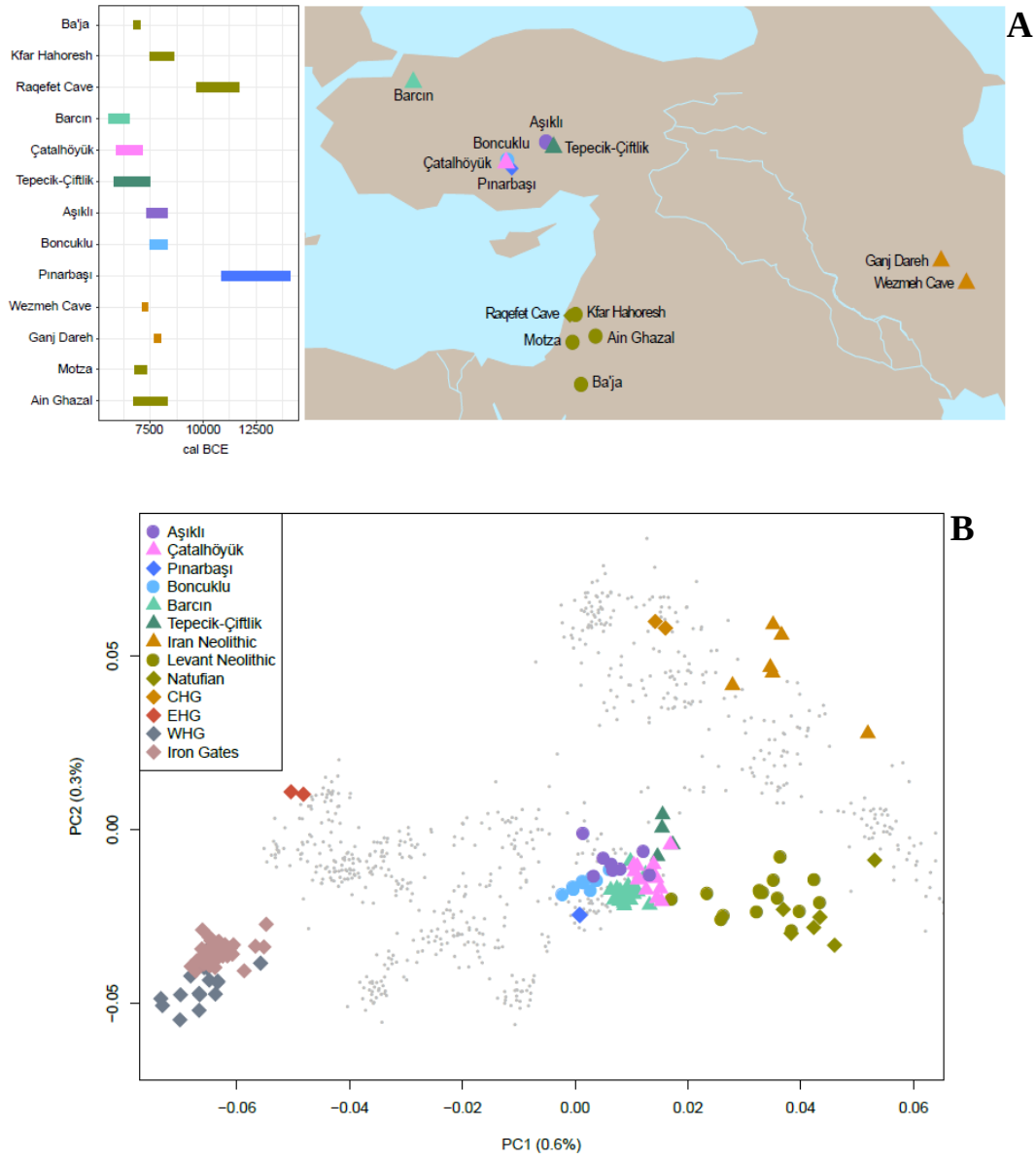


Figure 4.1 (**A**) Geographic map presenting ancient individuals from West Eurasia including newly generated data in this study and timeline showing occupation period of each site (cal BCE). Radiocarbon dates of the sites were adopted from Yaka et al. (under review in Curr.Biol.). (**B**) PCA plot indicating the genetic affinities among ancient individuals from West Eurasia (Appendix Table B.3) including the studied Aşıklı (n=8) and Çatalhöyük (n=14) individuals. Modern-day populations are indicated with small gray points. Colored dots represent ancient individuals. Appendix Figure A.2 lists population labels of present-day individuals

(gray dots). See Table 4.1 for genome data quality of Aşıklı and Çatalhöyük individuals. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

Second, we investigated genetic relationships among ancient individuals within each Anatolian settlement, conducting individual-level *D*-statistics. Specifically, we conducted *D*-tests of the form *D(Yoruba, BoncukluX; BoncukluY, Anatolia)*, *D(Yoruba, AşıklıX; AşıklıY, Anatolia), D(Yoruba, ÇatalhöyükX; ÇatalhöyükY, Anatolia), D(Yoruba, BarcınX; BarcınY, Anatolia)* and *D(Yoruba, Tepecik-ÇiftlikX; Tepecik-ÇiftlikY, Anatolia),* where *X* and *Y* are different individuals from the same population, and *Anatolia* is an individual from a different Anatolian Neolithic settlement. Our results showed that Aşıklı individuals were genetically closer to each other than they were to individuals from other Neolithic and late Epipaleolithic Anatolian individuals (Aşıklı individuals chose each other in the 73% of the comparisons and 24% of those are nominally significant) (Figure 4.2). Similarly, Çatalhöyük individuals showed more genetic affinity to their own group than individuals from Neolithic and late Epipaleolithic Anatolian populations, including both Boncuklu and Aşıklı (Çatalhöyük individuals chose each other in the 63% of the comparisons and 2% of those are nominally significant) (Figure 4.2). We also computed *D*-statistics at the individual-level for Boncuklu, Barcın and Tepecik-Çiftlik, using the same approach and the same pattern was observed for each Neolithic groups from Central and Northwest Anatolia (Figure 4.2). For these individual-level comparisons, we conducted 576-11,780 tests for each Anatolian Neolithic group and from those 2-51% of cases were nominally significant. Only among comparisons of Tepecik-Çiftlik individuals did we find no nominally significant results. These *D*-statistics results were also consistent with the PCA, in that individuals from each of these populations are genetically closer to their own group, which is observed as partly overlapping clusters in PCA.

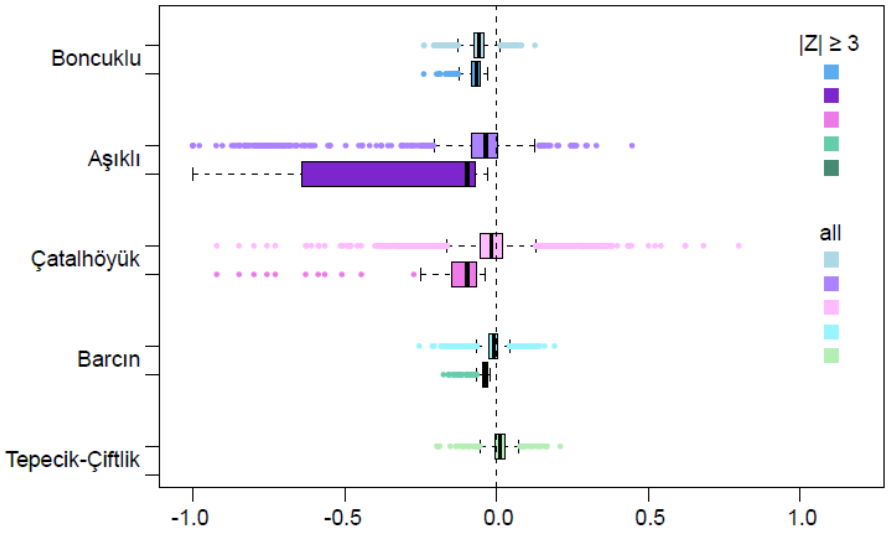
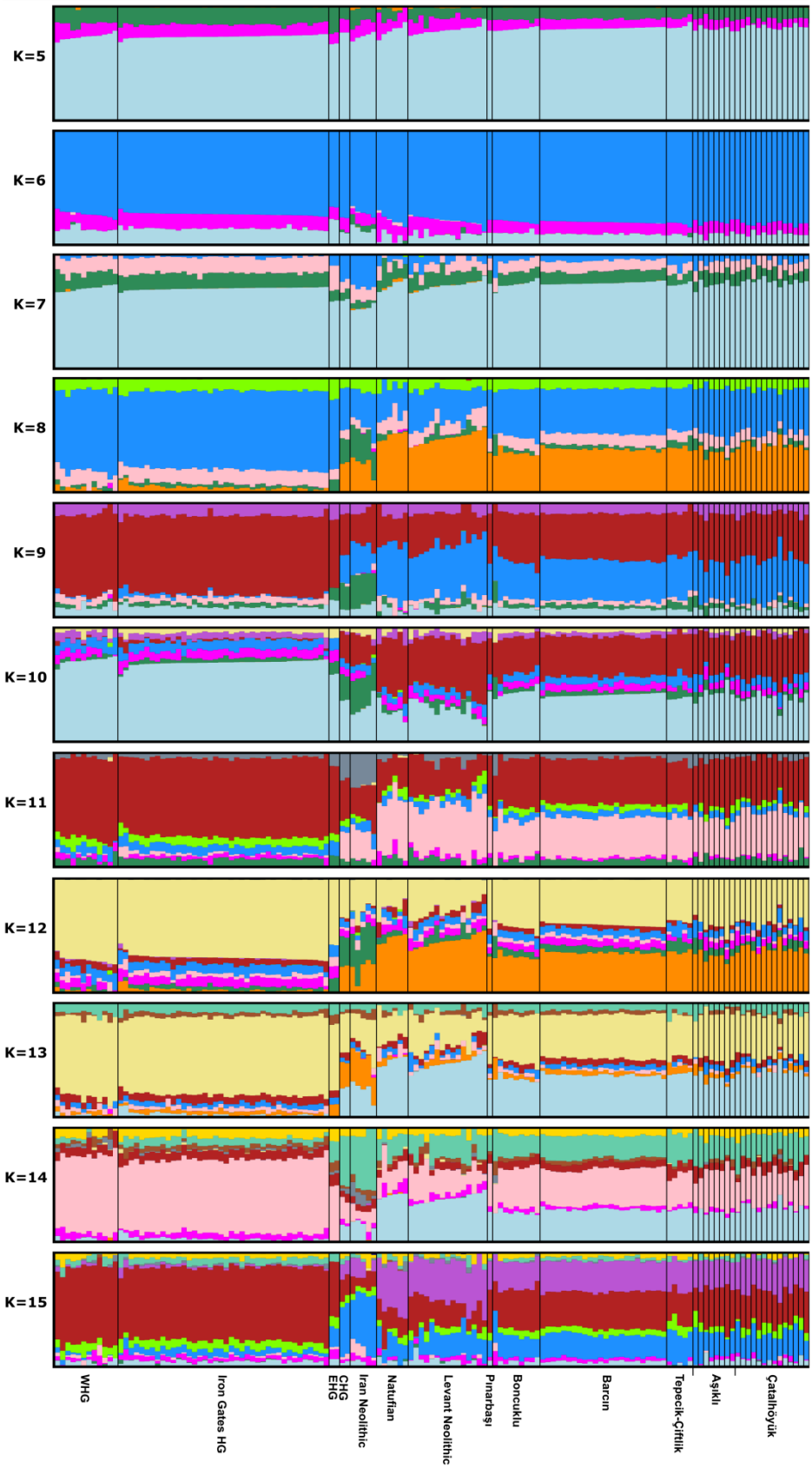


Figure 4.2 Distributions of *D*-statistics calculated among individuals within each population with the topologies; *D(Yoruba, BoncukluX; BoncukluY, Anatolia), D(Yoruba, AşıklıX; AşıklıY, Anatolia), D(Yoruba, ÇatalhöyükX; ÇatalhöyükY, Anatolia), D(Yoruba, BarcınX; BarcınY, Anatolia)* and *D(Yoruba, Tepecik-ÇiftlikX; Tepecik-ÇiftlikY, Anatolia)*. *X* and *Y* indicate different individuals from the same, and *Anatolia* indicates an individual from a different Neolithic Anatolian group. Darker and lighter colors show nominally significant results (|*Z*|>3) and all results, respectively. There were no nominally significant results among the Tepecik-Çiftlik tests. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

Next, we performed *ADMIXTURE* analysis to estimate ancestry components of Aşıklı and Çatalhöyük individuals, and to cluster these individuals based on their genetic profiles. In *ADMIXTURE* analysis we used genomic data from modern-day populations (n=231) (Human Origins dataset) to calculate ancestral components and projected early Holocene individuals (n=148) from Southwest Asia, including Aşıklı and Çatalhöyük (Appendix Table B.3) (See Materials and Methods for details). *ADMIXTURE* analysis showed similarity between the distribution of ancestry components of individuals from Aşıklı and Çatalhöyük, and those of other Anatolian Neolithic populations (Boncuklu, Barcın and Tepecik-Çiftlik) (Figure 4.3).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K=14
K=15
WHG
Iron Gates HG
CHG
EHG
Iran Neolithic
Natufian
Levant Neolithic
Pınarbaşı
Boncuklu
Barcın
Tepecik-Çiftlik
Aşıklı
Çatalhöyük

Figure 4.3 Unsupervised *ADMIXTURE* analysis based on *K*=5 to *K*=15 ancestry components for ancient individuals from Aşıklı (n=8) and Çatalhöyük (n=14) and from published studies (n=126). Çatalhöyük individuals from right to left-hand side, respectively: 20217, 11739, 21981, 8587, 2779, 2033, 1885, 21855, 2017, 5357, 5747, 2842, 2728 and 30006. Aşıklı individuals from right to left-hand side, respectively: 136, 131, 129, 133, 128, 40, 33 and 2. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

We further studied population-level genetic similarity between Aşıklı and Çatalhöyük, and early Holocene populations of Southwest Asia including individuals from Anatolian late Epipaleolithic (Pınarbaşı) and Neolithic groups (Boncuklu, Barcın and Tepecik-Çiftlik). We included one individual from any genetically related pairs (identified in the Chapter 8) to avoid bias (i.e. non-independent data points) in population genetic analysis. First, we calculated pairwise f_3-statistics to investigate genetic affinity between the Neolithic and late Epipaleolithic groups from Anatolia, Levant and Iran. f_3-statistics results were converted into a genetic distance matrix using 1-f_3 values and summarized in two-dimensions with multidimensional scaling (MDS) analysis. In the *MDS* Aşıklı showed higher genetic similarity to Boncuklu which is another early Neolithic population from Central Anatolia, compared to other early Holocene groups from Anatolia, Levant and Iran. Our *MDS* results revealed genetic affinity between Çatalhöyük and both Barcın and Tepecik-Çiflik (Figure 4.4). Despite the different clustering of Aceramic and Ceramic Neolithic populations observed in the *MDS*, early Holocene groups from Anatolia are genetically closer to each other than to those of Levant and Iran.

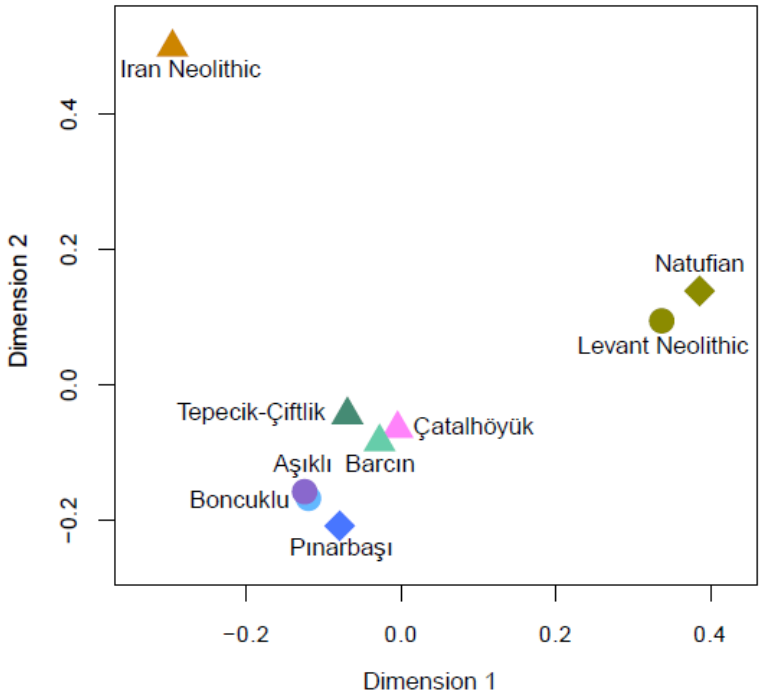

Figure 4.4 Multidimensional scaling (*MDS*) plot based on genetic distances among late Epipaleolithic and Neolithic populations from Anatolia, Levant and Iran, calculated with outgroup *f3*-statistics (1-*f3*). The *MDS* goodness of fit was calculated as 0.92. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

Next, we computed *D*-statistics at the population-level to test genetic affinity between studied Aşıklı and Çatalhöyük, and other Neolithic and late Epipaleolithic groups from Anatolia, Balkans, Europe, Levant, Iran and Caucasus. In this analysis the Yoruba population from 1000 Genomes Project (phase 3) (Auton et al., 2015) was used as outgroup. *D*-statistics results indicated that Aşıklı has higher genetic affinity to Boncuklu relative to other Neolithic populations (Çatalhöyük, Barcın and Tepecik-Çiftlik) and a late Epipaleolithic individual (Pınarbaşı) from Anatolia (Figure 4.5A). Likewise, Boncuklu is genetically closer to Aşıklı compared to other ancient populations (Figure 4.5B). In addition, the Pınarbaşı individual (Anatolian late Epipaleolithic) showed equal genetic affinity to both Aşıklı and Boncuklu,

which is also observed for other early Holocene populations from West Eurasia (Figure 4.5C). Meanwhile, Aşıklı is genetically closer to Çatalhöyük and Barcın than to Tepecik-Çiftlik across among Anatolian Ceramic Neolithic populations (Figure 4.6C).

Our *D*-statistics analysis at population-level revealed that Çatalhöyük had a strong genetic affinity to Barcın compared to Tepecik-Çiftlik across among Anatolian Ceramic Neolithic populations (Figures 4.6A and 4.6B). Moreover, Çatalhöyük, Barcın and Tepecik-Çiftlik, representing later periods of Neolithic Anatolia, also have an equal genetic affinity to Aşıklı and Boncuklu (non-significantly closer to Aşıklı) (Figure 4.5C). Our *D*-statistics results also showed that genetic affinities among early Holocene Anatolian populations, including late Epipaleolithic and both Aceramic and Ceramic Neolithic period Anatolian populations is higher, compared to their affinities to other West Eurasian early Holocene populations (e.g., Neolithic populations from Levant and Iran, hunter-gatherer groups from Europe and Caucasus) (Figures 4.1B, 4.4, 4.5A-B, 4.6).

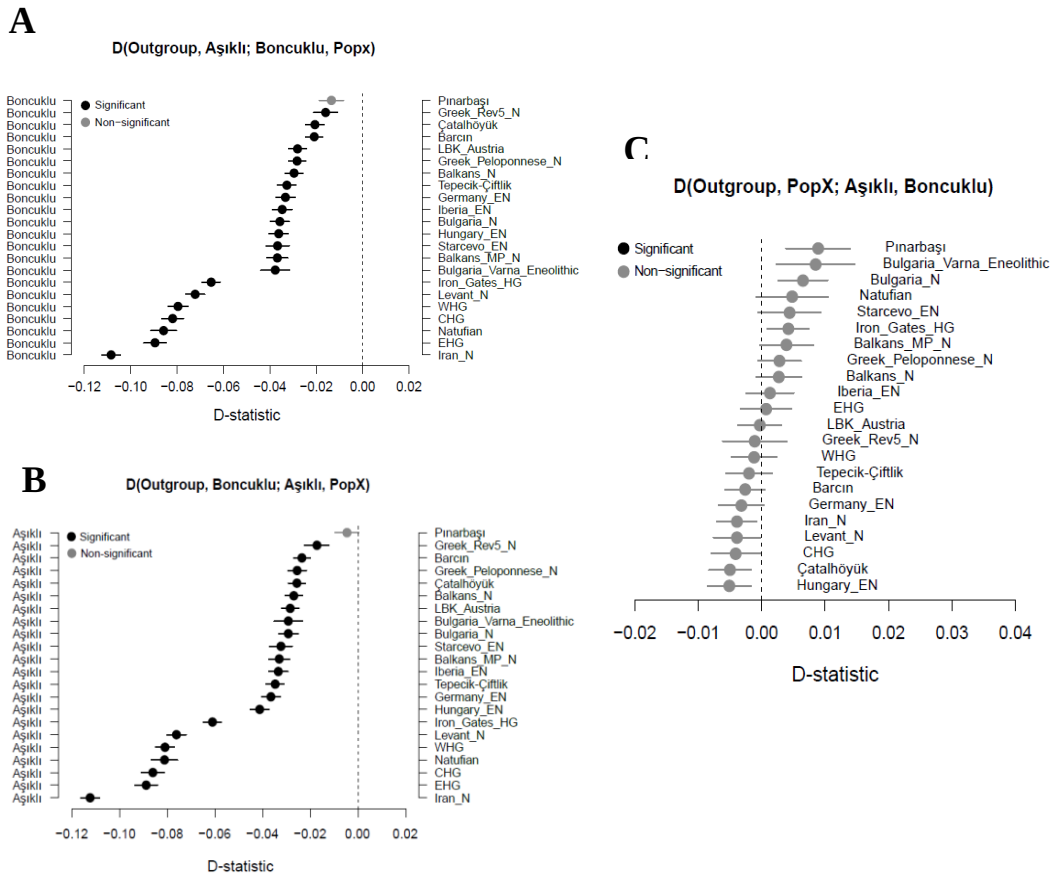

Figure 4.5 (**A**) Population level *D*-statistics for the topology *D(Yoruba, Aşıklı; Boncuklu, PopX);* (**B**) Population level *D*-statistic for the topology *D(Yoruba, Boncuklu; Aşıklı, PopX).* (**C**) Population level *D*-statistics for the topology *D(Yoruba, PopX; Aşıklı, Boncuklu)* where Aşıklı and Boncuklu are present on the left- and right-hand y-axis, respectively. In all panels *PopX* stands for ancient individuals/populations from Anatolian, Near Eastern, Balkans and European Neolithic and hunter-gatherer groups. $D < 0$ represents genetic affinity between Aşıklı and the test population (*PopX*), and $D > 0$ represents genetic affinity between Boncuklu and the test population (*PopX*) (see Appendix Table B.3 for population details). In each comparison, black color indicates nominally significant (*i.e.*, not corrected for multiple testing) *D*-statistics with $|Z| \geq 3$ and grey-color shows non-significant values. Horizontal lines show standard errors. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

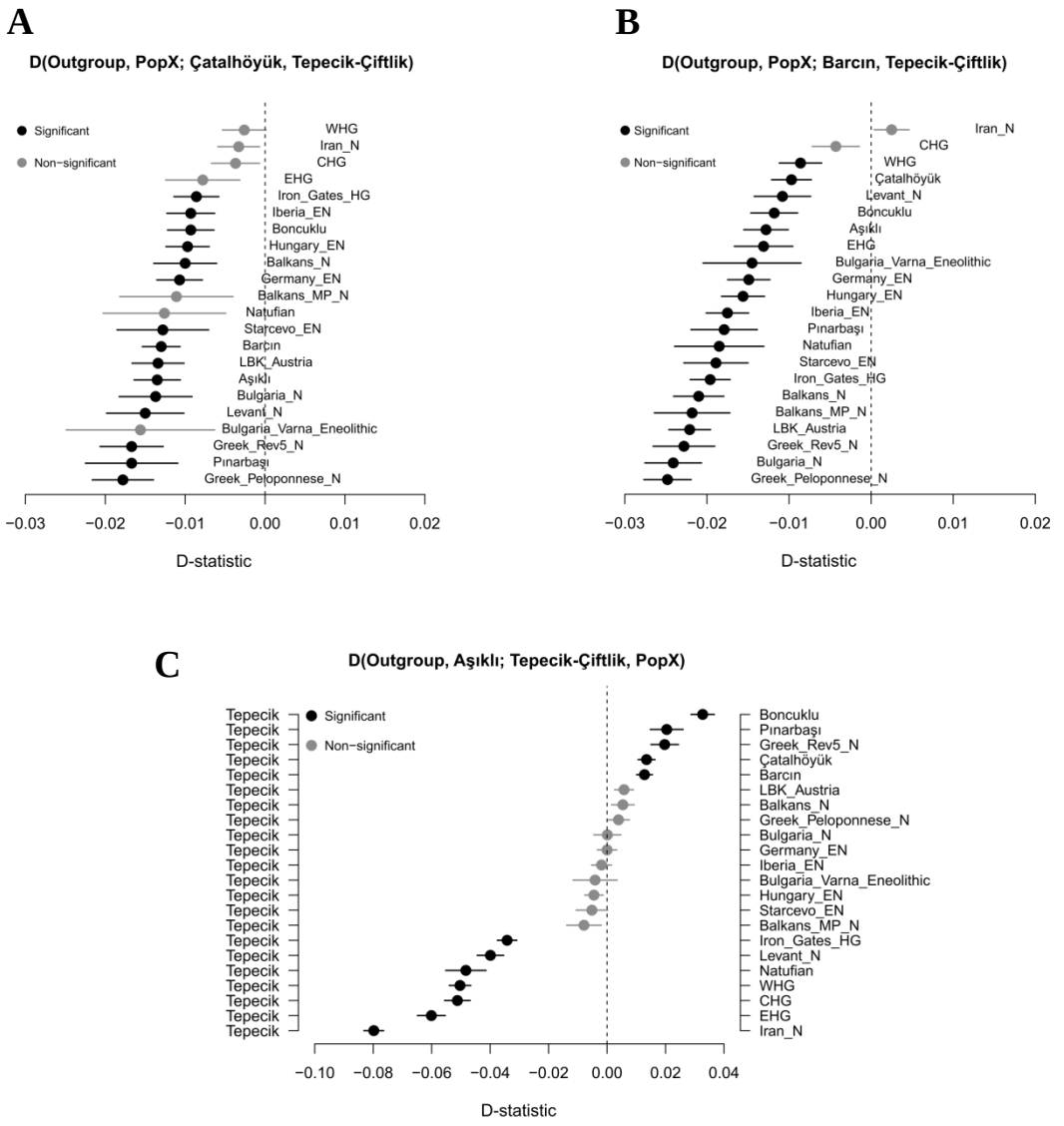

Figure 4.6 Population-level *D*-statistics calculated for the topology **(A)** *D(Yoruba, PopX; Çatalhöyük, Tepecik-Çiftlik)*, **(B)** *D(Yoruba, PopX; Barcın, Tepecik-Çiftlik)*, **(C)** *D(Yoruba, Aşıklı; Tepecik-Çiftlik, PopX);* where *PopX* stands for ancient individuals/populations from Anatolian, Near Eastern, Balkans and European Neolithic and hunter-gatherer groups, indicated in the middle and/or on the right-hand y-axis. In each comparison, black-color shows nominally significant *D*-statistics with $|Z| \geq 3$ and grey-color shows non-significant values. Horizontal lines show standard errors.

We further present *D*-statistics comparisons among early Holocene groups from Anatolia, Levant and Iran, to address genetic affinity between Anatolian Neolithic vs. Levantine and Iranian Neolithic populations. We tested the notion of possible regional migration and gene flow from Levant/Iran into Central Anatolia during the shift from Aceramic to Ceramic Neolithic in Anatolia which may have led to increase in genetic diversity in Ceramic Neolithic populations, suggested by earlier reports (Kılınç et al., 2016, 2017; Feldman et al., 2019). Here we increased the sample size by including Aşıklı and Çatalhöyük as parts of the picture. We performed *D*-statistics using the topology *D(Yoruba, X; Aceramic Anatolian, Ceramic Anatolian)*; where *Aceramic Anatolian* is represented by Aşıklı and Boncuklu, and *Ceramic Anatolian* is represented by Çatalhöyük, Barcın, Tepecik-Çiftlik, and *X* indicates early Holocene populations from Levant and Iran (Natufian, Levant and Iran Neolithic) (Figure 4.7). We observed that Neolithic populations from Levant and Iran, are genetically closer to Çatalhöyük (non-significant for Aşıklı-Iran and Boncuklu-Natufian comparisons), Barcın (non-significant for Iran comparisons) and Tepecik-Çiftlik (only significant for Boncuklu-Iran comparison) relative to Aşıklı and Boncuklu (Figure 4.7). Thus, our results indicate higher genetic affinity between Neolithic groups from Levant/Iran and Ceramic Neolithic populations from Anatolia compared to those of Aceramic Neolithic. This supports previous studies which had shown that there occurred gene flow from Near East to Anatolia during the transition from Aceramic to Ceramic Neolithic period, after c.7,500 BCE (Kılınç et al., 2017; Feldman et al., 2019).

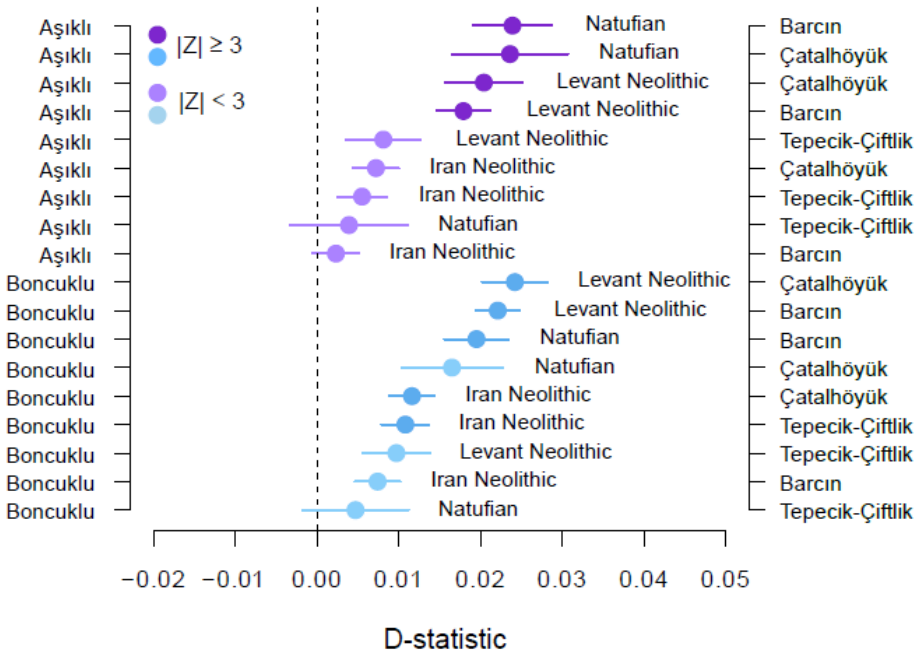

Figure 4.7 *D*-statistics based on populations calculated as *D(Yoruba, X; Aceramic Anatolian, Ceramic Anatolian)*; Aşıklı and Boncuklu are present on the left-side of y-axis as *Aceramic Anatolian*; Çatalhöyük, Barcın, Tepecik-Çiftlik as *Ceramic Anatolian* on the right-hand y-axis; *X* stands for ancient populations from Levant and Iran, given in the middle. $D < 0$ shows higher genetic affinity between the test population (*X*) and *Aceramic Anatolian*, and $D > 0$ shows higher genetic affinity between the test population (*X*) and *Ceramic Anatolian*. In each comparison, darker-colors show nominally significant *D*-statistics with $|Z| \geq 3$, and lighter colors show non-significant values. Horizontal lines show standard error. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

4.3 Genetic diversity in Anatolian Neolithic populations

Here our goal was to investigate within-group genetic diversity for each Anatolian Neolithic population and to compare the diversity levels of these populations. We carried out this analysis to test the hypothesis that a possible gene flow could cause an increase in genetic diversity during the transition from Aceramic to Ceramic

Neolithic in Anatolia (Kılınç et al., 2016, 2017; Feldman et al., 2019). For this we computed outgroup f_3-statistics based on individuals from each population, including one individual from genetically kin-related pairs of each population. We ran f_3-statistics either on the entire group per site (Figure 4.8B), or level by level within each site (Figure 4.8A), and calculated genetic distance using 1- f_3 for all comparisons. We found that Aceramic Neolithic populations from Central Anatolia, Aşıklı and Boncuklu, are genetically more homogeneous and represent lower within-group genetic diversity compared to Ceramic Neolithic populations (Çatalhöyük, Barcın and Tepecik-Çiftlik) in both types of comparisons (Figure 4.8, Appendix Table B.4). At the same time, Çatalhöyük, Barcın and Tepecik-Çiftlik, representing Ceramic Neolithic in Central and Northwest Anatolia, display similar within-group genetic diversity levels which are higher than those of Anatolian Aceramic Neolithic. These results support the aforementioned observation of external gene flow into Anatolia that increased genetic diversity levels of Anatolian Neolithic populations through time. The statistical significance of differences between genetic diversity levels of Anatolian Neolithic populations was tested using a random permutation test with 10,000 repeats in R (v.3.5) (see Appendix Table B.4 for details).

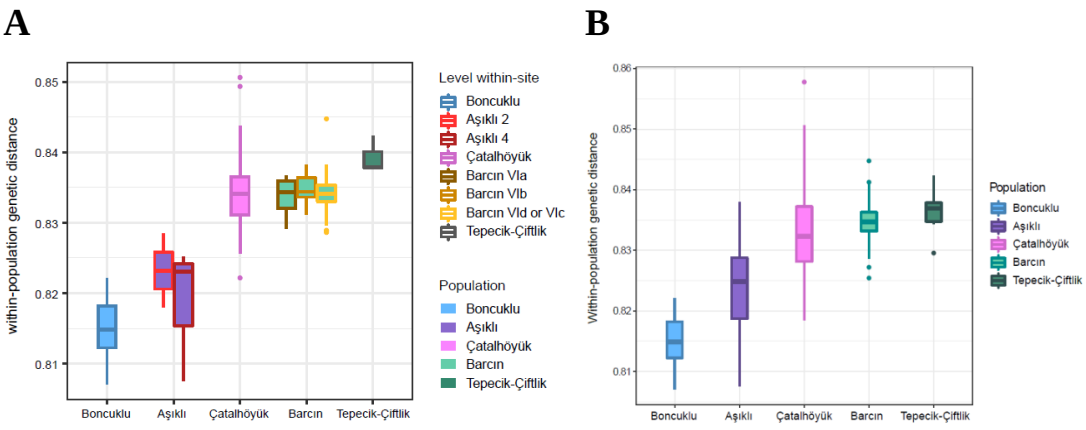

Figure 4.8 Boxplots indicating within-population genetic distances based on outgroup f_3-statistics calculated using contemporaneous individuals from each

population with the topologies f_3*(Yoruba, AşıklıX; AşıklıY),* f_3*(Yoruba, BoncukluX; BoncukluY),* f_3*(Yoruba, ÇatalhöyükX; ÇatalhöyükY),* f_3*(Yoruba, BarcınX; BarcınY),* f_3*(Yoruba, Tepecik-ÇiftlikX; Tepecik-ÇiftlikY)* where *X* and *Y* stand for different individuals from each population. The figure shows two types of comparisons; (**A**) level by level within each site and (**B**) on the entire group per site. Two Çatalhöyük individuals younger than 6,400 BCE were excluded due to not being contemporaneous with other Çatalhöyük individuals. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

4.4 Inbreeding estimation and phenotypic traits analysis for Aşıklı 128

4.4.1 Analysis of runs of homozygosity (ROH)

Runs of homozygosity (*ROH)* are uninterrupted DNA segments which are inherited from both parents, and thus they are homozygous in an individual, but polymorphic in populations (Keller et al., 2011; Broman and Weber, 1999). The short and intermediate length *ROHs* provide information about the demographic history of population (i.e., effective population size); while the long *ROHs* are used to estimate inbreeding in an individual (Ceballos et al., 2018; Pemberton et al., 2012; Keller et al., 2011). For instance, *ROH* are observed at a lower frequency and shorter in admixed and larger populations, whereas in smaller and isolate/bottlenecked populations *ROH* are observed as longer and at a higher frequency (Ceballos et al., 2018; Pemberton et al., 2012; Keller et al., 2011).

We analysed *ROH* for seven relatively high-coverage published ancient genomes (Bon002 (Boncuklu ZHB), Bar8 (Barcın M10-106), Loschbour, Stuttgart, NE1 and WC1) (Figure 4.9) (see Appendix Table B.3 for details about individuals) and our highest quality genome (Aşıklı 128), following the method described in Kılınç et al. (2016). We restricted *ROH* analysis with high-coverage genomes (>5x) to avoid excessive amounts of missing data and to be able to call diploid genotypes with at least modest confidence. *ROH* analysis per sample was carried out with *PLINK* (v.

1.9) (Purcell et al., 2007) using transversion SNPs (Dataset 2) (Figure 4.9A). We retrieved number of SNPs for these seven individuals ranging between 1,798,444 and 1,893,648. We also calculated *ROH* with the subsampled genome of each individual down to 5x coverage and found consistent results with the previous analysis (Figure 4.9B).

It can be observed from Figure 4.9 that the European hunter-gatherer, Loschbour, has a high level of short- and intermediate-*ROHs* implying a small ancestral population size as described in previous reports (Cassidy et al., 2016; Broushaki et al., 2016). This result is expected due to higher homogeneity in hunter-gatherer groups. We also estimated inbreeding in an early Neolithic individual from Iran observing higher level of long *ROHs* (>1.6 Mb), which was earlier reported in Broushaki et al. (2016). Our *ROH* results showed that Aşıklı 128 had fewer short- and intermediate-*ROHs* than Loschbour, but more of those compared to published high-quality genomes from Anatolian Ceramic Neolithic (Barcin, Bar8) (Figure 4.9), and this pattern was earlier reported for the Boncuklu individual (Bon002) (Kılınç et al., 2016). The estimated distribution of *ROHs* for Aşıklı 128 and Boncuklu ZHB (Bon002) are similar and this finding is consistent with our results of genetic diversity analysis, implying low genetic diversity in Central Anatolian early Neolithic groups than in those of later period (i.e., Barcın-Bar8) (Figures 4.8 and 4.9). *ROH* results of the Aşıklı and Boncuklu individuals suggested smaller population size in Boncuklu relative to Aşıklı, supporting the archaeological evidence that Boncuklu was a small early Neolithic village in Central Anatolia (Baird, 2012).

We further calculated genomic inbreeding coefficient (*F*) using the F_{ROH} to estimate the level of inbreeding, as described in previous section (Materials and Methods). F_{ROH} is an estimate of inbreeding coefficient, calculated as the proportion of the genome covered by *ROH* > 1.5 Mb (McQuillan et al., 2008; Keller et al., 2011). For example, for offspring of 4th degree relatives (e.g., offsprings of the individual's first cousins) the expected $F = 0.0312$, while for offspring of 3th degree relatives (e.g., first cousins) the expected $F = 0.0625$. We

estimated F_{ROH}=0.023 for Aşıklı 128 and F_{ROH}=0.031 for Boncuklu ZHB (Bon002) (Table 4.2), suggesting that the parents of Aşıklı 128 and of Boncuklu ZHB must have been not closer than 4th degree relatives.

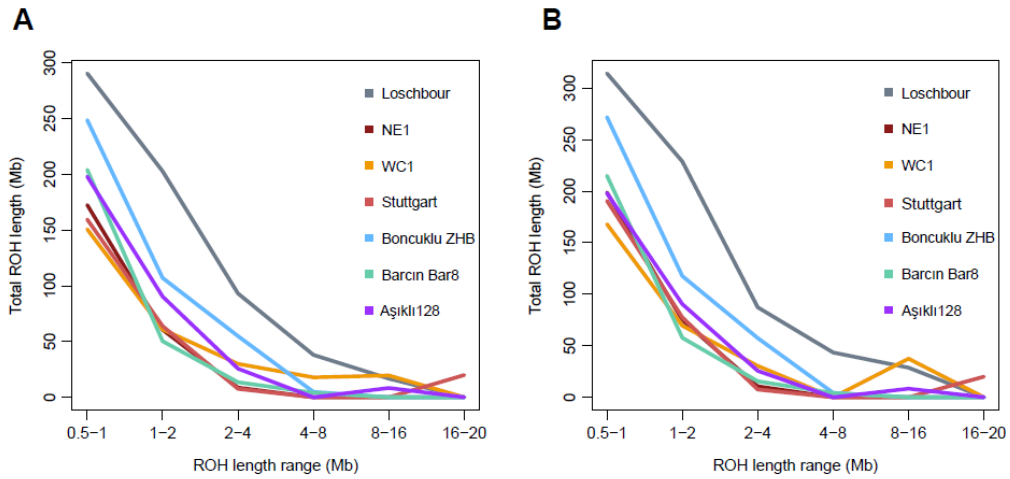

Figure 4.9 (**A**) Runs of homozygosity (*ROH*) distributions for Aşıklı 128 (Aceramic Neolithic from Central Anatolia, 8,400-7,400 cal BCE), Bon002 (Boncuklu, Aceramic Neolithic from Central Anatolia, 8300-7500 cal BCE), Loschbour (European hunter gatherer, 6,210-5,990 cal BCE), Bar8 (Ceramic Neolithic from Northwest Anatolia, 6,212-6,030 cal BCE), Stuttgart (early Neolithic from Central Europe, 5,310-5,070 cal BCE), NE1 (early European Neolithic, Hungary, 5,310-5,070 cal BCE) and WC1 (Wezmeh Cave, early Neolithic from Iran-Zagros, 7,455-7,082 cal BCE). The x-axis shows total *ROH* length of each individual in Mb, while the y-axis shows *ROH* length range in Mb. F_{ROH} values calculated from these individuals are presented in Table 4.2. (**B**) *ROH* distributions with each genome subsampled down to 5x coverage. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

Table 4.2 The table indicates the proportion of the genome covered by runs of homozygosity (*ROH*) >1.5 Mb (F_{ROH}), which is used to estimate inbreeding coefficient. Offspring of 3rd degree relatives (*e.g.*, first cousins) have expected F = 0.0625, while offspring of 4th degree relatives (e.g., offsprings of the individual's first cousins) have expected F = 0.0312. The rows are ordered from highest to lowest F_{ROH} (see Appendix Table B.3 for details about individuals). The table is adopted from Yaka et al. (under review in Curr.Biol.).

Individual	**Period and region**	F_{ROH}
Loschbour	Mesolithic C Europe	0.080
WC1	Neolithic Iran	0.035
Boncuklu ZHB (Bon002)	Neolithic Anatolia	0.031
Aşıklı 128	Neolithic Anatolia	0.023
Stuttgart	Neolithic Europe	0.016
Barcın Bar8 (M10-106)	Neolithic Anatolia	0.011
NE1	Neolithic Europe	0.010

4.4.2 Phenotypic trait analysis

A set of functional SNPs associated with phenotypes of interest (e.g., lactose tolerance, skin pigmentation, eyes color) were analysed for the Aşıklı individual with the highest coverage (5x) genome, Aşıklı 128. The reason for restricting this analysis to the highest coverage genome was to avoid excessive amounts of missing data and to be able to call diploid genotypes with at least modest confidence. We retrieved the alleles associated with predicting skin, eyes and hair color (van de Loosdrecht et al., 2018), and analysed with the HIrisPlex tool (http://hirisplex.erasmusmc.nl/) to compute the probability of skin, eyes and hair shade for the Aşıklı 128 individual. A probability of 0.968, 0.019 and 0.009 for having intermediate, very pale and pale skin colors were obtained, respectively, including higher probability of having light hair color (>0.99). Regarding the eyes color prediction, a probability of 0.547, 0.338 and 0.115 was obtained for brown, blue and intermediate colors, respectively.

Next, we analysed the derived allele variants in *MCM6* gene associated with predicting lactose tolerance in Europeans (*rs4988235*) (Enattah et al., 2002), Africans (*rs41456145, rs145946881*) (Jones et al., 2013, Ranciaro et al., 2014) and Middle Easterners (*rs41380347*) (Jones et al., 2013, Ranciaro et al., 2014) for the Aşıklı 128 individual. Aşıklı 128 shows a homozygous ancestral genotype at all four loci. Thus, Aşıklı 128 was likely lactose intolerant and could not digest milk as an adult. This result is consistent with the notion that appearance of lactose tolerance alleles occurred much later than the Neolithic (Allentoft et al., 2015, Mathieson et al., 2015).

CHAPTER 5

DISCUSSION

Through the analysis of ancient genomes from Aşıklı and Çatalhöyük, we provide new insights into the demographic dynamics and genetic composition of human populations from Central Anatolian Neolithic. We observed a low genetic diversity within the Aşıklı population and suggested that the Central Anatolian Aceramic Neolithic settlement Aşıklı, represented by eight individuals, showed a genetically homogenous population (Figures 4.8 and 4.9). This low genetic diversity and homogeneity pattern is similar to those of another Aceramic Neolithic population from Central Anatolia, Boncuklu, and these cases of Aşıklı and Boncuklu resemble to those of European hunter-gatherers (Figure 4.9), as reported in earlier studies (Kılınç et al., 2016, 2017; Feldman et al., 2019). This result, using a larger sample set with the inclusion of new genomic data from Aşıklı, supports the suggestion that population size was small in early Neolithic societies from Central Anatolia (Figure 4.9).

In contrast, the Çatalhöyük population, from Ceramic Neolithic period of Central Anatolia and represented by 14 individuals' genomes, showed higher within-group genetic diversity than Aceramic Neolithic populations (Figure 4.8). This result is consistent with previous reports that compared Boncuklu with Barcın and Tepecik-Çiftlik, and found higher genetic diversity in the latter (Kılınç et al., 2016; Kılınç et al., 2017), and also the report by Chylenski et al. (2019) who found a high diversity in maternal lineages within a group of individuals from Çatalhöyük.

What could cause increase in genetic diversity over time? We observed that the Neolithic populations from Levant and Iran showed higher genetic affinity to Ceramic Neolithic populations, Çatalhöyük, Barcın and Tepecik-Çiftlik than to Aceramic Neolithic, Aşıklı and Boncuklu (Figure 4.7). This result was again

reported by the previous studies, but using smaller sample size from Central Anatolian Neolithic sites (Kılınç et al., 2017; Feldman et al., 2019). Here, by adding genomic data from Aşıklı and Çatalhöyük, our results support the notion of a regional gene flow into Central and Northwest Anatolia between approximately 7,500-6,500 BCE. The effect of this gene flow could explain the increase in genetic diversity levels of Neolithic populations during the transition from Aceramic to Ceramic Neolithic period in Anatolia, and this shift is also contemporaneous with the changes from small villages to more complex social organizations (Özbaşaran, 2011; Baird, 2012).

The population-level analysis presented here indicated a clear genetic affinity between two Anatolian Aceramic Neolithic populations, Aşıklı and Boncuklu, relative to those of Ceramic Neolithic. In addition, Central Anatolian late Epipaleolithic, Pınarbaşı, showed equal genetic affinity to Aşıklı and Boncuklu (Figure 4.5C). These two findings suggested that the Central Anatolian early (Aceramic) Neolithic human populations had a single origin for both the Cappadocia and Konya subregions. Our results thus provide further genetic support to the archaeological hypothesis that the Central Anatolian early Neolithic groups, rather than being immigrants from the Fertile Crescent, were the descendants of local late Epipaleolithics who adopted sedentary life and farming, as previously reported in Feldman et al. (2019). In contrast, in the case of Central Europe, Hofmanová et al. (2016) showed lack of higher genetic affinity between local hunter-gatherers and early farmers in Central Europe, suggesting Central European early farmers were a mixture of local hunter-gatherers and early Neolithic groups migrated from Southwest Asia.

Whether Çatalhöyük could be the continuation of one of those Anatolian Aceramic Neolithic populations, Boncuklu or Aşıklı, has been an important question for the archaeologists working in the region due to material culture and building structure similarities among those three settlements. For example, Boncuklu and Çatalhöyük share cultural features such as lack of public buildings and division of building's floors as *"dirty"* and *"clean"* areas; while Aşıklı and Çatalhöyük are similar to

each other based on clustered building tradition in the settlements (Baird, 2012; Özbaşaran, 2011; Hodder, 2007). Our results thus provide a new insight into this discussion by showing that the Çatalhöyük population (at least the middle and late levels) was genetically equally close to the Aşıklı and Boncuklu populations (Figure 4.5C). Instead, one may hypothesize that Boncuklu and Aşıklı both genetically contributed to the Çatalhöyük population.

Another question is whether there was a genetic structure between two subregions of Central Anatolia, the Cappadocia region and the Konya Plain, which might have led to genetic continuity between Aceramic and Ceramic Neolithic populations within each subregion? If there was a genetic continuity within each part of Central Anatolia between Aceramic and Ceramic Neolithic, we would expect to observe a strong genetic affinity between Aşıklı and Tepecik-Çiftlik, and also between Boncuklu and Çatalhöyük, in the Cappadocia region and in the Konya Plain, respectively. In addition, if there was a genetic structure between these two subregions of Central Anatolia, we would also expect higher genetic affinity between Pınarbaşı and Boncuklu. However, our findings suggested no continuity within Konya and/or Cappadocia regions, as we do not observe any obvious genetic affinity between Pınarbaşı and Boncuklu (e.g. relative to Aşıklı), and also between Boncuklu and Çatalhöyük (e.g. relative to Aşıklı), or between Aşıklı and Tepecik-Çiftlik (e.g. relative to Çatalhöyük), albeit these pairs of settlements being geographically closer.

At the same time, we observed that both Çatalhöyük and Tepecik-Çiftlik showed an equal genetic affinity to Aşıklı and Boncuklu, while Aşıklı and Boncuklu are genetically closer to Çatalhöyük and Barcın, than to Tepecik-Çiftlik (Figure 4.5C and II.6C). Likewise, Çatalhöyük had more genetic affinity to Barcın relative to Tepecik-Çiftlik; while Aşıklı and Boncuklu showed a strong genetic affinity to each other. Thus, the overall results suggest that genetic differentiation between Aceramic and Ceramic Neolithic populations from Anatolia can be explained mostly temporally rather than by spatial proximity, at least in the case of Central Anatolia.

We also note that among the Anatolian Ceramic Neolithic populations, Çatalhöyük and Barcın are genetically closer to each other compared to Tepecik-Çiftlik (Figure 4.6A-B), suggesting Tepecik-Çiftlik is an outlier across among these contemporaneous Ceramic Neolithic groups. We also observed this result at individual-level *D*-statistics comparisons within each population where Tepecik-Çiftlik showed only non-significant results (Figure 4.2). The reasons for this case could be either low-quality of the Tepecik-Çiftlik genomic data or the genetic characterization of the Tepecik-Çiftlik population. On the other hand, this genetic structure of the Tepecik-Çiftlik population may be supported by archaeological evidence suggesting that Tepecik-Çiftlik showed different material culture in some aspects, compared to its contemporary settlements (Bıçakçı et al., 2012).

GENETIC RELATEDNESS PATTERNS AMONG CO-BURIALS IN ANATOLIAN NEOLITHIC SOCIETIES

CHAPTER 6

INTRODUCTION

The transition from the hunting-gathering to sedentary lifeway named as Neolithic Transition, first took place in the Fertile Crescent (c.10th - early 9th millennium cal BCE) and later spread into neighbouring regions (Baird, 2012; Price and Bar-Yosef, 2011; Düring, 2010). The initial changes during this transition from hunter-gatherers to sedentary villagers involved changes in subsistence and as well as in social rules and arrangements such as emergence of villages and increase in population size. These shifts in the lifestyle must also have led to changes in the social organization of communities (Byrd, 1994, 2000, 2005; Baird, 2012). For instance, in modern-day mobile hunter-gatherer groups, as observed in ethnographic studies, there are less social restrictions and less inequality, everyday life is more collective, and sharing among individuals is an important tradition (Hill et al., 2011; Byrd, 1994). Therefore, it has been suggested that the social organization in pre-Neolithic forager communites may also be similar, and not strictly based on family ties and social groups were not bounded by autonomous biological families (Byrd, 1994; Gamble and Boismier, 1991; Lee, 1979; Wilson, 1988). It was thus hypothesized that genetic kin-relationships were not a fundamental part of forager community social organization before the Neolithic (Byrd, 1994).

A related hypothesis is that with the emergence of sedentary settlements and a farming-based lifestyle, community organization shifted from collective foraging

groups that were not strictly organised based on genetic kinship, to more genetic kinship-centred societies (Byrd, 1994, 2000). The small early sedentary societies, in contrast to forager groups, probably had more spatial and social boundaries. It was also hypothesized that in these small and early sedentary communities, households of genetically related individuals or a group of people consisting of multiple genetically related households, played a primary role in the organization of food production and consumption (Byrd, 1994). Thus, during the shift from hunting-gathering to a sedentary lifeway, genetically-related households may have emerged as the basic unit of community and they shared residence, food production and tasks (Byrd, 1994, 2000; Flannery, 1972, 2002). But later, by the emergence of more complex organizations, social arrangement of the early sedentary communites is also thought to have changed (Byrd, 1994, 2000; Flannery, 2002; During and Marciniak, 2005).

This hypothetical description of changing social organization is inspired by the observations on changing building structures in Neolithic Southwest Asia during the transition from the earlier to later phases of Neolithic. For example, Redman hypothesized that in early Neolithic societies each building was occupied by a family and thus, a household consisted of nuclear family members (Redman, 1978). However, Flannery (1972) suggested that extended families and also small nuclear families, as households, were living in the small curvilinear buildings in the early sedentary communities from Near East (e.g., in Natufians and PPNA periods dated to c.12,500-8,500 BCE) (Flannery, 1972, 2002). In this respect, it was hypothesized that content of households could be different: (i) extended families who showed a patrilineal and polygamous family type when a man had multiple wifes, and each wife had her own small curvilinear hut, (ii) nuclear families consisting of a couple of people (e.g., a couple and their offpring) were living in the small but relatively larger curvilinear huts when a man had single wife, (iii) likewise, widows and unmaried young adults (female or male) were living in their own small curvilinear huts (Flannery, 1972, 2002).

According to Flannery's (1972) hypothesis, during a later period in Near East (e.g., PPNB period dated to c.8,500-7,000 BCE) buildings were transformed from curvilinear to rectilinear and these large rectilinear buildings housed more autonomous households who could be either those nuclear or extended families (Flannery, 1972). Flannery (1972) suggested that the reason for this shift was an increase in population size. It was hypothesized that in early sedentary communities food storage was outside of the house and may have been shared by neighbours, but, when the population size increased and the society became larger, households started to store their food productions inside the houses (Flannery, 1972, 2002). Thus, this change led to families to move into rectilinear buildings which were larger than curvilinear ones and each family became autonomous to privatize its storage (Flannery, 1972, 2002). In addition, Flannery suggested that this shift in food arrangement caused an increase in household family members, e.g., in some cases married sons of a household continued to live attached to their parents' household instead of moving to another house due to economic factors such as taking part in food production (e.g., cereal agriculture) and daily activities (e.g., to grazing herd animals) (Flannery, 2002). Therefore, it was hypothesized that nuclear family households were also transformed to extended family households during the transition from curvilinear to rectilinear buidings (Flannery, 2002). In this respect, it was suggested that households, either nuclear or extended families, were more autonomous when the houses were rectilinear, compared to households who lived in curvilinear houses (Flannery, 1972, 2002). On the other hand, another hypothesis suggested that each household consisted of nuclear family members and lived in small curvilinear buildings which may have been quite small for an extended family, and with the shift from curvilinear to rectilinear structure, these larger rectilinear buildings housed extended families (Byrd, 2000; Bar-Yosef, 2001).

Although this transition is observed across the Fertile Crescent, in Central Anatolian Neolithic sites they occurred later than in other regions, and involved new elements. During and Marciniak (2005) point out that temporal changes in the

shape of the buildings from curvilinear (sub-oval) to rectilinear (rectangular) were observed in a later period in Central Anatolia compared to those of Near East, such that during the transition from Aceramic to Ceramic Neolithic in Central Anatolia (c.mid-8th millennium cal BCE). They also note that this shift was contemporaneous with the changes in the spatial and social organization of communities such as appearance of more institutionalized societies which represent clustered neighbourhood patterns observed both in the Aceramic and Ceramic Neolithic periods in Central Anatolia (at later levels of Aşıklı and in Çatalhöyük, respectively) (During and Marciniak, 2005).

Specifically, in Central Anatolia, small curvilinear buildings were typical in the early (Aceramic) Neolithic sites (c.9th millennium cal BCE) (e.g., Boncuklu Höyük and earliest level of Aşıklı Höyük), while larger rectilinear buildings were observed in the late (Ceramic) Neolithic sites (c.8th-7th millennium cal BCE) (e.g., Çatalhöyük and Tepecik-Çiftlik Höyük) (Baird, 2012; Özbaşaran, 2011; Düring, 2010; Byrd, 2005). These curvilinear buildings were small (e.g., 13m^2 in Boncuklu Höyük and at average 9m^2 in Aşıklı Höyük) and suitable for a maximum five individuals to live, while rectilinear buildings were larger (i.e., with additional rooms) than curvilinear ones and available for more individuals (e.g., ~21m^2 in Çatalhöyük) (During and Marciniak, 2005). It was suggested that in early Neolithic societies, households may have been practising their daily activities in open areas and common spaces due to small-size of their curvilinear houses, and during these activities they were also socially interacting as a inter-households network (Baird et al., 2017; Özbaşaran, 2011; During and Marciniak, 2005). However, in the later Neolithic period, households may have started to practise daily activities and store food productions inside their rectilinear houses (During and Marciniak, 2005). At this point, some Central Anatolian Neolithic sites have a distinct feature not observed in other Fertile Crescent sites: the clustered neighbourhood pattern, which consists of multiple rectilinear buildings with the doors from roof levels and tightly clustered architecture, which is best known in Çatalhöyük (Özbaşaran, 2011; During and Marciniak, 2005). This tradition could also be observed at the later

levels of Aşıklı Höyük, and Aşıklı Höyük represents a gradual change on the building structure from curvilinear to clustered rectilinear patterns (Özbaşaran, 2011). On the other hand, rectilinear buildings dominate Çatalhöyük, representing the clustered neighbourhood tradition (Baird, 2012). Additionally, in most cases, interior doors were observed between the houses within a clustered neighbourhood (e.g., Çatalhöyük), suggesting that households may have interacted with each other through these doors. Thus, it was hypothesized that households within a clustered neighbourhood were socially bonded with each other and took part in food production together, because agriculture and food producing took a long time and required more labor (During and Marciniak, 2005).

During and Marciniak also hypothesized that household members were likely biologically related in early Neolithic communities in Central Anatolia, when architecture was curvilinear, while household members were more *socially* related, rather than organized only on genetic kin-ties, in some late (Ceramic) Neolithic period settlements in the region, at least in Çatalhöyük (During and Marciniak, 2005). This case was hypothetically described as people practicing their activities and food storage within the large rectilinear buildings, and taking part in these activities together with households from the same clustered neighbourhoods (e.g., Çatalhöyük) (During and Marciniak, 2005). Thus, this social organization could have led to a decrease in the importance of genetic kin-relations among household members in Çatalhöyük (During and Marciniak, 2005) and possibly other Ceramic Neolithic societies in the region. However, despite this archaeological and ethnographic evidence, it has still remained unclear whether household members in Central Anatolian Neolithic societies, consisted of genetically related individuals, as either nuclear or extended family, or they were bound by other factors, and how these changed over time in the region.

These aforementioned hypotheses about the social structures within sedentary communities await to be tested - but how? Herein, a tradition of burying individuals under buildings' floors, also called in-house burials, provides an opportunity using ancient genome analysis on those burials. This in-house burial

tradition is observed in most of the Anatolian Neolithic settlements, as well as in some Neolithic sites from Southwest Asia (e.g., Levant and North Mesopotamia) (Hodder, 2007; Baird, 2012). It has long been asked whether the in-house burials were people living in the house as household members, and also, whether those burials were genetically kin-related or related in a non-genetic but social way (Flannery, 2002; Byrd, 1994; Düring and Marciniak, 2005). Since these sequential changes in the social organization of Neolithic communities from early to later periods could be clearly observed in Central Anatolia, the region becomes quite important. The tradition of in-house burials provides an opportunity for genetic kinship studies in Central Anatolian Neolithic societies using ancient genome analysis. Thus, it becomes possible to test genetic kinship patterns among individuals buried in the same building who could be household.

Recent aDNA studies used a similar approach to understand familial relationships among individuals buried in the Neolithic and Bronze Age cemeteries from Europe. For example, recent ancient genome studies from European Neolithic and Bronze Age cemeteries revealed genetic kin-relationships among individuals buried in megaliths and suggested a patrilocal tradition in these populations (Mittnik et al., 2019; Sánchez-Quinto et al., 2019). Mittnik et al. (2019) studied genetic kinship among individuals buried within different sites from the Lech River valley in the southern German and identified close genetic kin-relationships (e.g., first- and second-degree) among the individuals buried within the same site compared to burials from different sites in the region. On the other hand, a group of burials who presumed to live together with those familial households, were identified as genetically unrelated, and this finding suggested institutionalized social structure (Mittnik et al., 2019). In another recent study, burials from five megalith sites in North and West Europe were studied using genetic kinship analysis. In Sánchez-Quinto et al.'s (2019) study, close genetic kin-relations were found among pairs of individuals buried in the same megaliths, except one genetically related pair (second-degree) who were buried in different megalith sites (Sánchez-Quinto et al., 2019). In addition, these megaliths contained more male

burials than female ones. Thus, in the light of these findings, Sánchez-Quinto et al. (2019) suggested patrilineal population structure and also the geographical expansion of these populations.

In the case of Central Anatolia, to date, there have been a number of studies based on mtDNA and dental morphology analysis, investigating genetic kin-relations among households in a Ceramic Neolithic society from Central Anatolia, specifically in Çatalhöyük (Chyleński et al., 2019; Pilloud and Larsen, 2011). In the study using dental morphology, biological distance between co-burials were calculated using dental samples (n=226) from Çatalhöyük, employing both metric and nonmentric measurements (Pilloud and Larsen, 2011). Pilloud and Larsen's (2011) study revealed that co-buried individuals were not biologically more closely related to each other compared to burials from other buildings, suggesting Çatalhöyük may have been a society that was organized based on social relationships rather than genetic kin-relations (Pilloud and Larsen, 2011). Another study on genetic kinship among co-burials from Çatalhöyük, analyzed mitochondrial genomes of co-buried individuals (n=10) in the buildings of a clustered neighbourhood structure (Chyleński et al., 2019). Chyleński et al. (2019) showed a high mtDNA diversity among co-buried individuals in Çatalhöyük. The findings of Chyleński et al.'s (2019) study suggested that individuals buried in the same building in Çatalhöyük do not have to be genetically related, at least maternally, consistent with the earlier report on dental data (Pilloud and Larsen, 2011).

However, these aforementioned studies from Çatalhöyük had limited resolution to examine genetic kin-relationships among co-burials who could be households. First, mtDNA is only inherited from the mother and only reveals genetic information about the maternal lineage. Second, dental morphology analysis provides limited genetic information to infer genetic kinship among individuals. The reasons for this could be: (i) dental morphological traits represent only phenotypic data and sharing a dental characteristic between genetically unrelated individuals may also be coincidental in some cases, (ii) dental traits show polygenic

genetic nature and environmental factors may also affect the expression of these genes (Vai et al., 2020). Thus, the question about genetic kinship patterns among co-buried individuals in Anatolian Neolithic settlements has awaited to be addressed by ancient genome analysis. In this study, we aim (i) to test genetic kin-relationships among co-buried individuals in Anatolian Neolithic societies using ancient genomes and (ii) also to investigate possible shifts of genetic kinship patterns among co-burials in Anatolian Neolithic communities through time.For this, we analysed burials from five Neolithic sites located in Central and Northwest Anatolia including both earlier and later periods. As explained in the Chapter 4, we generated new genomes from Aşıklı Höyük (n=8) and Çatalhöyük (n=14) in this study, and combined these with published ancient genomes from Boncuklu Höyük (n=9), Barcın Höyük (n=23) and Tepecik-Çiftlik Höyük (n=5), and also with archaeological/anthropological information to confirm pedigree relationships among co-buried individuals. As explained in the Chapter 1 Section 1.3 in detail, Aşıklı Höyük and Boncuklu Höyük are Aceramic Neolithic sites from Central Anatolia dated to c.9th to mid-8th millennium cal BCE, and the remaining three sites belong to Ceramic Neolithic period dated to c.7th-6th millennium cal BCE; Çatalhöyük and Tepecik-Çiftlik Höyük are from Central Anatolia, while Barcın Höyük is from Northwest Anatolia. In this study, for the first time, genomic data from Neolithic Anatolia was used to investigate genetic kinship among intramural co-buried individuals, to better understand the social structures and traditions of Neolithic societies from this region.

CHAPTER 7

MATERIALS AND METHODS

For this part of the thesis, we used the same newly generated genomes from Aşıklı (n=8) and Çatalhöyük (n=14) (Table 4.1 and Appendix Table B.2) (see Chapter 4 for details about the generated data) and combined these with published genomic data from other Neolithic settlements in Central and Northwest Anatolia; Boncuklu (n=9), Barcın (n=23) and Tepecik-Çiftlik (n=5) (see Appendix Table B.2 for details).

7.1 Estimating genetic relatedness

We estimated genetic relatedness among individuals buried in the same and/or proximate buildings from each Anatolian Neolithic settlement using ancient genomes. For this, we first estimated the kinship coefficient (θ) between each pair of individuals from Anatolian Neolithic communities (Appendix Table B.3). The kinship coefficient is defined as the probability of identical-by-descent (*IBD*) allele sharing between a pair of individuals, more specifically, the probability that one allele randomly selected from each individual is derived from a recent common ancestor (Speed and Balding, 2014; Hanghøj et al., 2019; Lipatov et al., 2015). The kinship coefficient (θ) can be estimated from allele sharing data using Cotterman coefficients (k_0, k_1, k_2), which are the probabilities of sharing 0, 1 and 2 alleles via identity-by-descent between a pair of individuals, such that $k_0 + k_1 + k_2 = 1$. These values can be estimated and used to calculate the kinship coefficient, as $\theta = k_1 / 4 + k_2 / 2$ (Hanghøj et al., 2019).

Here, we used three different software to estimate genetic relatedness for each pair; *NgsRelate* (Hanghøj et al., 2019) and *lcMLkin* (Lipatov et al., 2015), which

estimate θ by measuring allele sharing relative to a population background using maximum likelihood, and *READ* (Monroy Kuhn et al., 2018), which is based on a heuristic approach that calculates allele sharing across a pair of genomes, and then normalizing this sharing statistic with the allele sharing between likely unrelated individual pairs. Although, there are other commonly used software for genetic kinship estimation (e.g., *KING* and *PLINK*) (Manichaikul et al., 2010; Purcell et al., 2007, respectively), the reasons for preferring these three software were: (i) these three software can estimate genetic relatedness accurately for low-coverage genomes (e.g., 1x or lower) and ancient genomes are mostly low-coverage, while *KING* and *PLINK* are used for high-coverage genomic data where fully diploid genotypes can be reliably called (e.g., 20x) (Hanghøj et al., 2019; Lipatov et al., 2015), (ii) Instead of SNP-calling, which is not accurate when genome coverage is low, *NgsRelate* and *lcMLkin* use genotype likelihoods to calculate the kinship coefficient, which makes them suitable for ancient genome data (Hanghøj et al., 2019; Lipatov et al., 2015). In addition, *NgsRelate* takes into account the possibility that the studied individuals may have been inbred when calculating θ, while the other softwares cannot estimate inbreeding. Meanwhile, *NgsRelate* (Hanghøj et al., 2019) and *lcMLkin* (Lipatov et al., 2015) use maximum likelihood with expectation maximization to estimate θ, while the *READ* software (Monroy Kuhn et al., 2018) uses a non-parametric approach. We thus used these three software, which use partly different approaches (see the next sections for details about three software) to identify genetic kin-relationships between co-buried individuals, and we used consistency among the three software's estimates as a measure of confidence in our genetic kinship estimates.

We estimated pedigree relationships among individuals down to the 3rd-degree relatedness. We restricted our analysis to at least 5,000 overlapping SNPs between each pair of individuals, because we observed that lower numbers of overlapping SNPs could yield unreliable results (Figure 8.1).

We further estimated X-chromosomal θ using *NgsRelate* as described below. In the genetic kinship analyses the *BAM* files as input data were used for all three

software with the autosomal (Dataset2) and X-chromosomal (Dataset3) datasets (Chapter 3, Materials and Methods).

Moreover, we used additional information (e.g., mtDNA haplogroups, age-at-death and radiocarbon dates) for the evaluation of pedigree relationships inferred for pairs estimated to be genetically closely related such as first-degree relatives. For this, we combined genomic data with anthropological and archaeological evidence (Table 8.2). For example, a female-male adult pair who was estimated as parent-offspring using genomic data could be either mother-son or father-daughter. In this case, we used mtDNA haplogroup sharing between these individuals to identify their pedigree relationships, such that if they share the same mtDNA haplogroup, they are probably mother and son. To confirm that they are likely mother-son, we also used consistency between the radiocarbon dates of individuals. In another example, we used age-at-death information to infer that a female first-degree pair were sisters, given that they were subadults (child and infant) and could not be mother and daughter.

7.1.1 *NgsRelate* analysis

To identify genetic kinship among co-buried individuals from Anatolian Neolithic populations, including Aşıklı and Çatalhöyük, we first used *NgsRelate* software (version 2, Hanghøj et al., 2019). *NgsRelate* uses genotypes of two individuals and population allele frequencies as background as input, and employs maximum likelihood and the expectation maximization algorithm to estimate Cotterman coefficients. It is reported to estimate genetic relatedness with high accuracy between pairs of individuals down to 5th degree (i.e., grandparents-grandchilds are second-degree, first cousins are third-degree, etc.) from low coverage data down to 1x (Hanghøj et al., 2019). *NgsRelate* takes into account possible inbreeding between two individuals when calculating the kinship coefficient and estimates the inbreeding coefficient within individuals along with Cotterman coefficients. In brief, *NgsRelate* calculates the kinship coefficient θ (theta) using identity-by-

descent (*IBD*) approach, as $\theta = k_1 / 4 + k_2 / 2$ (as described above). *NgsRelate* can use genotype likelihoods as input, instead of genotype calls, which presents lower accuracy for low-coverage ancient genomic data. Here, we used genotype likelihoods calculated by the *ANGSD* program (http://www.popgen.dk/angsd/). As background, we used population allele frequencies calculated from 60 Anatolian Neolithic individuals (Appendix Tables B.2 and B.3).

7.1.2 *lcMLkin* analysis

lcMLkin was used as the second software. Similar to *NgsRelate*, *lcMLkin* also implements a maximum likelihood approach to estimate genetic kinship, again using population allele frequencies as background. It is also based on genotype likelihoods instead of calling a single best genotype for each individual. According to the authors, *lcMLkin* can accurately estimate genetic relatedness down to 3rd degree (i.e., avuncular and half-siblings are second-degree, cousins are third-degree) from low-coverage data (minimum 2x per individual of a pair) (Lipatov et al., 2015). *lcMLkin* calculates the relatedness coefficient (r), which is twice the kinship coefficient (θ), again using population allele frequencies. Another difference from *NgsRelate* is that *lcMLkin* assumes no inbreeding within individuals (Lipatov et al., 2015). *lcMLkin* also estimates k_0, k_1 and k_2 (Cotterman coefficients) using identical-by-descent (*IBD*) approach between two individuals and calculates the relatedness coefficient, as $r = k_1 / 2 + k_2$. Thus, the $r/2$ value is equivalent to kinship coefficient (θ) (Figure 8.2A). Here, again 60 Anatolian Neolithic individuals were used to calculate population allele frequencies (Appendix Tables B.2 and B.3).

7.1.3 *READ* analysis

As the third approach, we used *READ* software to estimate genetic relatedness between studied pairs of individuals. *READ* calculates and normalizes the mismatch rate (nonmatching alleles' proportion, *P0*) in non-overlapping windows

of 1 Mbps across the genome using pseudohaploid data (Chapter 3, section 2.8 SNP-calling and dataset preparation). First, *P0* is calculated for each individual-pair and normalized by the median *P0* accross the pairs. Thus, this analysis is run including at least two unrelated pairs from the same group (Monroy Kuhn et al., 2018). Next, *READ* uses these values to infer the degree of relationship. The normalized 1-*P0* values are theoretically equivalent to the kinship coefficient (θ) (Figure 8.2A). *READ* thus classifies *P0* values above 0.625 as first-degree (e.g., parent-offspring and siblings), and 0.8125-0.90625 as second degree (e.g., grandparent-grandchild, uncles/aunts-niece/nephew and half-siblings), and above 0.90625 as more distant / unrelated (Monroy Kuhn et al., 2018). The authors of *READ* do not suggest inferring kinship beyond 2nd degree (Monroy Kuhn et al., 2018).

7.1.4 Genetic relatedness estimation on X chromosome

To help distinguishing different types of pedigree relationships (e.g., sisters, mother-son) among the individual pairs that were estimated as first-degree related, we calculated X-chromosomal θ using *NgsRelate* with Dataset3 (Chapter 3, Materials and Methods). Next, the ratio of autosomal versus X-chromosomal θ for each first-degree related pair was calculated and compared (Figure 8.2). We also applied the same method to one second-degree pair. We used these ratios to infer pedigree relationships, based on different theoretically expected values, e.g. this ratio is 0.5 for a mother-son pair, and 1 for a brother-sister pair (Table 7.1) (Thornton et al., 2012).

Table 7.1 Table shows expected autosomal and X chromosomal (chrX) kinship coefficients (θ) including Cotterman coefficients for first-degree relatives without inbreeding in the pedigree (Thornton et al., 2012). The table is adopted from Yaka et al. (under review in Curr.Biol.).

Kin-relationship	**Autosomal θ**	**chrX θ**	k_0	k_1	k_2
Mother-daughter	0.25	0.25	0	1	0
Mother-son	0.25	0.5	0	1	0
Father-daughter	0.25	0.5	0	1	0
Father-son	0.25	0	0	1	0
Sisters	0.25	0.375	0.25	0.5	0.25
Brothers	0.25	0.5	0.25	0.5	0.25
Brother-sister	0.25	0.25	0.25	0.5	0.25

CHAPTER 8

RESULTS

In this part of the study, we carried out genetic relatedness analysis among n=32 co-buried individuals, i.e. the individuals buried in association with (under the floors of or near by) the same building or proximite buildings, in different Neolithic settlements from Anatolia, to gain understanding into social organization of these communities (Table 8.1 and Appendix Table B.2). We studied and compared genetic kinship patterns using data generated from Aşıklı and Çatalhöyük in this study, and also using published genome data from Boncuklu, Barcın and Tepecik-Çiftlik (Appendix Table B.2). Three software were used to estimate genetic relatedness among individuals down to 3rd-degree: *NgsRelate* (Hanghøj et al., 2019), *lcMLkin* (Lipatov et al., 2015), and *READ* (Monroy Kuhn et al., 2018) (see Materials and Methods for details). We also used additional sources of information to decide on pedigree relationships.

We performed genetic kinship analysis on pairs of individuals and used a minimum of 5,000 overlapping SNPs for each pair. The reason for this choice was based on empirical observations, shown in Figure 8.1, where we estimated θ between all 1770 pairs of Anatolian Neolithic individuals. Studying the results we observed a number of implausible cases. For example, θ was calculated as 0.02 (4th-5th degree related) by *NgsRelate* based on 3,044 ovelapping SNPs between the Aşıklı 2 and Barcın M10_352 individuals, who are from different populations with approximately 1000 years (~40 generations) between them. In another case, the kinship coefficient (θ) was estimated as 0.71 (first-degree with inbreding) based on 1,662 overlapping SNPs for the Aşıklı 2 - Aşıklı 40 pair, although these two individuals are separated by more than 300 years according to their radiocarbon dates. Meanwhile, all our estimates of first- and second-degree relatives with

>5,000 SNPs involved pairs who could plausibly be closely related (i.e. from the same settlement and period). Given these results, we suggest that a threshold of 5,000 overlapping SNPs should be a reliable cutoff.

Using the same data we inferred that with minimum 5,000 overlapping SNPs we should able to estimate genetic relatedness down to 3rd-degree related pairs (Figure 8.1). The pairs that are more distantly related than 3rd-degree and/or were unrelated were identified as "unrelated".

We further estimated X-chromosomal θ and compared this with autosomal θ to be able to identify pedigree relationships among genetically related pairs (Figure 8.2 and Table 8.1). Beside this, we used probabilities of sharing 0, 1 or 2 alleles identical-by-descent (Cotterman coefficients) estimated based on autosomal data, as well as the information from mitochondrial and Y-chromosome haplogroups of pairs, anthropological age-at-death estimates and consistency of radiocarbon dates (Table 8.1). These additional information sources allowed us to differentiate first-degree kin-relationships such as mother-son and brother-sister pairs (Figure 8.2B-C and Table 8.2).

In addition, we made use of results of pedigree simulations, where θ was estimated using *NgsRelate* and 5,000 overlapping SNPs for simulated pairs of known relationships (simulations were carried out by Igor Mapelli from our group) (Yaka et al. under review in Curr.Biol.). The simulations were performed using genomic data of modern-day Tuscany individuals (n=107) (1000 Genomes dataset phase 3) (Auton et al., 2015) who are not genetically related, and using n=2,000 simulations to estimate autosomal kinship coefficients for first-, second-, third-degree related and unrelated pairs. Next, the ranges of expected autosomal θ for different pedigree relationships (e.g., parent-offspring, siblings) were calculated using the 0.025 and 0.975 quantiles of the autosomal θ distributions (by Igor Mapelli from our group). We used these autosomal θ ranges as reference for the estimated θ from our low-coverage ancient data (Yaka et al., under review in Curr.Biol.). These simulated data was used to evaluate the accuracy of kinship coefficient estimations from low-

quality data generated in this study. In addition, the expected Cotterman coefficients were also calculated for each degree of pairs using n=700 simulations (Figure 8.2C) (for details about the simulations, see Yaka et al., under review in Curr.Biol.)

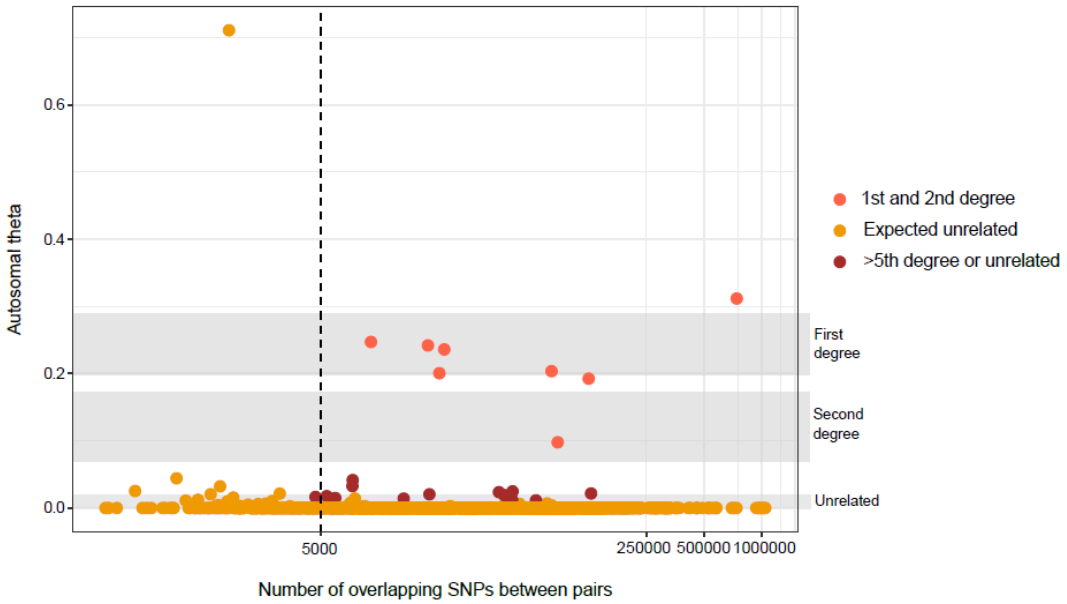

Figure 8.1 The influence of overlapping SNP numbers on the reliability of kinship estimates. The figure shows a threshold of overlapping SNPs between a pair of individuals to estimate the autosomal kinship coefficient with a higher accuracy. In total 1770 individual pairs from 60 Anatolian Neolithic individuals were used. The x-axis is the number of overlapping autosomal SNPs and the y-axis is autosomal kinship coefficient estimated for pairs of individuals using *NgsRelate* (see Materials and Methods for details). The orange points indicate pairs expected to be unrelated given their distance in time and space. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

8.1 Genetic relatedness patterns among co-buried individuals in Anatolian Neolithic populations

Aşıklı Höyük: Our kinship analyses revealed first-degree genetic relatedness among contemporaneous five individuals from the same and earliest level of Aşıklı Höyük. These individuals were all females and were co-buried in two proximate buildings (Figure 8.3). A child (Aşıklı 128) and an old-adult (Aşıklı 133), buried in separate, but close, buildings, were identified as sisters (Figures 8.2 and 8.3, Table 8.2). Another pair of individuals (Aşıklı 131 and 136) buried in the same building, was likely a sister pair. Other pairs of Aşıklı individuals were determined as unrelated.

In addition, statistical calculation of radiocarbon ages showed that those five Aşıklı individuals lived at the same time period ($\chi 2=7.6$, $\chi 2(5\%)=9.5$, $\nu=4$; Appendix Table B.2; calculated by Alex Bayliss). Although, Aşıklı 128-129 were buried in the same building and Aşıklı 133 was from a proximite building, we did not identify any genetic kin-relationships between either Aşıklı 128-129 or Aşıklı 129-133 pairs (Figure 8.3). Meanwhile, Aşıklı 128-129 were buried in the same building and also belonged to the same mtDNA haplogroup, but a genetic kin-relation was not observed for the Aşıklı 128-129 pair down to third-degree, based on their genomic data (Figure 8.3).

Boncuklu Höyük: We studied published genomes of nine contemporaneous individuals from Boncuklu Höyük buried in three buildings or in external spaces. Here, two pairs of first-degree related individuals were determined among five co-buried individuals from close proximate buildings. One female-male old adult-adult pair, buried in the same building, was identified as possibly mother and son; while another female-male adult pair, buried in two neighboring buildings, was likely a brother-sister pair (Figures 8.2 and 8.3, Table 8.2).

Here, it was calculated from radiocarbon dates that with 90% probability old-adult female (Boncuklu ZHJ) died earlier than the adult male, suggesting that the former

might have been the mother of the latter (Appendix Table B.2; calculated by Alex Bayliss). Interestingly, although a female baby (Boncuklu ZHAG) was found co-buried with the adult female (Boncuklu ZHAF) of the brother-sister pair, and also had the same mtDNA haplogroup with this brother-sister pair, we did not find any genetic relatedness between the baby and the brother-sister pair down to third-degree based on genomic data analysis (Figure 8.3).

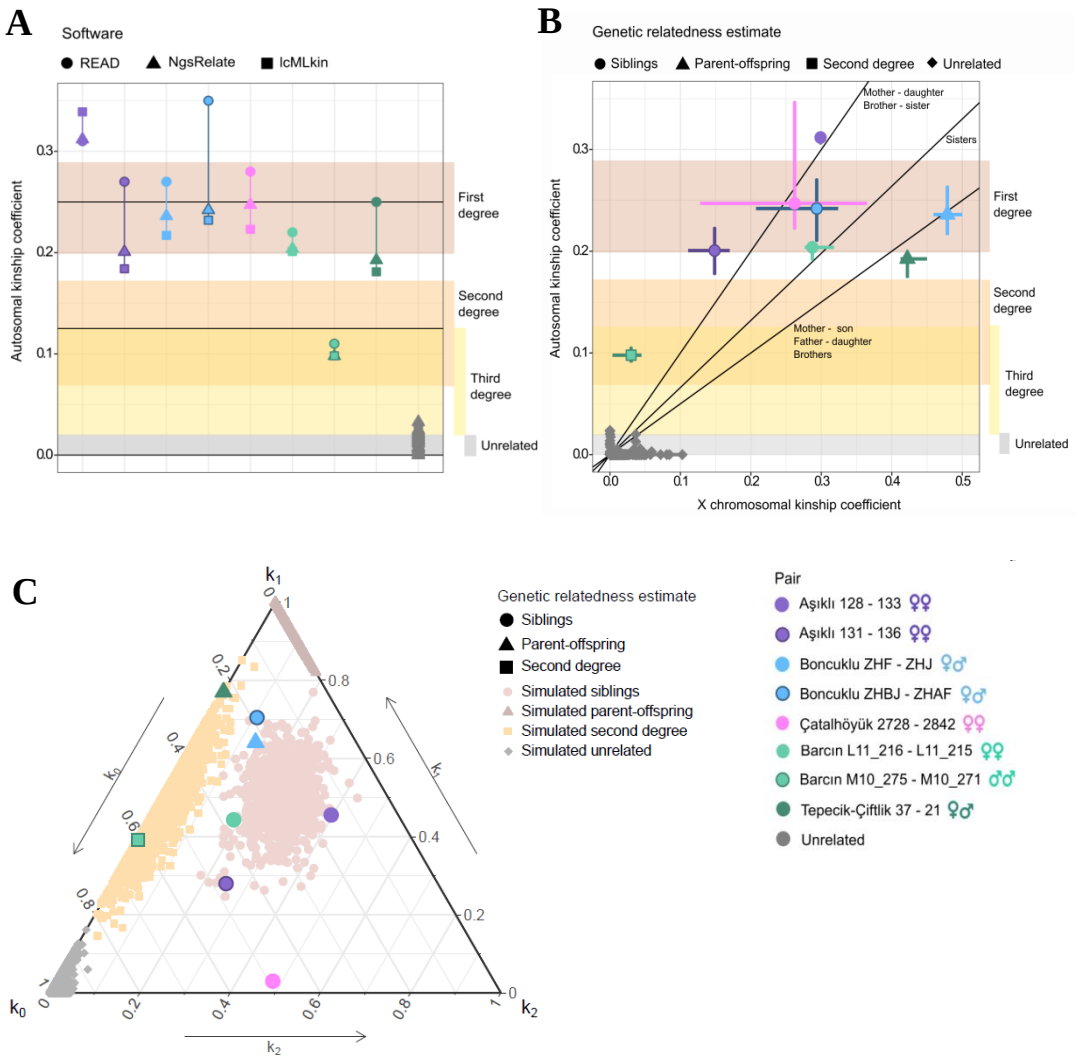

Figure 8.2 Genetic kinship estimates for co-buried pairs of individuals from Neolithic Anatolia using genomic data. (**A**) The figure shows the distribution of autosomal kinship coefficient (θ) estimated for each genetically related pair using three different software. The horizontal black lines represent expected autosomal θ values for the first- and second-degree related and unrelated pairs. (**B**) Kinship coefficients calculated between pairs of individuals using both autosomal and X chromosomal data with *NgsRelate* software. The horizontal and vertical point-bars show autosomal and X chromosomal θ estimates based on ten bootstraps, respectively. The black lines show theoretically expected ratios calculated between autosomal and X-chromosomal θ values for first-degree pedigree relationships of different sex (e.g., mother-son, sister, brother-sister etc.). In both panels **A** and **B**, the horizontal coloured bars show expected θ ranges based on simulation using

NgsRelate and 5,000 SNPs. **(C)** Probabilities of sharing 0, 1 or 2 alleles identical-by-descent (Cotterman coefficients) calculated within each individual-pair using autosomal data and *NgsRelate.* The coloured small dots indicate expected values based on the simulations. The figure is adopted from Yaka et al. (under review in Curr.Biol.).

Çatalhöyük: We studied genome data of 14 Çatalhöyük individuals from different levels, consisting of one adult and 13 subadults (child or infants) including both sexes, females (n=10) and males (n=4). Twelve individuals were contemporaneous subadult burials from five buildings (Appendix Table B.2). One female subadult pair (an infant and a child) buried in the same building was determined as first-degree related and inferred as sisters (Figures 8.2 and 8.3, Table 8.2). Radiocarbon dates of the related pairs provided consistent results ($\chi2$=0.0, $\chi2$(5%)=3.8, ν=1; Appendix Table B.2; calculated by Alex Bayliss). Other pairs of individuals except the mentioned subadult sister pair were identified as genetically unrelated.

Barcın Höyük: For Barcın Höyük, we analysed published genomes of 23 individuals from different levels (VIa, VIb and VIc or VId2/3); ten of those were associated with three or possibly four buildings. Two pairs of close relatives were determined among 23 individuals; one female-female subadult pair was identified as sisters (first-degree) and one male-male subadult pair was found to be second-degree related, as half-siblings or uncle-nephew (Figures 8.2 and 8.3, Table 8.2). Those related pairs were also buried close to each other (Figure 8.3). Radiocarbon dates of the both related pairs provided consistent results (L11_216 and L11_215, $\chi2$=0.7; M10_271 and M10_ 275, $\chi2$=0.2, $\chi2$(5%)=3.8, ν=1 for both; Appendix Table B.2; calculated by Alex Bayliss). Other individual pairs were not identified as genetically related, including four individuals who were infants and buried in the same building (Building-4) (Figure 8.3).

Tepecik-Çiftlik: Published genomes of five individuals from two levels in Tepecik-Çiftlik Höyük were analysed. One female-male adult pair, buried in the

same building, were identified as first-degree related, probably a mother and son pair (Figures 8.2 and Table 8.2). It was calculated from radiocarbon dates that with 96% probability the adult female died before the adult male (Appendix Table B.2; calculated by Alex Bayliss). Other pairs of individuals were not genetically related.

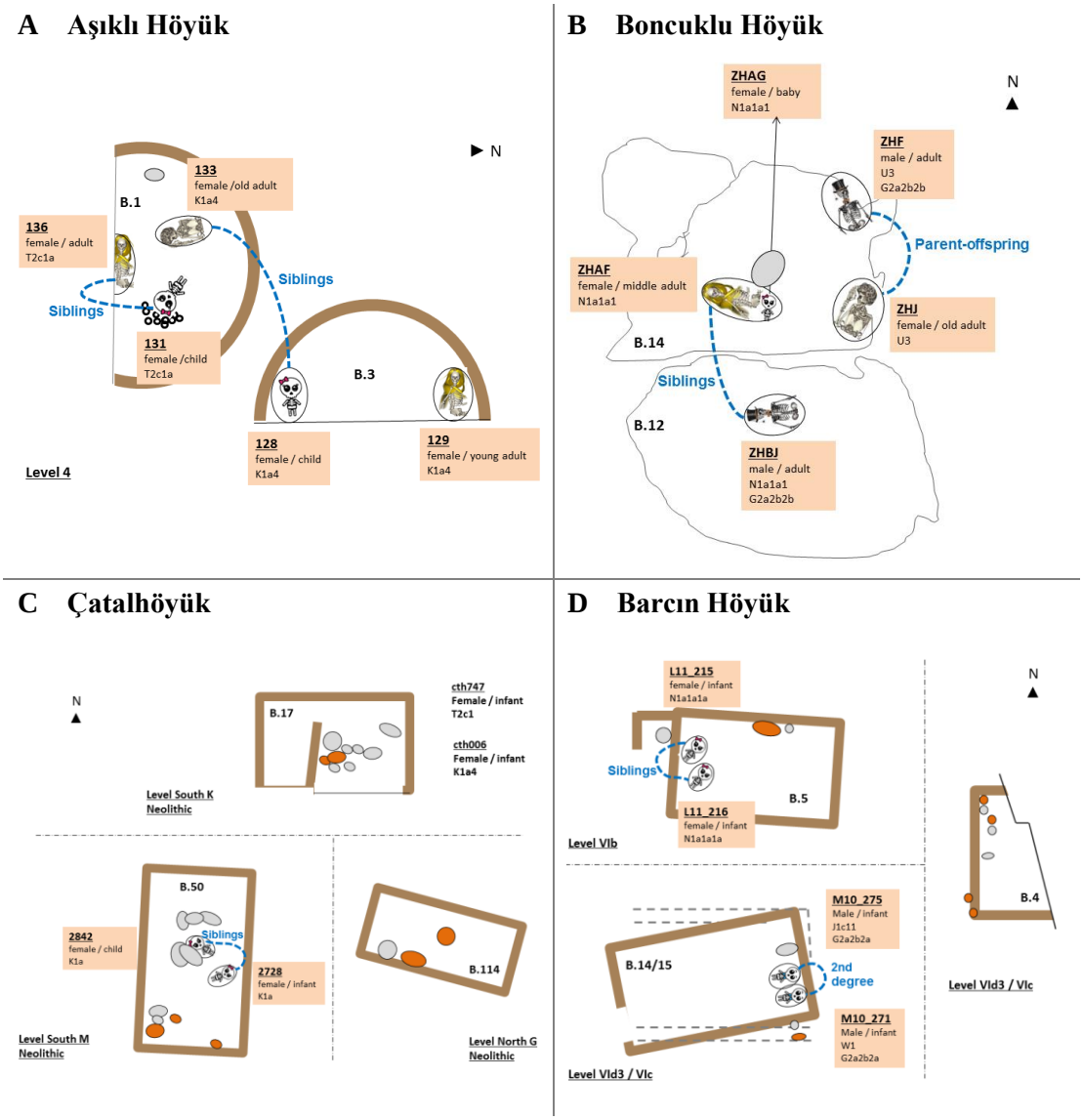

Figure 8.3 Schematic representation of contemporaneous co-buried individuals from (**A**) Aşıklı (n=5), (**B**) Boncuklu (n=5), (**C**) Çatalhöyük (n=9) and (**D**) Barcın (n=10). Brown-color lines represent the buildings with different shapes. Orange-color and grey-color circles show genetically unrelated with DNA data and no

DNA data individuals, respectively. Pinky-color boxes present the information of each individual; individual ID, sex, age-at-death and mtDNA haplogroup, respectively, and additionally Y chromosome haplogroup for males. The figure was prepared based on the design from Yaka et al. (under review in Curr.Biol.).

Our findings on the different patterns of genetic kin-relationships among co-burials from the same or proximate buildings in Neolithic Anatolian societies, suggest that these groups of people, who could potentially be household members, included pairs who showed close genetic kinship. This pattern was especially clear in the Aceramic Neolithic populations from Central Anatolia, Aşıklı and Boncuklu.

On the other hand, we identified only one pair as first-degree related (an infant-child pair buried in the same building) among co-burials in Çatalhöyük (1/17) (Table 8.1, Figure 8.3). A similar pattern of low rate of genetic kinship was observed where we identified only two pairs of close relatives as first- and second-degree relatedness among co-buried individuals in Barcın (2/12) (Table 8.1, Figure 8.3). The high frequency of co-buried pairs from Çatalhöyük (94%) and Barcın (84%) were genetically unrelated (Table 8.1). This suggests that co-burials, who are possible households (in this case, the children of households), might not consist of genetically close relatives in these societies. This finding suggests that frequencies of genetic kinship among co-buried individuals could be different between Anatolian Aceramic and Ceramic Neolithic societies, and indeed, the frequencies of co-burials with genetic kin are different when we compare Aşıklı and Boncuklu to Çatalhöyük and Barcın (Fisher's exact test P=0.019, Table 8.1).

Table 8.1 Table shows information about the studied individual and pairs from each site. "Contemporaneous pairs" are calculated based on number of individuals from the same or similar archaeological levels. "Co-buried pairs" are calculated using number of co-buried individuals in the same or proximate buildings. The table is adopted from Yaka et al. (under review in Curr.Biol.)

Site	**Individuals studied**	**Contemporaneous pairs**	**Co-buried pairs**	**Genetically related pairs**		**Kin pairs among co-burials (%)**	**Co-buried individuals**	**Genetic kin**
				First degree	**Second degree**			
Aşıklı	8	10	10	2	0	20 (2/10)	5	4
Boncuklu	9	36	10	2	0	20 (2/10)	5	4
Çatalhöyük	14	66	17	1	0	6 (1/17)	10	2
Barcın	23	107	12	1	1	16 (2/12)	10	4
Tepecik-Çiftlik	5	4	1	1	0	100 (1/1)	2	2
TOTAL	59	223	50	7	1	-	32	16

Table 8.2 The evaluation of pedigree relationships inferred for pairs estimated to be genetically closely related. The evaluation was done combining genomic data with anthropological and archaeological evidence. In the table a brief discussion is provided for each pair about how the most likely relationship was inferred. Cotterman coefficients (k_0, k_1, k_2) indicate the probabilities of sharing 0, 1 or 2 alleles identical-by-descent for each pair and expected values for these statistics are given in Table 7.1. The table is adopted from Yaka et al. (under review in Curr.Biol.) (Radiocarbon dates were recalibrated and the probability of death-dates for pairs were calculated by Alex Bayliss, see Appendix Table B.2 for details).

Pair	**Most likely pedigree relationship**	**Autosomal θ**	**chrX θ**	**Autosomal Cotterman coefficients**			**MtDNA haplo group**	**chrY haplo group**	**Osteological age-at-death**	**Autosomal number of SNPs**	**chr X number of SNPs**	**C14 date (95% cal BCE)**
				k_0	k_1	k_2						
Aşıklı 128-133	Sisters	0.31	0.29	0.15	0.45	0.40	Same	-	Child and old adult	741,193	21,452	8225–7955 8170-7735
	We inferred this pair as likely sisters (as opposed to mother-daughter) due to their low k_1 values, although their low X chromosomal θ could also be consistent with a mother-daughter pair. Notably, the Çatalhöyük and Barcın sister pairs also display similar statistics. They may have been contemporary based on their radiocarbon data.											
Aşıklı 131-136	Sisters	0.20	0.15	0.46	0.28	0.25	Same	-	Child and adult	20,810	790	8200-7740 8175-7655
	We inferred this pair as likely sisters (as opposed to mother-daughter) due to their low k_1 values, although their low X chromosomal θ could also be consistent with a mother-daughter pair. Notably, the Çatalhöyük and Barcın sister pairs also display similar statistics. They may have been contemporary based on their radiocarbon data.											

Table 8.2 (continued)

Boncuklu ZHJ-ZHF	Mother- son	0.24	0.48	0.22	0.62	0.13	Same	-	Old adult and adult	22,076	566	8295-8240 8225-7940
	We inferred this pair as parent-offspring (as opposed to brother-sister) due to their relatively high X chromosomal θ, their relatively high autosomal k_1 values and their age-at-death and C14 dates. A mother-son relationship is more likely than a father-daughter relationship given mtDNA haplogroup sharing and their age-at-death and C14 dates (Table 4.1). Their radiocarbon data suggests that it is 90% probable that the woman (ZHJ) died first.											
Boncuklu ZHBJ-ZHAF	Brother-sister	0.24	0.29	0.18	0.67	0.10	Same	-	Middle adults	18,136	563	>7952 8285-8010
	We inferred this pair as likely brother-sister (as opposed to parent-offspring) due to their low X chromosomal θ, but their relatively high autosomal k_1 could also be consistent with a mother-son pair. A father-daughter pair is also possible but even less likely due to mtDNA haplogroup sharing. They may have been contemporary based on their radiocarbon data.											
Çatalhöyük 2728-2842	Sisters	0.25	0.26	0.49	0.03	0.48	Same	-	Infant and child	9,151	238	6695-6506 6690-6506
	We inferred this pair as sisters (as opposed to mother-daughter) due to their subadult age. Their radiocarbon data also suggests they may have been contemporary.											
Barcın L11_216-L11_215	Sisters	0.20	0.29	0.37	0.44	0.19	Same	-	Both infants	80,115	5,195	c.6200-6100 6320-6080
	We inferred this pair as sisters (as opposed to mother-daughter) due to their subadult age. Their radiocarbon data also suggests they may have been contemporary.											

Table 8.2 (continued)

Barcın M10_275-M10_271	Paternal half- siblings or uncle-nephew	0.09	0.03	0.61	0.39	0.0	Different	Same	Both infants	86,247	3,313	6405-6240 6425-6250
	We inferred this pair as paternal half-siblings or an uncle-nephew pair (as opposed to maternal half-siblings or a grandparent-grandchild pair) due to their sharing of Y chromosomal but not mtDNA haplogroups and their young age. A third-degree relationship (paternal cousins) is also possible. Their radiocarbon data suggests they may have been contemporary.											
Tepecik-Çiftlik 37-21	Mother- son	0.19	0.42	0.23	0.77	0.00	Same	-	Adult and old adult	125,110	3,204	6385-6100 6225-6070
	We inferred this pair as likely mother-son (as opposed to brother-sister) due to their high X chromosomal θ, their high autosomal k_1 value and their C14 dates, although a father-daughter pair is also possible, but less likely. Their radiocarbon data also suggests that it is 96% probable that the woman (Individual 37) died first.											

CHAPTER 9

DISCUSSION

In this study, we provide the first insights into genetic relatedness patterns among co-buried individuals in Aceramic and Ceramic Neolithic societies from Central and Northwest Anatolia. We used newly generated genomes from Aşıklı Höyük and Çatalhöyük, and combined this with published genomic data from Boncuklu Höyük, Barcın Höyük and Tepecik-Çiftlik Höyük. Most notably, we identified close genetic kin-relations (e.g., first- and second-degree) among individuals buried in or around the same and close proximity buildings, i.e. co-buried groups, in both Aceramic and Ceramic Neolithic populations from Anatolia (Figure 8.2 and 8.3).

This result is important in the context of the hypothesis, partly based on ethnographic evidence, that curvilinear buildings in early Neolithic societies were occupied by genetically kin-related households, but household members became less kin-tied by the shift from curvilinear to rectilinear buildings during the transition from early to later Neolithic in Central Anatolia (Byrd, 1994, 2000; During and Marciniak, 2005). In line with this hypothesis, we found that the proportion of the first-degree relatives (e.g., siblings and mother-son) between Aceramic and Ceramic Neolithic is different (Table 8.1). We identified first-degree pedigree relationships at a higher frequency among co-buried individuals in the Aceramic Neolithic societies from Central Anatolia (Aşıklı and Boncuklu), while the frequency of close relatives is lower in Ceramic Neolithic societies from both Central and Northwest Anatolia (Çatalhöyük and Barcın, respectively) (Table 8.1), and this difference between Aşıklı and Boncuklu versus Çatalhöyük and Barcın was significant (Fisher's exact test P=0.019, Table 8.1). Thus, our findings provide genetic support to the aforementioned hypothesis that the possible household members in the earliest Neolithic societies frequently showed close genetic kin-

relations to each other, at least in Central Anatolia. In addition, our results are in line with the notion that this pattern may have changed during the population growth and the transition from curvilinear to rectilinear building architecture, from early to later Neolithic period in Anatolia (During and Marciniak, 2005).

In earlier studies it was suggested that co-burials who could be household members in Çatalhöyük were not genetically more related to each other than to individuals buried in another buildings (Pilloud and Larsen, 2011; Chyleński et al., 2019). Pilloud and Larsen (2011) used dental morphology data and examined biological distance among co-burials using metric and nonmentric analysis. Chyleński et al. (2019), using mtDNA analysis on co-buried individuals in Çatalhöyük, observed high diversity in mtDNA lineages of individuals and found no genetic kin-relation among co-burials, which is consistent with the previous dental morphology-based study (Pilloud and Larsen, 2011). Here, our findings confirm these earlier reports using ancient genomic data. We report that the most of the co-burials in Çatalhöyük (94%) were not genetically related to each other, which is consistent with previous reports using other data types (Pilloud and Larsen, 2011; Chyleński et al., 2019). In total, we identified only three pairs that are genetically first- and second-degree related among a total of 29 co-buried pairs from Çatalhöyük and Barcın (94-84%, Table 8.1). This finding thus provides genetic support in line with the aforementioned hypothesis that co-burials, who could be household members, may have not been necessarily related to each other by close genetic kin-ties, at least in some Ceramic Neolithic Anatolian societies.

Another interesting point we noticed in this study was about sex-related kinship patterns identified in the Neolithic Anatolian societies investigated, which were in contrast to those observed in Neolithic and Bronze Age cemeteries in Europe (Appendix Table B.5). The latter represent clear patrilocal burial traditions with an excess of male individuals and/or adult females coming from outside the group (Sanchez et al., 2019; Mittnik et al., 2019). Although we identified sister pedigree relationships at a high frequency among first-degree related co-buried individuals (57%) (Appendix Table B.5), our sample size is too limited to suggest any

matrilocality in the Neolithic Anatolian societies; also two pairs of sisters were subadults (in Çatalhöyük and Barcın). On the other hand, the presence of adult female siblings in Aşıklı and Boncuklu pairs suggested that adult females did not necessarily always move away and/or were not buried away from their family group in these societies.

Importantly, we observed that the age of the related individual pairs also differ between Aceramic and Ceramic period populations, considering that the co-buried close relatives in Çatalhöyük and Barcın are all subadults (child or infant) (Figure 8.2 and 8.3). In fact, most of the in-house burials in these Ceramic Neolithic societies (Çatalhöyük and Barcın) are also subadults, compared to Aşıklı and Boncuklu (Haddow and Knüsel, 2017; Haddow, 2020) (Appendix Figure A.3, Appendix Table B.2). In contrast, genetically related individuals in Boncuklu are all adults; while the both related pairs in Aşıklı, consist of one subadult and one adult. This finding suggests that females were living in their village or maintaining social relationships with their home village. This also supports the notion of different in-house burial traditions in early and later period Anatolian Neolithic societies (Haddow and Knüsel, 2017; Haddow, 2020).

In contrast to Anatolian Ceramic Neolithic societies where genetically related co-burials we could identify were all subadults, we observed close genetic kin-relations among co-buried adult-adult and adult-subadult pairs in Anatolian Aceramic Neolithic societies, Boncuklu and Aşıklı. In Boncuklu genetically related co-burials were adult female-male pairs, a sibling pair and a mother-son pair, while the relative pairs in Aşıklı consisted of two adult-subadult female pairs, who were estimated to be sisters (Figure 8.3). This pattern of co-burials of genetically related adults in Aşıklı and Boncuklu could be interpreted in the light of a hypothetical model based on ethnographic observations (Flannery, 1972). Flannery's model suggests that the content of households could vary in early Neolithic societies. For example, household members might be the following cases: (i) a couple with their offspring, (ii) unmarried men, (iii) widows and unmarried women. Herein, assuming the co-buried pairs were households, our finding that an adult-subadult

female pair buried in the same building in Aşıklı were sisters (Aşıklı 131-136) could fit the third case. We also identified an old-adult female (Aşıklı 133) co-buried in the same building genetically unrelated with this pair (Aşıklı 131-136) (Figure 8.3). Thus, we speculate that the old-adult female (Aşıklı 133) could have been a widow living together with unmarried adult and subadult females (Aşıklı 131-136) as household members.

Considering Flannery's same model, we may also speculate that the unmarried-man scenario may explain the adult male-female pair in Boncuklu (Boncuklu ZHBJ-ZHAF), who were identified as siblings, and the male individual (Boncuklu ZHBJ) buried in a building (B.12) in close proximity to his sister's building (Boncuklu ZHAF in the B.14) (Figure 8.3). Likewise, we may be observing the first case of the aforementioned model in the Boncuklu Building 14 (B.14), where an old-adult female (Boncuklu ZHJ) and adult male (Boncuklu ZHF), who were identified as mother-son (Figure 8.3), may have lived together. On the other hand, this Boncuklu case may also be explained by another model, which suggests that in some cases when offspring of a household got married, they continued to live attached to their parental house instead of moving to another house (Flannery, 2002). This might explain the case of the Boncuklu B.14 (Building 14) such that in the Building 14, this mother-son pair (Boncuklu ZHJ-ZHF) was buried together with an adult female (Boncuklu ZHAF), who could be a possible wife and may have been living in the neigbouring building with her brother (Boncuklu ZHBJ) before the marriage (Figure 8.3).

Interestingly, the case of Aşıklı may alternatively be explained by another model, which posits that in early Neolithic societies households consisted of genetically related family members who were living in curvilinear separated buildings and practised their daily activities in open spaces with other households (Byrd, 2000; Baird et al., 2017; Özbaşaran, 2011). Meanwhile, archaeological evidence showed that the Aşıklı Building 1 (B.1) was constructed later than the Building 3 (B.3) (Özbaşaran et al. 2018) (Figure 8.3). Thus, the Aşıklı scenario may be that old-adult female (Aşıklı 133) were living with her sister (Aşıklı 128) together in their

building (B.3), and after Aşıklı 128 died, Aşıklı 133 moved to the close neighbouring building (B.1) and started to live together with neighbouring household based on inter-households social relatedness, without any genetic kin-relationships (Figure 8.3).

Another interesting point of this study is that we did not find any genetic kin-relationships among certain individuals who shared the same mtDNA haplogroup and were co-buried with other kin-related pairs in the same or in proximate buildings in both Aşıklı and Boncuklu (e.g., Aşıklı 129 in the Building 3 and Boncuklu ZHAG (the baby) in the Building 14) (Figure 8.3). The reasons for this could be either: (i) that those individuals may be genetically unrelated, but carry the most common haplogroup observed in the population, or (ii) that they may be genetically related to the others but more distantly than third-degree (e.g., >4th-5th degree and/or extended family members), so that we could not identify their relationships with our low-coverage data and the methods used in this study. On the other hand, these cases, along with results from Barcın and Çatalhöyük, may be explained by another archaeological hypothesis, which suggests that household members could be related based on other factors (e.g., socially) instead of genetic kin-ties (Baird et al., 2017; During and Marciniak, 2005). Meanwhile, the possible scenarios for the case of the Boncuklu baby (ZHAG) could be: (i) the mother of the baby (ZHAG) lived in the same building (Building 14) together with other adults (ZHAF, ZHF, ZHJ) and had no genetic kin-ties to any of them, (ii) through any relationships between the mother or the baby (ZHAG) and adult female (ZHAF) from the same building (Building 14), the baby (ZHAG) was buried with this adult female (ZHAF) (Figure 8.3).

CHAPTER 10

CONCLUSION AND FUTURE DIRECTIONS

In this study, first, I investigated genetic relationships among Aceramic and Ceramic Neolithic populations from Central and Northwest Anatolia using ancient genomes and population genetic analysis. Our results show high genetic affinity between two Aceramic Neolithic, Boncuklu and Aşıklı, suggesting descendants of same gene pool in early Neolithic Central Anatolia. Likewise, Anatolian Ceramic Neolithic populations (Çatalhöyük, Barcın and Tepecik-Çiftlik) are also genetically closer to each other, and display higher within-population genetic diversity relative to Aceramic Neolithic groups. Our findings suggest temporal changes on genetic characterization of populations from Aceramic to Ceramic Neolithic periods in Anatolia and this could be explained by possible gene flow from other Near Eastern Neolithic groups after c.8th millennium BCE.

In the future of this chapter, by combining these data with new genomes from early Neolithic settlements in Northern Mesopotamia (e.g., Çayönü), genetic relationships among Neolithic populations in Anatolia, especially the specificity of Central Anatolia, can be better understood.

Second, we studied genetic kinship patterns among co-buried individuals in Aceramic and Ceramic Neolithic societies from Central and Northwest Anatolia using ancient genomes and genetic relatedness analysis. Our overall findings suggest that close genetic kin-relationships among a group of people who could be households in Aceramic Neolithic societies from Central Anatolia, Boncuklu and Aşıklı. However, we observe close genetic kinship at a low frequency among co-burials, who are possible household members, in both Çatalhöyük and Barcın, suggesting genetic kin-relations may not be a fundamental component in the social

organization of these Anatolian Ceramic Neolithic societies. Our results thus suggest that genetic kin-relations patterns among possible household members could have changed over time during the transition from Aceramic to Ceramic Neolithic in Anatolia.

We observed a low frequency of close relatives among co-buried individuals in both Çatalhöyük and Barcın, which is in contrast to the hypothesis that households living in rectilinear buildings represent extended family members who were related with genetic kin-ties (Byrd, 2000; Bar-Yosef, 2001; Flannery, 2002). Despite the presence of several co-buried individuals in Çatalhöyük houses, the possible reasons for not observing the evidence of kinship-centred social organization could be: (i) small sample size of co-burials from each building, e.g. we could only sample 29% of burials across only three Çatalhöyük buildings for genomic analysis, (ii) the limited data generated in this study, such that we were not even able to study all individuals buried in the buildings studied, (iii) the fact that most of the co-burials that we could genetically sample from Çatalhöyük and Barcın were subadults. Thus, in future studies by generating more ancient genomes, kinship patterns in Anatolian Ceramic Neolithic societies, such as Çatalhöyük and Barcın, may be more clearly identified. In addition, we could not identify any social structure based on genetic kinship among co-burials in Tepecik-Çiftlik, representing Central Anatolian Ceramic Neolithic, due to the extremely small sample size. Indeed, only a pair of individuals among five studied individuals was co-buried and this pair was identified as first-degree relative (mother and son) (Figure 8.2).

Importantly, most of the co-burials from Ceramic Neolithic societies (e.g., Çatalhöyük) studied here were subadults compared to those of Aceramic Neolithic (e.g., Aşıklı) (Appendix Figure A.3, Appendix Table B.2). In the future, if genomic data could be obtained from co-buried adults from Çatalhöyük, Barcın and other Ceramic Neolithic sites, it is not impossible that close genetic kinship may be identified among those co-burials, who could also be households. On the other hand, in future studies, if co-buried adults are identified as genetically related,

while co-buried subadults are not relatives, this case may be interpreted in terms of adoptive or foster kin relationships among household members (Hodder, 2016).

Meanwhile, in future studies, to test whether these co-buried individuals in the same or close proximity buildings represent households, stable isotope analysis could be used. For example, nitrogen/carbon isotopes might be helpful to test whether co-buried individuals may have had similar diets (Irvine and Erdal, 2020; Larsen et al., 2019; Miller et al., 2019). Likewise, strontium and oxygen isotopes analysis on early and late molar teeth of co-burials could be used to understand whether they were local or not (Mittnik et al., 2019; Larsen et al., 2019).

Another limitation of the study is that there were other individuals co-buried with studied individuals, but we did not sample from those burials or could not generate enough genomic data for the analysis (e.g., Aşıklı B.1, Boncuklu B.14, Çatalhöyük B.17). Thus, in future studies genomic data could be obtained from those co-burials and genetic kinship patterns in Anatolian Neolithic societies could be more clearly understood.

Moreover, we could not test genetic kinship among burials within "*house series*" buildings (aforementioned in Chapter 1, General Introduction) in Aşıklı Höyük and Çatalhöyük, due to limited number of genomes generated from these sites and lack of the samples from those structures studied here. Therefore, in future studies it can be possible to test genetic relatedness among burials from these building sequences in Aşıklı Höyük and Çatalhöyük, by increasing sample size.

Despite these shortcomings, this work opens the way toward a deeper understanding into genetic relationships among house-related individuals and also the social structures of Neolithic societies in Central Anatolia and in Southwest Asia.

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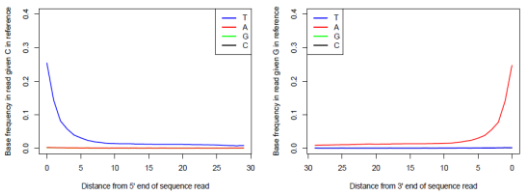
APPENDICES

A. Appendix A

Aşıklı 2



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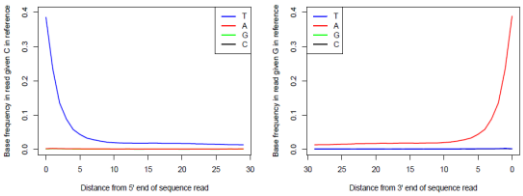

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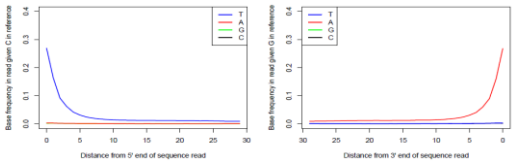

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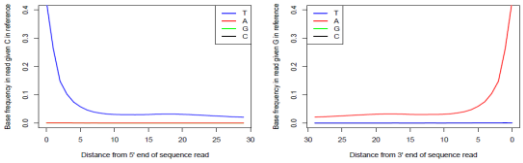

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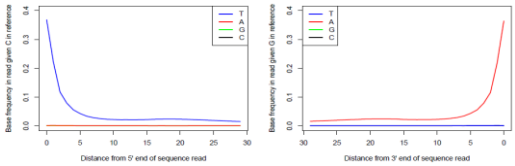

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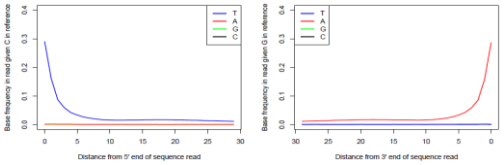

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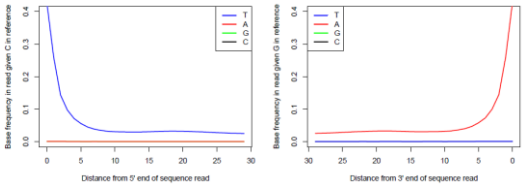

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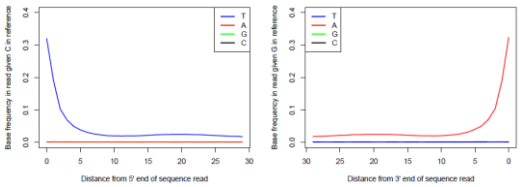

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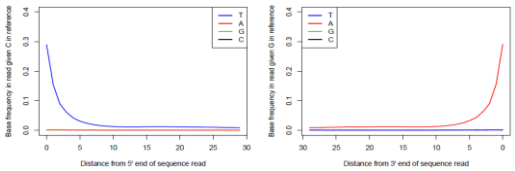

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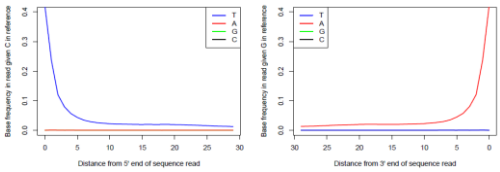

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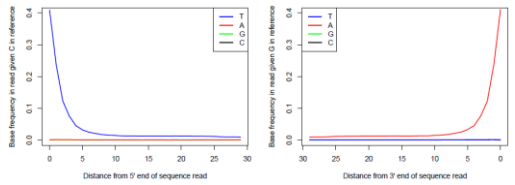

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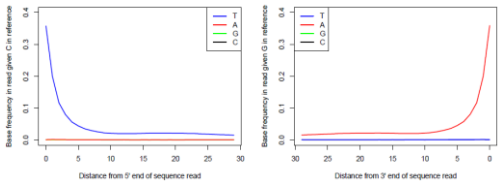

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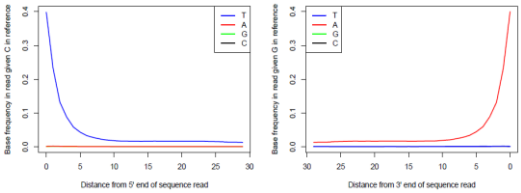

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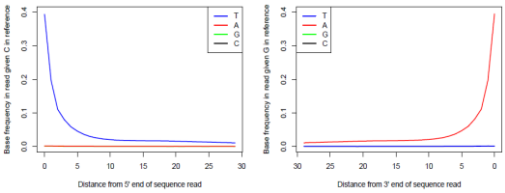

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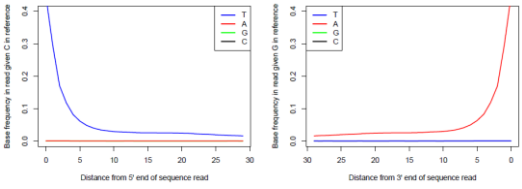

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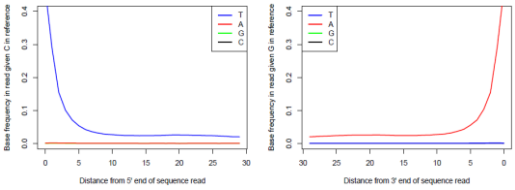

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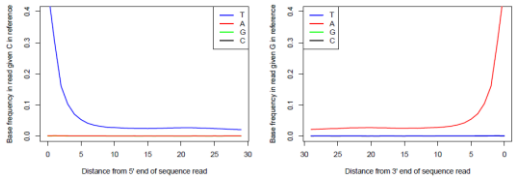

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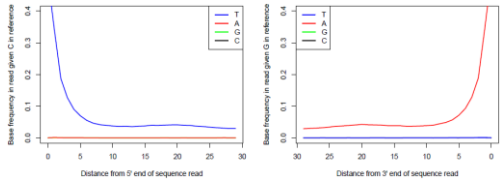

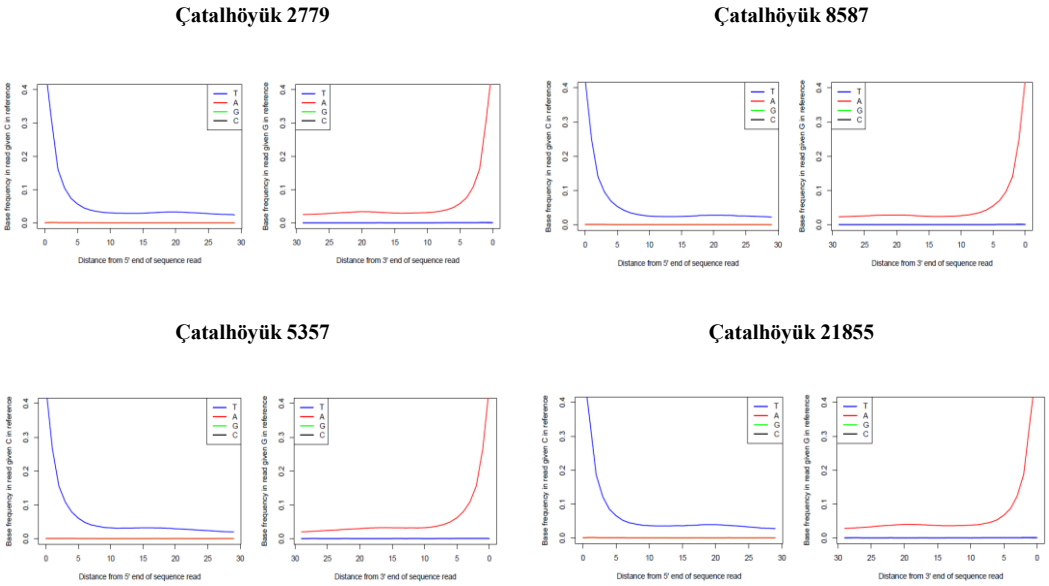

Figure A.1 Postmortem damage (deamination) patterns of Aşıklı and Çatalhöyük individuals.

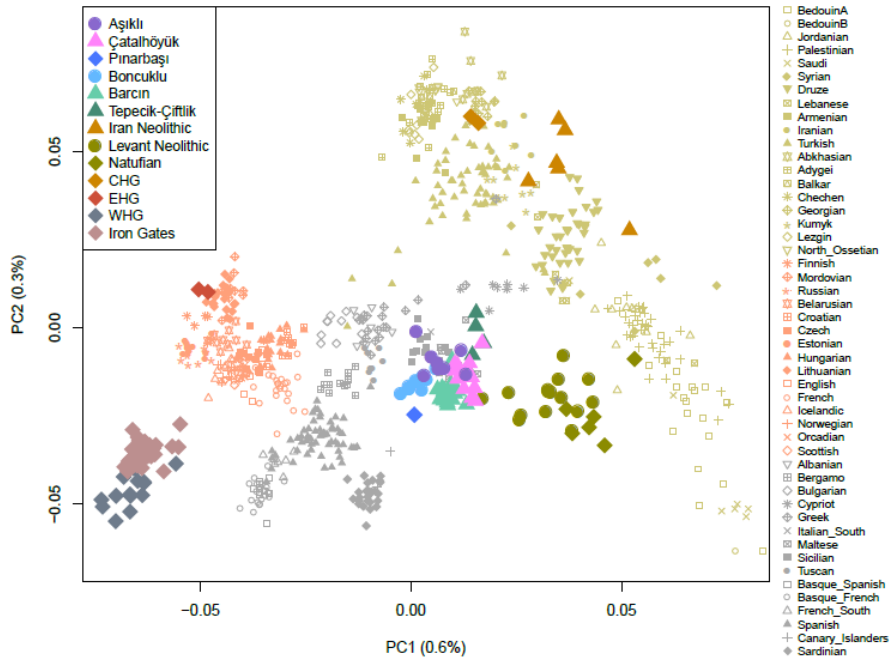

Figure A.2 Principle components analysis (PCA) on early Holocene individuals from Anatolia including ancient and modern-day individuals from West Eurasian populations. Colored dots show different ancient and modern-day individuals as shown by the key (on the left- and right-hand y-axis, respectively).

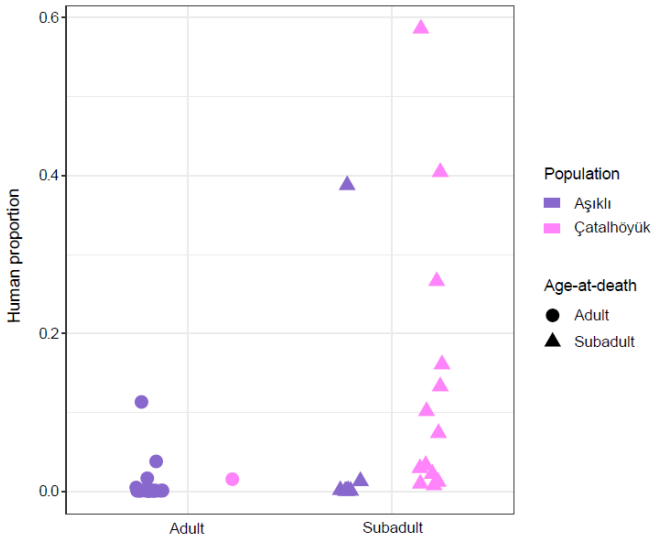

Figure A.3 Figure show endogenous human DNA proportions of studied samples from Aşıklı and Çatalhöyük with the age-at-death information of individuals.

B. Appendix B

Table B.1 Sequencing statistics of genomes produced in this study from Aşıklı and Çatalhöyük.

Site	Excavation ID	Lab ID of the sample	Library	Mapped reads after duplicate removal	Read length	Clonality (%)	Genome coverage	Mitochondrial coverage	Screening (median of all libraries)		Whole genome capture enrichment (median of all libraries)		Postmortem damage profiles		Mitochondrial contamination estimates (Proportion Authentic %)		X-chromosome contamination %(SE)	Autosomal SNPs (Human Origins)	Autosomal SNPs (1000 Genomes Yoruba population)	X chromosome SNPs (1000 Genomes Yoruba population)	Library indexing	ENA sample ID
									Human proportion	Clonality (%)	Human proportion	Clonality (%)	5' end	3' end	Transitions and transversions	Only transversions						
Aşıklı Höyük	2	Ash002	Ash002_all	6.471.092	54,03	84,98	0,023	3,044	0,007	14,15	0,009	36,73	0,25434	0,24737	93,99	94,32	NA	8.154	39.559	2.816	Single	ERS4811035
Aşıklı Höyük	33	Ash033	Ash033_all	23.386.349	53,06	79,49	0,069	34,283	0,018	20,50	0,047	79,08	0,38575	0,38892	96,04	99,06	13.00 (0.01)	19.678	113.867	4.269	Single	ERS4811084
Aşıklı Höyük	40	Ash040	Ash040_all	9.442.394	54,75	83,37	0,027	6,094	0,001	44,01	0,027	77,42	0,26914	0,26817	97,65	97,26	NA	7.363	46.705	3.223	Single	ERS4811085
Aşıklı Höyük	128	Ash128	Ash128_all	302.487.376	65,27	41,91	5,032	258,203	0,147	33,03	0,363	19,48	0,42948	0,42990	98,95	99,68	NA	588.693	1.783.750	133.575	Single	ERS4811086
Aşıklı Höyük	129	Ash129	Ash129_all	76.891.816	62,81	53,36	0,786	237,608	0,113	25,68	0,289	46,62	0,36820	0,36423	97,35	99,86	NA	190.651	945.381	72.072	Single	ERS4811087
Aşıklı Höyük	131	Ash131	Ash131_all	16.781.594	66,94	88,99	0,092	63,941	0,014	55,68	0,138	89,48	0,29009	0,28668	98,14	98,82	NA	22.900	156.261	13.332	Single	ERS4811088
Aşıklı Höyük	133	Ash133	Ash133_all	62.454.527	72,54	46,91	1,164	59,776	0,013	18,40	-	-	0,42654	0,42786	99,92	99,43	NA	256.779	1.215.960	90.643	Single	ERS4811089
Aşıklı Höyük	136	Ash136	Ash136_all	19.835.438	76,18	80,30	0,149	140,904	0,014	41,86	0,340	79,71	0,32047	0,32425	98,91	99,96	NA	29.976	239.029	20.493	Single	ERS4811090
Çatalhöyük	30006	cth006	cth006_all	16.351.320	59,97	85,99	0,074	47,734	0,011	25,63	0,275	85,72	0,28946	0,29084	98,96	96,28	NA	21.198	127.455	10.180	Single	ERS4811091
Çatalhöyük	2728	cth728	cth728_all	6.209.127	57,00	18,22	0,082	7,446	0,034	25,99	-	-	0,41923	0,42104	99,70	94,91	NA	37.122	142.610	10.158	Single	ERS4811092
Çatalhöyük	2842	cth842	cth842_all	19.618.647	54,46	67,08	0,086	31,049	0,011	23,91	0,099	85,22	0,40874	0,41093	97,27	99,00	NA	47.877	141.855	11.081	Single	ERS4811093
Çatalhöyük	5747	cth747	cth747_all	18.850.253	62,15	67,51	0,122	52,986	0,008	23,91	0,135	62,23	0,35759	0,35889	96,89	98,85	NA	57.375	202.081	14.299	Single	ERS4811094

Çatalhöyük	21981	pch034	pch034_all	6.722.479	57,28	11,22	0,089	3,807	0,266	11,22	-	-	0,44504	0,44591	99,17	94,91	NA	35.321	151.218	11.190	Single	ERS4811095
Çatalhöyük	5357	CCH144	CCH144_L1	4.212.571	59,19	16,48	0,059	2,476	0,102	16,48	-	-	0,44924	0,44924	99,76	95,91	2.00 (0.02)	23.006	106.752	3.830	Double	ERS4811096
Çatalhöyük	21855	CCH285	CCH285_L1	4.879.361	64,52	17,45	0,074	2,140	0,133	17,45	-	-	0,51226	0,51132	99,64	93,74	NA	26.439	127.257	9.131	Double	ERS4811098
Çatalhöyük	2017	CCH163	CCH163_L1	2.428.370	58,63	18,35	0,032	3,308	0,074	18,35	-	-	0,46549	0,46590	98,80	98,72	NA	11.871	57.529	4.151	Double	ERS4811097
Çatalhöyük	1885	CCH289	CCH289_L1	5.013.829	59,63	15,66	0,069	2,540	0,161	15,66	-	-	0,47588	0,47818	97,80	95,11	1.00 (0.01)	25.583	121.559	4.449	Double	ERS4811099
Çatalhöyük	2033	CCH290	CCH290_L1	765.274	69,09	14,98	0,012	0,366	0,029	14,98	-	-	0,50570	0,51094	99,70	93,35	--	5.009	22.975	868	Double	ERS4811100
Çatalhöyük	2779	CCH294	CCH294_L1	15.356.366	71,57	16,31	0,270	11,255	0,586	16,31	-	-	0,47957	0,48054	99,79	97,48	0.10 (0.004)	95.152	435.398	16.951	Double	ERS4811101
Çatalhöyük	8587	CCH311	CCH311_L1	7.539.845	71,96	15,37	0,137	8,437	0,404	15,37	-	-	0,42611	0,42373	99,86	99,25	NA	52.660	236.570	17.726	Double	ERS4811102
Çatalhöyük	11739	cth739	cth739_all	45.491.923	54,21	67,57	0,201	58,120	0,016	25,06	0,121	72,23	0,39831	0,39999	94,34	99,38	NA	90.164	305.318	23.771	Single	ERS4811103
Çatalhöyük	20217	cth217	cth217_all	4.481.358	54,88	18,28	0,055	6,073	0,021	25,86	-	-	0,39365	0,39487	97,36	93,93	NA	25.633	97.175	7.090	Single	ERS4811104

Table B.2 Archaeological, anthropological and genetic information of individuals from Anatolian Neolithic sites used in this study.

Excavation ID	Lab ID of the sample	Sample type	Site	Period	Country	Level	Building	Age-at-death (Morphological)	Morphological Sex	Molecular sex	Literature	C14 age (BP)	Weighted mean (BP)	Calibrated C14 age BCE	Contextual date	mtDNA haplogroup	Y chr haplogroup
2	Ash002	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	2A	AB	Young adult	F	F	This study	8454±35		7585–7475 (95%)	-	H2a	-
33	Ash033	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	2C	C	Child	Child (Unknown)	M	This study	8727±42		7945–7890 (9%), 7870–7595 (86%)	-	U3a	G2a2b
40	Ash040	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	2B	BH	Old adult	F	F	This study	8698±39		7935–7915 (1%), 7825–7590 (94%)	-	N1a1a1	-
128	Ash128	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	4	B3	Child	F	F	This study	8894±38 8895±39	8894±28; T′=0.0, T′(5%)=3.8, ν=1	8225–7955 (95%)	-	K1a4	-
129	Ash129	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	4	B3	Young adult	F	F	This study	8773±37 8840±40	8804±28; T′=1.5, T′(5%)=3.8, ν=1	8170–8115 (6%), 8060–8045 (1%), 8010–7985 (1%), 7970–7735 (86%)	-	K1a4	-
131	Ash131	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	4	B1	Child	Child	F	This study	8820±40		8200–8110 (16%), 8095–8035 (7%), 8015–7740 (72%)	-	T2c1a	-
133	Ash133	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	4	B1	Old adult	F	F	This study	8789±38 8828±42	8807±29; T′=0.5, T′(5%)=3.8, ν=1	8170–8115 (8%), 8060–8040 (1%), 8010–7980 (2%), 7975–7735 (84%)	-	K1a4	-
136	Ash136	Petrous	Aşıklı Höyük	Early Neolithic (Aceramic)	Central Anatolia	4	B1	Adult	F	F	This study	8794±40		8175–8110 (7%), 8090–8075 (1%), 8065–8040 (1%), 8015–7705 (84%), 7695–7655 (2%)	-	T2c1a	-
30006	cth006	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	North G Neolithic	114	Infant		F	This study	7795±40 7710±36	7748±27; T′=2.5, T′(5%)=3.8, ν=1	6645–6495 (94%), 6490–6480 (1%)	-	K1a4	-
2728	cth728	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Infant		F	This study	7799±39		6695–6505 (95%)	-	K1a	-

2842	cth842	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Child		F	This study	7792±40		6690–6505 (95%)	-	K1a	-
5747	cth747	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	91	Infant		F	This study	7685±45 7770±37	7736±29; T′=2.1, T′(5%)=3.8, ν=1	6640–6490 (95%)	-	T2c1	-
21981	pch034	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South N Neolithic	89	Infant		F	This study	-		-	-	K1a17	-
5357	CCH144	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South K Neolithic	17	Infant		M	This study	7930±40		7035–6680 (93%), 6670–6650 (2%)	-	N1a1a1	C1a2
21855	CCH285	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South K Neolithic	17	Child		F	This study	-		-	-	H2a2a1	-
2017	CCH163	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Neonate		F	This study	7850±30		6815–6790 (2%), 6775–6595 (93%)	-	T2	-
1885	CCH289	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Child		M	This study	7865±30		6905–6885 (1%), 6825–6635 (92%), 6625–6600 (2%)	-	K1a	G2a2a1
2033	CCH290	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Child		M	This study	7805±20		6690–6590 (95%)	-	H2a2a1d	H3a1
2779	CCH294	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	South M Neolithic	50	Infant		M	This study	-		-	-	H2a2a	C1a2
8587	CCH311	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	North G Neolithic	114	Neonate		F	This study	-		-	-	T2e	-
11739	cth739	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	TP Late Neolithic	NA	Middle adult		F	This study	7311±36		6235–6075 (95%)	-	K1b1	-

20217	cth217	Petrous	Çatalhöyük	Neolithic (Ceramic)	Central Anatolia	TPC Late Neolithic	NA	Child		F	This study	7461±36		6415–6240 (95%)	-	K1a4b	-
ZHF	Bon001	M1 Tooth	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		14	Adult	-	M	Kılınç et al., 2016	8875±25		8225–7940 (95%)	-	U3	G2a2b2b
ZHB	Bon002	Petrous	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		-	Middle adult	F	F	Kılınç et al., 2016	8965±37		8280–8165 (57%), 8120–7960 (38%)	-	K1a	-
ZHBJ	Bon004	M3 Tooth	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		12	Middle adult	M	M	Kılınç et al., 2016	-		-	Pre-dates ZHF, thus > 7952	N1a1a1	G2a2b2b
ZHAF	Bon005	M3 Tooth	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		14	Middle adult	F	F	Kılınç et al., 2016	9054±24		8285–8175 (83%), 8115–8090 (4%), 8040–8010 (8%)	-	N1a1a1	-
ZHAG	ZHAG_BON004	Petrous	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		14	Baby		F	Feldman et al., 2019	-		-	Pre-dates ZHJ, thus > 8200	N1a1a1	-
ZHAJ	ZHAJ_BON034	Petrous	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		-	Middle adult		F	Feldman et al., 2019	-		-	Pre-dates ZHJ, thus > 8200	U3	-
ZHJ	ZHJ_BON024	Third molar	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		14	Old adult		F	Feldman et al., 2019	8980±25		8295–8240 (95%)	-	U3	-
ZKO	ZKO_BON001	Petrous	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		9	Old adult		M	Feldman et al., 2019	-		-	8300-7800	U3	G2a2b2b
ZMOJ	ZMOJ_BON014	Third molar	Boncuklu Höyük	Early Neolithic (Aceramic)	Central Anatolia		-	Young adult		M	Feldman et al., 2019	-		-	8300-7800	K1a	C1a2
BAR2 / L11-213	I0707	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIb	5	infant		F	Mathieson et al., 2015	9935±35 7272±34	PSUAMS-3180 is considered anomalous, and so TÜBİTAK-455 alone is calibrated	6220–6060 (95%)	c. 6200-6100	K1a4	-
BAR6 / L11-439	I0708	Petrous core (Cochle+	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIb	-	Adult		M	Mathieson et al., 2015	7285±30		6225–6070 (95%)	-	N1b1a	J2a

		Semicircular canal)															
BAR20/ M13-170	I0709	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIb	-	Child		M	Mathieson et al., 2015	7255± 30		6215–6055 (95%)	-	U3	H2
L11-216	I0736	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIb	5	Infant		F	Mathieson et al., 2015	-		-	c. 6200-6100	N1a1a1a	-
M10-275	I0744	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	14 or 15	Infant		M	Mathieson et al., 2015	7455± 30		6405–6240 (95%)	-	J1c11	G2a2b2a
M11-363	I0745	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	4	Infant		M	Mathieson et al., 2015	7405± 30		6375–6225 (95%)	-	U8b1b1	H2
L11-322	I0746	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIa	-	Infant		M	Mathieson et al., 2015	7110± 50		6070–5890 (95%)	-	K1a	G2a2b2a 1c
L11-215	I0854	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIb	5	Infant		F	Mathieson et al., 2015	7310± 30		6320–6080 (95%)	-	N1a1a1a	-
BAR26 / M10-76	I1096	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	-	Infant		M	Mathieson et al., 2015	-		-	c. 6400-6200	N1a1a1	I2c
BAR271 / M10-271	I1097	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	14 or 15	Infant		M	Mathieson et al., 2015	7475± 30		6425–6325 (59%), 6320–6250 (36%)	-	W1	G2a2b2a
BAR99 / M10-352	I1098	Petrous core (Cochle+	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId2 or VId3	associated with 14 or	Infant		F	Mathieson et al., 2015	7475± 25		6425–6330 (60%), 6320–6250 (35%)	-	X2d2	-

		Semicircular canal)					15										
L11-S-488	I1099	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIc	-	Infant		M	Mathieson et al., 2015	-		-	c. 6300-6200	T2b	G2a2a1b
M11-351	I1100	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	4	Infant		F	Mathieson et al., 2015	-		-	c. 6350-6200	K1a	-
M11-352a	I1101	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	4	Infant		M	Mathieson et al., 2015	-		-	c. 6350-6200	T2b	H
M11-354	I1102	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	4	Infant		M	Mathieson et al., 2015	-		-	c. 6350-6200	K1a3a	C1a2
M11-S-350	I1103	Petrous core (Cochle+ Semicircular canal)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	-	Infant		M	Mathieson et al., 2015	-		-	c. 6350-6200	K1b1b1	G2a2a1b1
M13-72	I1579	Petrous core (Cochlea)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIa	-	Middle adult		F	Mathieson et al., 2015	7245±25		6210–6050 (95%)	-	K1a	-
L12-393	I1580	Petrous core (Cochlea)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIc	-	Adult		F	Mathieson et al., 2015	7385±40		6390–6205 (88%), 6170–6155 (1%), 6145–6100 (6%)	-	H5	-
L12-502	I1581	Petrous core (Cochlea)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId3 or VIc	-	Adult		F	Mathieson et al., 2015	7415±30		6380–6280 (95%)	-	U3	-
L14-200	I1583	Petrous core (Cochlea)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VId2 or VId3	-	Child		M	Mathieson et al., 2015	7460±50		6425–6235 (95%)	-	K1a2	G2a2a1b
M11-59	I1585	Petrous core (Cochlea)	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIa	-	Middle/Old Adult		F	Mathieson et al., 2015	7215±30		6210–6135 (14%), 6115–6010 (81%)	-	J1	-
M10-106	Bar8	-	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIa	-	Adult		F	Hofmanová et al., 2016	7238±38		6215–6025 (95%)	-	K1a2	-

L11W-546	Bar31	-	Barcın Höyük	Neolithic (Ceramic)	Northwest Anatolia	VIc	-	Adult		M	Hofmanová et al., 2016	7457±44		6420–6235 (95%)	-	X2m	G2a2b
TP'10 BB 4-23	Tep001	Petrous	Tepecik-Çiftlik Höyük	Neolithic (Ceramic)	Central Anatolia	5	BB	Young adult	M	M	Kılınç et al., 2016	7750±30		6645–6495 (94%), 6490–6480 (1%)	-	K1a	J
TP'10 SK 40	Tep002	M2 Tooth	Tepecik-Çiftlik Höyük	Neolithic (Ceramic)	Central Anatolia	5	-	Adult	-	F	Kılınç et al., 2016	7710±30		6640–6625 (2%), 6600–6465 (93%)	-	K1a12a	-
TP'09 16 K	Tep003	Petrous	Tepecik-Çıftlik Höyük	Neolithic (Ceramic)	Central Anatolia	5	16K	Adult	-	M	Kılınç et al., 2016	7630±30		6570–6545 (5%), 6515–6420 (90%)	-	N1b1a	G2a2a
TP'10 SK 37	Tep004	M3 Tooth	Tepecik-Çiftlik Höyük	Neolithic (Ceramic)	Central Anatolia	4	AK	Adult	F	F	Kılınç et al., 2016	7400±30		6385–6220 (92%), 6130–6100 (3%)	-	N1a1a1	-
TP'10 SK 21	Tep006	Petrous	Tepecik-Çiftlik Höyük	Neolithic (Ceramic)	Central Anatolia	4	AK	Old adult	M	M	Kılınç et al., 2016	7280±30		6225–6070 (95%)	-	N1a1a1	E1a2a1b1

Table B.3 Archaeological and genetic information of published early Holocene genomes from West Eurasia used in this study.

Individual ID	Population	Period	Location	Country	Molecular sex	mtDNA haplogroup	Y haplogroup	Reference	Used in kinship analysis
ZBC_IPB001	Pınarbaşı	Anatolian HG	Central Anatolia	Turkey	M	K2b	C1a2	Feldman et al., 2019	yes
Bon001	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	M	U3	G2a2b2b	Kılınç et al., 2016	yes
Bon002	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	F	K1a	-	Kılınç et al., 2016	yes
Bon004	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	M	N1a1a1	G2a2b2b	Kılınç et al., 2016	yes
Bon005	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	F	N1a1a1	-	Kılınç et al., 2016	yes
ZHAG_BON004	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	F	N1a1a1	-	Feldman et al., 2019	yes
ZHAJ_BON034	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	F	U3	-	Feldman et al., 2019	yes
ZHJ_BON024	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	F	U3	-	Feldman et al., 2019	yes
ZKO_BON001	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	M	U3	G2a2b2b	Feldman et al., 2019	yes
ZMOJ_BON014	Boncuklu	Anatolian Early Neolithic	Central Anatolia	Turkey	M	K1a	C1a2	Feldman et al., 2019	yes
I0707	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	K1a4	-	Mathieson et al., 2015	yes
I0708	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	N1b1a	J2a	Mathieson et al., 2015	yes
I0709	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	U3	H2	Mathieson et al., 2015	yes
I0736	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	N1a1a1a	-	Mathieson et al., 2015	yes
I0744	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	J1c11	G2a2b2a	Mathieson et al., 2015	yes
I0745	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	U8b1b1	H2	Mathieson et al., 2015	yes
I0746	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	K1a	G2a2b2a1c	Mathieson et al., 2015	yes
I0854	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	N1a1a1a	-	Mathieson et al., 2015	yes
I1096	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	N1a1a1	I2c	Mathieson et al., 2015	yes
I1097	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	W1	G2a2b2a	Mathieson et al., 2015	yes

I1098	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	X2d2	-	Mathieson et al., 2015	yes
I1099	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	T2b	G2a2a1b	Mathieson et al., 2015	yes
I1100	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	K1a	-	Mathieson et al., 2015	yes
I1101	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	T2b	H	Mathieson et al., 2015	yes
I1102	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	K1a3a	C1a2	Mathieson et al., 2015	yes
I1103	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	K1b1b1	G2a2a1b1	Mathieson et al., 2015	yes
I1579	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	K1a	-	Mathieson et al., 2015	yes
I1580	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	H5	-	Mathieson et al., 2015	yes
I1581	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	U3	-	Mathieson et al., 2015	yes
I1583	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	K1a2	G2a2a1b	Mathieson et al., 2015	yes
I1585	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	J1	-	Mathieson et al., 2015	yes
Bar8	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	F	K1a2	-	Hofmanová et al., 2016	yes
Bar31	Barcın	Anatolian Neolithic	Northwest Anatolia	Turkey	M	X2m	G2a2b	Hofmanová et al., 2016	yes
Tep001	Tepecik-Çiftlik	Anatolian Neolithic	Central Anatolia	Turkey	M	K1a		Kılınç et al., 2016	yes
Tep002	Tepecik-Çiftlik	Anatolian Neolithic	Central Anatolia	Turkey	F	K1a12a	-	Kılınç et al., 2016	yes
Tep003	Tepecik-Çiftlik	Anatolian Neolithic	Central Anatolia	Turkey	M	N1b1a		Kılınç et al., 2016	yes
Tep004	Tepecik-Çiftlik	Anatolian Neolithic	Central Anatolia	Turkey	F	N1a1a1	-	Kılınç et al., 2016	yes
Tep006	Tepecik-Çiftlik	Anatolian Neolithic	Central Anatolia	Turkey	M	N1a1a1		Kılınç et al., 2016	yes
I0861	Natufian	Natufian	Raqefet Cave	Israel	M	J2a2	E1b1b1b2	Lazaridis et al., 2016	no
I1069	Natufian	Natufian	Raqefet Cave	Israel	M	H5b1	E1b1	Lazaridis et al., 2016	no
I1072	Natufian	Natufian	Raqefet Cave	Israel	M	N1b	E1b1b1b2	Lazaridis et al., 2016	no
I1685	Natufian	Natufian	Raqefet Cave	Israel	M	J2a2	CT	Lazaridis et al., 2016	no
I1687	Natufian	Natufian	Raqefet Cave	Israel	F	..	..	Lazaridis et al., 2016	no
I1690	Natufian	Natufian	Raqefet Cave	Israel	M	H	CT	Lazaridis et al., 2016	no
I0867	Levant_N	Levant Neolithic	Motza	Israel	M	K1a4b		Lazaridis et al., 2016	no
I1414	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	K1a18		Lazaridis et al., 2016	no
I1415	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	..		Lazaridis et al., 2016	no

I1416	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	..		Lazaridis et al., 2016	no
I1679	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	F	I		Lazaridis et al., 2016	no
I1699	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	F	R0a2		Lazaridis et al., 2016	no
I1700	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	T1a2		Lazaridis et al., 2016	no
I1701	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	F	K1a18		Lazaridis et al., 2016	no
I1704	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	F	T1a		Lazaridis et al., 2016	no
I1707	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	R0a		Lazaridis et al., 2016	no
I1709	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	..		Lazaridis et al., 2016	no
I1710	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	T1a2		Lazaridis et al., 2016	no
I1727	Levant_N	Levant Neolithic	Ain Ghazal	Jordan	M	T1a2		Lazaridis et al., 2016	no
KFH2	Levant_N	Levant Neolithic	Kfar Hahoresh	Israel	F	N1a1b	..	Feldman et al., 2019	no
BAJ001	Levant_N	Levant Neolithic	Ba'ja	Jordan	F	N1b1a	..	Feldman et al., 2019	no
WC1	Iran_N	Iran Neolithic	Wezmeh Cave	Iran	M	J1d6	G2b	Broushaki et al., 2016	no
I1290	Iran_N	Iran Neolithic	Ganj Dareh	Iran	F	X2	..	Lazaridis et al., 2016	no
I1944	Iran_N	Iran Neolithic	Ganj Dareh	Iran	F	R2	..	Lazaridis et al., 2016	no
I1945	Iran_N	Iran Neolithic	Ganj Dareh	Iran	M	J1c10	R	Lazaridis et al., 2016	no
I1949	Iran_N	Iran Neolithic	Ganj Dareh	Iran	M	..	R	Lazaridis et al., 2016	no
I1951	Iran_N	Iran Neolithic	Ganj Dareh	Iran	F	HV0f	..	Lazaridis et al., 2016	no
I1671	Iran_N	Iran Late Neolithic	Seh Gabi	Iran	M	K1a12a	G2a1a	Lazaridis et al., 2016	no
I4081	Iron_Gates_HG	Iron Gates HG	Ostrovul Corbului	Romania	M	H13	R1b1a	Mathieson et al., 2018	no
I4582	Iron_Gates_HG	Iron Gates HG	Ostrovul Corbului	Romania	F	K1	..	Mathieson et al., 2018	no
I4607	Iron_Gates_HG	Iron Gates HG	Schela Cladovei	Romania	M	U5a2	I2	Mathieson et al., 2018	no
I4655	Iron_Gates_HG	Iron Gates HG	Schela Cladovei	Romania	M	K1	R	Mathieson et al., 2018	no
I4657	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	K1c	..	Mathieson et al., 2018	no
I4660	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U8b1b	..	Mathieson et al., 2018	no
I4870	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	K1c	I2	Mathieson et al., 2018	no

I4871	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5b2a1a	..	Mathieson et al., 2018	no
I4872	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5a1c	..	Mathieson et al., 2018	no
I4873	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5a2a	..	Mathieson et al., 2018	no
I4874	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5b2a1a	..	Mathieson et al., 2018	no
I4875	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5b1d1a	..	Mathieson et al., 2018	no
I4876	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5a2d	..	Mathieson et al., 2018	no
I4877	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	F	U5b1d1	..	Mathieson et al., 2018	no
I4878	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U4a	I2a2a	Mathieson et al., 2018	no
I4880	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U4b1b1	I2a2a1b2	Mathieson et al., 2018	no
I4881	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U4b1b1	I2a2a1b2	Mathieson et al., 2018	no
I4914	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	M	U5a1c1	I2a2a1b2	Mathieson et al., 2018	no
I4915	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	M	U5b2b	I2a2	Mathieson et al., 2018	no
I4916	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	M	U5b2b	R1b1a (xR1b1a1,xR1b1a 1a,xR1b1a1a2)	Mathieson et al., 2018	no
I4917	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	F	U5a1c	..	Mathieson et al., 2018	no
I5233	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U5b1d1	..	Mathieson et al., 2018	no
I5234	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U4a	..	Mathieson et al., 2018	no
I5235	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	M	U5b2c	R1b1a(xR1b1a1a,x R1b1a1a2)	Mathieson et al., 2018	no
I5236	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	M	U5a2d	I2a1	Mathieson et al., 2018	no
I5237	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	M	U5a2a	R1b1a(xR1b1a1a,x R1b1a1a2)	Mathieson et al., 2018	no
I5238	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	K1c	..	Mathieson et al., 2018	no
I5239	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U5a2d	..	Mathieson et al., 2018	no
I5240	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	M	U5a1c	R1b1a(xR1b1a1a,x R1b1a1a2)	Mathieson et al., 2018	no
I5242	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U5a1	..	Mathieson et al., 2018	no
I5244	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	K1f	..	Mathieson et al., 2018	no
I5401	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	M	U5a1	I2a2	Mathieson et al., 2018	no

I5402	Iron_Gates_HG	Iron Gates HG	Hadučka Vodenica	Serbia	M	U5a1c1	I2a2a1b	Mathieson et al., 2018	no
I5408	Iron_Gates_HG	Iron Gates HG	Ostrovul Corbului	Romania	M	K1c	R1b1a(xR1b1a1a,xR1b1a1a2)	Mathieson et al., 2018	no
I5409	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U5a1c	..	Mathieson et al., 2018	no
I5411	Iron_Gates_HG	Iron Gates HG	Schela Cladovei	Romania	M	U5a1c1	R1b1a(xR1b1a1a,xR1b1a1a2)	Mathieson et al., 2018	no
I5436	Iron_Gates_HG	Iron Gates HG	Schela Cladovei	Romania	F	U5a2	..	Mathieson et al., 2018	no
I5771	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U5a1c1	I	Mathieson et al., 2018	no
I5772	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U5a2a	R1b1a(xR1b1a1a,xR1b1a1a2)	Mathieson et al., 2018	no
I5773	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U4a	I	Mathieson et al., 2018	no
I5232	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	M	K1a	R1b1a(xR1b1a1a,xR1b1a1a2)	Mathieson et al., 2018	no
I4882	Iron_Gates_HG	Iron Gates HG	Vlasac	Serbia	M	U4b1b1	I2a2a1b	Mathieson et al., 2018	no
I5241	Iron_Gates_HG	Iron Gates HG	Padina	Serbia	F	U5a2a	..	Mathieson et al., 2018	no
Satsurblia	CHG	Paleolithic	Satsurblia cave	Georgia	M	..	J1	Jones et al., 2015	no
Kotias	CHG	Mesolithic	Kotias Klde	Georgia	M	H13c	J2a1h1	Jones et al., 2015	no
Karelia (I0061)	EHG	Mesolithic	Yuzhnyy Oleni Ostrov, Karelia	Russia	M	C1	R1a1a1	Mathieson et al., 2015	no
Samara (I0124)	EHG	Mesolithic	Lebyanzhinka IV, Sok River, Samara	Russia	M	U5a1d	R1b1a1a	Mathieson et al., 2015	no
Chaudardes1	WHG	Villabruna	Chaudardes	France	M	U5b1b	I	Fu et al., 2016	no
LesCloseaux13	WHG	Villabruna	Les Closeaux	France	M	U5a2	..	Fu et al., 2016	no
Ofnet	WHG	Villabruna	Ofnet	Germany	F	U5b1d1	..	Fu et al., 2016	no
Ranchot88	WHG	Villabruna	Ranchot	France	F	U5b1	..	Fu et al., 2016	no
Villabruna	WHG	Villabruna	Villabruna	Italy	M	U5b2b	R1b1a	Fu et al., 2016	no
BerryAuBac	WHG	Villabruna	Berry au bac	France	M	U5b1a	I	Mathieson et al., 2018	no
Falkenstein	WHG	Villabruna	Falkenstein-Höhle, Swabian Jura, Baden-Württemberg	Germany	M	U5a2c	I2a2a	Mathieson et al., 2018	no
I1875	WHG	Villabruna	Vela Spila	Croatia	F	U5b2b	..	Mathieson et al., 2018	no

I2158	WHG	Villabruna	Grotta d'Oriente, Favignana island, Egadi islands, Sicily	Italy	F	U2'3'4'7'8'9	..	Mathieson et al., 2018	no
Iboussieres25-1	WHG	Villabruna	Aven des Iboussières à Malataverne, Rhône-Alpes	France	M	U5b2a	J?	Mathieson et al., 2018	no
Iboussieres31-2	WHG	Villabruna	Aven des Iboussières à Malataverne, Rhône-Alpes	France	M	U5b1	R	Mathieson et al., 2018	no
Rochedane	WHG	Villabruna	Rochedane	France	M	U5b2b	I	Mathieson et al., 2018	no
Loschbour	WHG	Mesolithic	Echternach	Luxembourg				Lazaridis et al., 2014	no
Stuttgart	Germany_EN	European early Neolithic	Viesenhaeuser Hof, Stuttgart-Muehlhausen	Germany		T2c1d1		Lipson et al., 2017	no
I0022	Germany_EN	European early Neolithic	Viesenhaeuser Hof, Stuttgart-Muehlhausen	Germany	F	T2e	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0025	Germany_EN	European early Neolithic	Viesenhaeuser Hof, Stuttgart-Muehlhausen	Germany	F	T2b	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0026	Germany_EN	European early Neolithic	Viesenhaeuser Hof, Stuttgart-Muehlhausen	Germany	F	T2b	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0046	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	F	T2c1	..	Lipson et al., 2017	no
I0048	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	M	K1a	G2a2a1	Lipson et al., 2017	no

I0054	Germany_EN	European early Neolithic	Unterwiederstedt	Germany	F	J1c17	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0056	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	M	T2b	G2a2a	Lipson et al., 2017	no
I0057	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	F	N1a1a1	..	Lipson et al., 2017	no
I0100	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	F	N1a1a1a	..	Lipson et al., 2017	no
I0659	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	M	N1a1a1a2	G2a2a1	Lipson et al., 2017	no
I0795	Germany_EN	European early Neolithic	Karsdorf	Germany	M	H1orH1au1b	CT	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0797	Germany_EN	European early Neolithic	Karsdorf	Germany	M	H46b	T1a	Mathieson et al., 2015	no
I0821	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	M	X2d1	G2a2a1	Lipson et al., 2017	no
I1550	Germany_EN	European early Neolithic	Halberstadt-Sonntagsfeld	Germany	F	K1a2	..	Lipson et al., 2017	no
I4918	Starcevo_EN	European early Neolithic	Saraorci-Jezava	Serbia	F	K1a4a1	..	Mathieson et al., 2018	no
I0174	Hungary_EN	European early Neolithic	Alsonyek-Bataszek, Mérnöki telep	Hungary	M	N1a1a1	H2	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0176	Hungary_EN	European early Neolithic	Szemely-Hegyes	Hungary	F	N1a1a1a3	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no

I1506 (NE1)	Hungary_EN	European early Neolithic	Polgar Ferenci hat	Hungary	F	U5b2c	..	Mathieson et al., 2015	no
I1498 (NE2)	Hungary_EN	European early Neolithic	Debrecen Tocopart Erdoalja	Hungary	F	H	..	Mathieson et al., 2015	no
I1499 (NE3)	Hungary_EN	European early Neolithic	Garadna	Hungary	F	X2b-T226C	..	Mathieson et al., 2015	no
I1505 (NE4)	Hungary_EN	European early Neolithic	Polgar Ferenci hat	Hungary	F	J1c5	..	Mathieson et al., 2015	no
I1500 (NE5)	Hungary_EN	European early Neolithic	Kompolt-Kigyoser	Hungary	M	J1c1	C1a2	Mathieson et al., 2015	no
I1496 (NE6)	Hungary_EN	European early Neolithic	Apc-Berekalya I	Hungary	M	K1a3a3	C1a2	Mathieson et al., 2015	no
Rev5	Greek_Rev5_N	Greece Early Neolithic	Revenia	Greece	F	X2b	..	Hofmanová et al., 2016	no
I2318	Greek_Peloponnese_N	Greece Peloponnese Neolithic	Franchthi Cave	Greece	F	H2	..	Mathieson et al., 2018	no
I3708	Greek_Peloponnese_N	Greece Peloponnese Neolithic	Diros, Alepotrypa Cave	Greece	F	T1a	..	Mathieson et al., 2018	no
I3709	Greek_Peloponnese_N	Greece Peloponnese Neolithic	Diros, Alepotrypa Cave	Greece	F	K1b1a	..	Mathieson et al., 2018	no
I3920	Greek_Peloponnese_N	Greece Peloponnese Neolithic	Diros, Alepotrypa Cave	Greece	F	H	..	Mathieson et al., 2018	no
I5427	Greek_Peloponnese_N	Greece Peloponnese Neolithic	Diros, Alepotrypa Cave	Greece	F	K1a24	..	Mathieson et al., 2018	no
I0633	Balkans_N	Balkans Neolithic	Gomolava, Hrtkovci, Vojvodina	Serbia	M	HV	G2a2a1	Mathieson et al., 2018	no
I0634	Balkans_N	Balkans Neolithic	Gomolava, Hrtkovci, Vojvodina	Serbia	M	K1a4	G2a2a1a	Mathieson et al., 2018	no
I0704	Balkans_N	Balkans Neolithic	Dzhulyunitsa	Bulgaria	F	T2b	..	Mathieson et al., 2018	no
I0706	Balkans_N	Balkans Neolithic	Dzhulyunitsa	Bulgaria	M	K1a4b	C	Mathieson et al., 2018	no
I1131	Balkans_N	Balkans Neolithic	Gomolava, Hrtkovci, Vojvodina	Serbia	M	H	G2a2a1a	Mathieson et al., 2018	no
I3498	Balkans_N	Balkans Neolithic	Beli Manastir-Popova zemlja	Croatia	M	U8b1b1	C	Mathieson et al., 2018	no

I1108	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	M	T2e	T1a1	Mathieson et al., 2018	no
I1109	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	F	J2b1	..	Mathieson et al., 2018	no
I1113	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	F	U5a1c	..	Mathieson et al., 2018	no
I1295	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	M	J1c	G2a2b2a	Mathieson et al., 2018	no
I1296	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	M	U5a2	C	Mathieson et al., 2018	no
I1297	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	F	H5b	..	Mathieson et al., 2018	no
I2215	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	U	T2b	..	Mathieson et al., 2018	no
I2216	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	F	J2b1	..	Mathieson et al., 2018	no
I3879	Balkans_MP_N	Balkans Neolithic	Malak Preslavets	Bulgaria	M	H	G2a2b2a	Mathieson et al., 2018	no
I0698	Bulgaria_N	Balkans Neolithic	Yabalkovo	Bulgaria	M	H	G2a2a1a2a	Mathieson et al., 2018	no
I2521	Bulgaria_N	Balkans Neolithic	Dzhulyunitsa	Bulgaria	M	H	G2a2b2b1a	Mathieson et al., 2018	no
I2529	Bulgaria_N	Balkans Neolithic	Yabalkovo	Bulgaria	M	T1a	I2a2	Mathieson et al., 2018	no
ANI152	Bulgaria_Varna_Eneolithic	Balkans Neolithic	Varna	Bulgaria	M	U2	CT	Mathieson et al., 2018	no
ANI153	Bulgaria_Varna_Eneolithic	Balkans Neolithic	Varna	Bulgaria	M	U4	R1	Mathieson et al., 2018	no
ANI159_ANI181	Bulgaria_Varna_Eneolithic	Balkans Neolithic	Varna	Bulgaria	M	T2b2b	G2a2b2b	Mathieson et al., 2018	no
ANI160	Bulgaria_Varna_Eneolithic	Balkans Neolithic	Varna	Bulgaria	M	H1ag	G2	Mathieson et al., 2018	no
CB13	Iberia_EN	European early Neolithic	Cova Bonica, Vallirana, Barcelona	Spain	F	K1a2a	..	Olalde et al., 2015	no
I0409	Iberia_EN	European early Neolithic	Els Trocs	Spain	F	J1c3	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0410	Iberia_EN	European early Neolithic	Els Trocs	Spain	M	T2c1dorT2c1d2	R1b1a(xR1b1a1a2)	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no

I0412	Iberia_EN	European early Neolithic	Els Trocs	Spain	M	N1a1a1	I2a1b1	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I0413	Iberia_EN	European early Neolithic	Els Trocs	Spain	F	V	..	Mathieson et al., 2015 (1240k of same sample with 390k in Haak Lazaridis, 2015)	no
I5068	LBK_Austria	European Neolithic	Kleinhadersdorf Flur Marchleiten	Austria	M	T2b23	J2	Mathieson et al., 2018	no
I5069	LBK_Austria	European Neolithic	Kleinhadersdorf Flur Marchleiten	Austria	F	N1a1	..	Mathieson et al., 2018	no
I5070	LBK_Austria	European Neolithic	Schletz	Austria	M	K1a1a	C1a2	Mathieson et al., 2018	no
I5204	LBK_Austria	European Neolithic	Schletz	Austria	M	J1c2	G2a2b2a3	Mathieson et al., 2018	no
I5205	LBK_Austria	European Neolithic	Schletz	Austria	F	H	..	Mathieson et al., 2018	no
I5206	LBK_Austria	European Neolithic	Schletz	Austria	F	T2b	..	Mathieson et al., 2018	no
I5207	LBK_Austria	European Neolithic	Schletz	Austria	M	H67	J2a	Mathieson et al., 2018	no
I5208	LBK_Austria	European Neolithic	Schletz	Austria	F	K1b1a	..	Mathieson et al., 2018	no

Table B.4 Comparisons of within-population genetic diversity based on outgroup f_3-statistics. The lower triangle shows *P* values.

	Aşıklı	**Boncuklu**	**Çatalhöyük**	**Barcın**	**Tepecik-Çiftlik**
Aşıklı	-				
Boncuklu	>0.1	-			
Çatalhöyük	0.0006	<0.0001	-		
Barcın	<0.0001	<0.0001	0.0978	-	
Tepecik-Çiftlik	0.0057	<0.0001	0.217	0.0725	-

	Aşıklı Level4	**Aşıklı Level2**	**Boncuklu**	**Çatalhöyük**	**Barcın Level VIa**	**Barcın Level VIb**	**Barcın Level VId_or_VIc**	**Tepecik-Çiftlik Level 5**
Aşıklı Level4	-							
Aşıklı Level2	0.405	-						
Boncuklu	0.8782	0.9749	-					
Çatalhöyük	0.0052	0.0175	<0.0001	-				
Barcın Level VIa	0.0118	0.0358	<0.0001	0.5944	-			
Barcın Level VIb	0.0131	0.0342	<0.0001	0.4729	0.7441	-		
Barcın Level VId_or_VIc	<0.0001	0.0005	<0.0001	0.694	0.6656	0.7293	-	
Tepecik-Çiftlik Level 5	0.0497	0.1032	<0.001	0.1608	0.0123	0.0375	0.0026	-

Table B.5 Table shows genetic pedigree relationships identified among co-burials from Neolithic Anatolian populations and comparisons with Neolithic and Bronze Age burials from Europe.

Period and Region	First-degree related pairs							Second-degree related pairs	Total pairs	Reference
	Mother-daughter	Mother-son	Father-daughter	Father-son	Sisters	Brothers	Brother-sister			
Neolithic Anatolia (c.8300 - 6000 BCE)	-	2	-	-	4	-	1	1	223	This study
Neolithic Europe (c.4825 - 2580 BCE)	-	-	1	1	-	-	-	4	76	Sánchez-Quinto et al. 2019
Late Neolithic and Bronze Age Europe (c.2750 - 1300 BCE)	-	6	-	4	2	8	1	19	799	Mittnik et al. 2019

CURRICULUM VITAE

PERSONAL INFORMATION

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EDUCATION

Degree	Institution	Year of Graduation
PHD	METU Molecular Biology and Genetics	2020
MS	METU Biology	2015
BS	Istanbul Uni. Molecular Biology and Genetics	2012
High School	Burdur Anadolu Teacher High School	2006

WORK EXPERIENCE

Year	Place	Enrollment
2020	Working at COVID-19 Aziz Sancar Test Center (Ankara/Turkey) as a volunteer for diagnostic tests in patients with COVID-19 for one month	Laboratory Group Leader
2018	EMBO Short-Term Fellowship (grant no. STF 7909) 21 October 2018 to 18 January 2019. "Development of an optimal ancient DNA capture method for samples with varying levels of preservation". Department of Archaeology and Classical Studies, Archaeological Research Laboratory, Stockholm University, Sweden	Visiting reseacher
2013 summer	Erasmus Summer Internship in Laboratory of Forensic Genetics and Molecular Archaeology Center for Archaeological Sciences, University of Leuven,Belgium	Intern Student

2010 fall semester	Molecular Biology Laboratory , Budapest Corvinus University, Hungary	Intern Student
2010 spring semester	Immunology Laboratory, Institute of Experimental Medicine, Istanbul University, Turkey	Intern Student
2009 summer	Human Gene Therapy Division of Medical Genetics, Akdeniz University, College of Medicine, Antalya, Turkey	Intern Student
2008 summer	Molecular Tests Lab of Biochemistry and Clinical Biochemistry Department, Isparta Süleyman Demirel University, College of Medicine, Turkey	Intern Student

FELLOWSHIPS AND AWARDS

1. Award of EMBO Short-Term Fellowship (grant no. STF 7909) 21 October 2018 to 18 January 2019. "Development of an optimal ancient DNA capture method for samples with varying levels of preservation". Department of Archaeology and Classical Studies, Archaeological Research Laboratory, Stockholm University, Sweden

2. Best Article Award by the Ecology and Evolutionary Biology Society of Turkey during 5th Ecology and Evolutionary Biology Symposium in Turkey 2018. "Yaka R, Birand A, Yılmaz Y, Caner C, Açan SC, Gündüzalp S, Parvizi P, Erim Özdoğan A, Togan İ, Somel M. 2018. Archaeogenetics of Late Iron Age Çemialo Sırtı, Batman: Investigating maternal genetic continuity in north Mesopotamia since the Neolithic. Am J Phys Anthropol. 166(1):196-207 DOI: 10.1002/ajpa.23423"

PUBLICATIONS

1. **Yaka R**, Mapelli I, Kaptan D, Doğu A, Chyleński M, Erdal Ö.D., Koptekin D, KB, Bayliss A, Mazzucato C, Fer E, Çokoğlu SS, Lagerholm VK, Krzewińska M, et al. Variable Kinship Patterns in Neolithic Anatolia Revealed by Ancient Genomes (Under review in Curr. Biol.).

2. Chylenski M, Ehler E, Somel M, **Yaka R**, Krzewinska M, Dabert M, Juras A. Marciniak A. 2019. Ancient Mitochondrial Genomes Reveal the Absence of Maternal Kinship in the Burials of Çatalhöyük People and Their Genetic Affinities. Genes 10, 207.

3. Kılınç GM*, Kashuba N*, **Yaka R***, Sümer AP, Yuncu E, Shergin D, Ivanov GL, Kichigin D, Pestereva K, Volkov D, Mandryka P, Kharinskii A, Tishkin A, Ineshin E, Kovychev E, Stepanov A, Alekseev A, Fedoseeva SA, Somel M, Jakobsson M, Krzewińska M, Stora J, Gotherstrom A. 2018. Investigating Holocene human population history in North Asia using ancient mitogenomes. Scientific Reports 8: 8969

4. Krzewinska M, Kjellström A, Günther T, Hedenstierna-Jonson C, Zachrisson T, Omrak A, **Yaka R**, Kılınç GM, Somel M, Sobrado V, Evans J, Knipper C, Jakobsson M, Stora J, Götherström A. 2018. From far and near: genomic and strontium isotope variation reveal immigration patterns in a Viking Age town. Current Biology (in press)

5. **Yaka R**, Birand A, Yılmaz Y, Caner C, Açan SC, Gündüzalp S, Parvizi P, Erim Özdoğan A, Togan İ, Somel M. 2018. Archaeogenetics of Late Iron Age Çemialo Sırtı, Batman: Investigating maternal genetic continuity in north Mesopotamia since the Neolithic. Am J Phys Anthropol. 166(1):196-207 DOI: 10.1002/ajpa.23423

6. Kılınç GM, Koptekin D, Atakuman Ç, Sümer AP, Dönertaş HM, **Yaka R**, Bilgin CC, Büyükkarakaya AM, Baird D, Altınışık E, Flegontov P, Götherström A, Togan İ, Somel M. 2017. Archaeogenomic analysis of the first steps of Neolithization in Anatolia and the Aegean. Proc Biol Sci. 284(1867) doi: 10.1098/rspb.2017.2064.

7. Kılınç, GM., Omrak A., Ozer F., Gunther T., Buyukkarakaya AM., Bıcakcı E., Baird D., Donertas HM., Ghalichi A., **Yaka R**., Koptekin D., Acan SC., Parvizi P., Krzewinska M., Daskalaki EA., Yuncu E., Dagtas ND., Fairbairn A., Pearson J., Mustafaoglu G., Erdal YS., Cakan YS., Togan I., Somel M., Stora J., Jakobsson M., Gotherstrom A. 2016. The Demographic Development of the First Farmers in Anatolia. Current Biology 26: 1–8.

8. Ottoni, C. et al. 2016. Comparing maternal genetic variation across two millennia reveals the demographic history of an ancient human population in southwest Turkey, R. Soc., 3: 150250. (**Reyhan YAKA** in the Acknowledgements)

9. **Yaka, R**. 2015. Ancient DNA Isolation and Mitochondrial DNA Analysis of Human Samples from Çemialo Sırtı, Batman in Southeast Anatolia. (Msc thesis). Middle East Technical University/Graduate School of Natural and Applied Sciences, Ankara Turkey.

PROJECTS CONTRIBUTED

1. 2018-present: ERC (Consolidator Grant no. 772390 to Mehmet Somel), Title: NEOGENE: Archaeogenomic analysis of genetic and cultural interactions in Neolithic Anatolian societies

2. 2018-present: The Scientific and Technological Research Council of Turkey (TUBITAK-3001), Project Title: Study of social structure in Central Anatolian Neolithic settlements using ancient genomics

3. 2015-2018: The Scientific and Technological Research Council of Turkey (TUBITAK-3501), Project Title: Characterization of Neolithic Anatolian populations by ancient DNA extraction and genome sequencing of individuals from Tepecik-Çiftlik (Nigde) and Çatalhöyük (Konya).

4. Workshop on Quantitative Evolutionary Biology, 14-21 September 2014, Şirince, İzmir/Turkey, http://qevolution2014.wordpress.com. Project title: SNP Calling from Whole Genome Sequence Data Project.

5. 2014-2016: The Scientific and Technological Research Council of Turkey (TUBITAK-3001), Project Title: A pilot study to obtain authentic aDNA (ancient DNA) from 2500-3000 years old human teeth samples obtained from the Çemialo Sırtı Excavations and determination of their mtDNA haplogroups.

6. 2014-2015: BAP-METU, Project Title: mtDNA extraction, aDNA sequencing and determining mtDNA haplogroups related to aDNA sequences from human teeth and bones samples from various excavation sites.

PRESENTATION AND CONGRESS REPORTS

1. 41st International Symposium Excavations, Surveys and Archaeometry, 17-21 June 2019, Dicle University Congress Center, Diyarbakır/Turkey. " First Insights into Genomic History of Aşıklı Höyük People using Ancient DNA " (Oral Presentation)

2. 5th Ecology and Evolutionary Biology Symposium (EEBST), 18-20 July 2018, Dokuz Eylul University, Continuing Education Center (DESEM), Izmir/Turkey. “New insights into the maternal history of Anatolian and north Mesopotamian populations since the Neolithic.” (Poster Presentation)

3. 40th International Symposium Excavations, Surveys and Archaeometry, 7-11 May 2018, Çanakkale Onsekiz Mart University, Troia Congress Center, Çanakkale/Turkey. "Maternal History of Anatolian and north Mesopotamian Populations since the Neolithic" (Oral Presentation)

4. 39th International Symposium Excavations, Surveys and Archaeometry, 22-26 May 2017, Uludağ University Atatürk Congress Center-Merinos, Bursa/Turkey. "Ancient DNA Isolation and mtDNA Haplogroup Analysis of Human Samples from Çemialo Sırtı, Batman in Southeast Anatolia" (Oral Presentation)

5. Biotechnology and Life Sciences Industry Exhibition, OpenLab Scientific Program, 20-22 April 2017, Istanbul Lutfi Kirdar Congress and Exhibition Center. "Ancient DNA and the Studies at METU in Turkey" (Oral Presentation)

6. 2nd Ecology and Evolutionary Biology Symposium (EEBST), 6-8 August 2015, Middle East Technical University, Ankara/Turkey. "Ancient DNA Isolation and mtDNA Analysis of approximately 2500-year-old Human Teeth and Phalanx Samples from Çemialo Sırtı/Batman in Southeastern Anatolia (Poster Presentation)

7. 37th International Symposium Excavations, Surveys and Archaeometry, 11-15 May 2015, Atatürk University Congress Center, Erzurum/Turkey. "Ancient DNA Isolation and mtDNA Haplogroup Analysis of Human Teeth and Bone Samples Dated to approximately 3000 BP from Çemialo Sırtı Excavation Site" (Oral Presentation)

8. Workshop on Quantitative Evolutionary Biology, 14-21 September 2014, Şirince, İzmir/Turkey, http://qevolution2014.wordpress.com. "Ancient and Modern mtDNA in Relation to Peopling of Anatolia" (Poster Presentation)

9. European Association of Archaeologists 20th Annual Meeting, 10-14 September 2014, İTÜ, İstanbul. "The Use of Ancient mtDNA to Infer Early Domestication History of Sheep in Anatolia" Population Genetics Research Laboratory (Togan Lab.), METU, Department of Biology (Poster Presentation)

10. The EMBO Conference on Human evolution in the genomic era: Origins, populations and phenotypes, 1–4 April 2014, Leicester/UK, http://events.embo.org/14-human-evo/programme.html. "The Asian Contribution to Turkish Population with Respect to Balkans: mtDNA Perspective" (Poster Presentation)

FOREIGN LANGUAGES

Advanced English, Begginer German