

AN ADMIXTURE SIGNAL IN ARMENIANS AROUND THE END OF THE BRONZE AGE REVEALS WIDESPREAD POPULATION MOVEMENT ACROSS THE MIDDLE EAST

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Abstract

The Armenians, a population inhabiting the region in West Asia known as the Armenian Highland, has been argued to show a remarkable degree of population continuity since the Early Neolithic. Here we test the degree of continuity of this population as well as its plausible origin, by collating modern and ancient genomic data, and adding a number of novel contemporary genomes. We show that Armenians have indeed remained unadmixed through the Neolithic and at least until the first part of the Bronze Age, and fail to find any support for historical suggestions by Herodotus of an input from the Balkans. However, we do detect a genetic input of Sardinian-like ancestry during or just after the Middle-Late Bronze Age. A similar input at approximately the same time was detected in East Africa, suggesting large-scale movement both North and South of the Middle East. Whether such large-scale population movement was a

result of climatic or cultural changes is unclear, as well as the true source of gene flow remains an open question that needs to be addressed in future ancient DNA studies.

Keywords

Genetic continuity, Armenian Highland, Armenians, Balkan theory, Middle East

Introduction

The Armenian population has historically inhabited the Armenian Highland, a mountainous region stretching from the Mediterranean northeast to the South Caucasus (Fig.1, A). Geographic isolation, distinct language, a strong national and cultural identity might have been conducive to the long-term genetic isolation of Armenians from the neighboring populations [1,2,3]. Indeed, a comparison of both ancient and modern Armenian mitochondrial genomes (mtDNA), across a time span of eight thousand years, revealed a remarkably high level of matrilineal genetic continuity in the region [1]. The picture of genetic isolation is further backed up by genome-wide autosomal studies of contemporary Armenians suggesting a lack of an external genetic influx at least since the Bronze Age [2,3]. However, contemporary genomes have limited power to draw inferences on the genetic continuity through time.

The origin of Armenians is highly debated. Several theories and legends exist as to the formation of the population, though two of them prevail. According to the broadly accepted Balkan theory based on the ancient Greek historian Herodotus' writings, the ancestors of the Armenians were Phrygian colonists who migrated to the Armenian Highland from the Balkans [4]. The conclusion was derived mainly from the fact that Armenians were armed in the Phrygian fashion when they were part of the Persian army. Common ancestry for the Armenians and the Phrygians is further suggested by some linguists, who speculate that proto-Armenian language belonged to a Thracio-Phrygian sub-group within the Indo-European language family [5]. However, an alternative view based on popular legend for the origins of Armenians suggests the local formation of the Armenian population [6]. Despite extensive excavations in the area, to date, no convincing archaeological evidence has been found to support either of these hypotheses. Furthermore, no thorough genetic study on the relationships between modern Armenians and the ancient and modern samples from the Balkans has been conducted.

The availability of ancient DNA (aDNA) from different geographical areas and time periods has brought direct evidence from the past. Through comparing ancient and modern samples, it has become possible to get detailed insights into the historical relationships between human populations and make powerful inferences on whether there is direct genetic continuity in the region or immigration by an exogenous population [7, 8]. Here, we collated all published genomic data for Armenians (both ancient and modern), as well as generated a number of whole-genome sequences of modern Armenians with all four grandparents (4GP) originating from western (n=2), central (n=2) and eastern (n=2) regions within the Armenian Highland (Fig. 1a,b). By putting together a dataset covering a broad timescale and range of ancestries, we were able to formally investigate the genetic origins of Armenians, their genetic continuity as well as check whether the Armenian population can indeed be considered as an isolate that was sheltered from the major migrations that shaped the rest of Western Eurasia.

Results

Relationships to modern and ancient populations: testing the Balkan theory

We first performed a principal component analysis (PCA) to assess the genetic affinities of the Armenian samples to a broad panel of ancient and modern-day populations (Fig.1c). We observed that the modern Armenians' cluster falls in-between the genetic variation of the modern Caucasus and the Middle East, which is in accordance with their geographic location. Moreover, the cluster partly overlaps with the ancient inhabitants from the Armenian Highland, thus suggesting a degree of regional genetic continuity since the Chalcolithic (the earliest samples available for this region). In stark contrast, modern and ancient samples from the Balkans appear significantly distant from the Armenian cluster and are drawn mostly toward other European populations. To further investigate this pattern, we used a D statistics in the form $D(\text{Modern_Armenians}, \text{Ancient_Armenian_Highland}; X_{\text{Balkan}}, \text{Mbuti})$ to formally test whether modern Armenians received any genetic input from ancient and modern samples from the Balkans (Table 1, Supplementary Table 1). To account for attraction in ancient samples due to damage, we performed our analysis with transversions only. We did not observe any significantly positive values for the D statistic, implying that there is no Balkan-related ancestry in modern Armenians. Furthermore, we found that ancient and modern samples from the Armenian Highland form a clade ($|Z| < 3$) to the exclusion of ancient and modern samples from

the Balkans. Thus, both PCA and D statistics support the distinctiveness of ancient and modern Armenian populations from any Balkan input.

Insights into the Armenian regional continuity

We next used a maximum-likelihood approach [9] to test for continuity of ancient and modern populations of the Armenian Highland. This model assumes a scenario where changes in allele frequency from the common ancestor forward in time are explained through drift alone. We performed our analysis on capture and shotgun ancient data, separately. The test suggests a very recent drift time in modern Armenians since the common ancestor with the ancient samples, thus supporting the close relationship between ancient and modern inhabitants of this region. However, the model rejects the regional continuity hypothesis, since the ancient populations appear to have significantly larger drift times (Supplementary Table 2). This result is compatible with a scenario of admixture into modern Armenians from an outside population, which diverged earlier than the split time between the modern and ancient inhabitants of the region. As a consequence, this admixture would have increased heterozygosity in modern Armenians and thus it would exaggerate the drift time in ancient Armenians (since no genetic influx is allowed according to the model), eventually resulting in the rejection of the regional continuity. We then investigated corresponding signatures of gene flow into the Armenian Highland by using D statistics in the form $D(\text{Modern_Armenians, Ancient_Armenian_Highland}; X_{\text{ancient_population}}, \text{Mbuti})$. We find that a number of Bronze Age samples from the region break the clade of ancient and modern Armenian (Table 2, Supplementary Table 3); in all cases, the Bronze Age samples from the region are more closely associated with the ancient Armenian ($Z < -3$), suggesting an influx into modern Armenians after the end of the Bronze Age from an unknown source which is not well represented by any of the available ancient samples.

Identifying the source and time of admixture

In order to determine the nature of the genetic influx, we calculated D statistics in the form $D(\text{Modern_Armenians, Ancient_Armenian_Highland}; X_{\text{modern_population}}, \text{Mbuti})$ to check whether some modern groups break the clade between modern Armenians and ancient samples from the region. We did not find any significant D values when using transversions only, though we were able to detect a suggestive signal ($Z > 2$) for introgression from a Sardinian-like

source after Early Bronze Age (Table 3). We then boosted our power by increasing the number of SNPs by including also transitions in our analysis. Using all SNPs, we found significant D values for a genetic influx into modern Armenians from a Sardinian-like source when using Early and Late Bronze Age Armenian samples as our ancient reference (Supplementary Table 4). However, the result for the Late Bronze Age samples should be treated with caution, as these samples were not UDG treated and thus using transitions might lead to artifacts arising from ancient DNA damage. The result for the Early Bronze Age, on the other hand, is much more reliable as these samples were UDG treated.

Among modern-day populations, Sardinians have the highest affinity to early European farmers, and often act as a proxy for that ancestry in population genetic analyses [10, 11]. We assume that the migration more likely came from the Middle East, rather than a relatively isolated Mediterranean island. However, we were not able to find any detectable signal of gene flow from an ancient Anatolian farmer-like source (Table 2, Supplementary Table 3). According to recent studies, 38-44% of the ancestry of modern Sardinians is derived from an Iranian, Steppe and North-African-related source [12]. Likewise, we did not reveal any Iranian-related ancestry in modern Armenians that may have altered them from their regional ancestors (Table 2, Supplementary Table 3). Thus, it is more likely that the true source of gene flow to the Armenian Highland is yet unsampled, and that the time span between the date of mixture (i.e. at some point during/after the Bronze age) and all available ancient individuals of the Middle Eastern region (which are much older) is too large for the latter to act as representative sources.

Finally, we attempted to date the genetic influx based on the patterns of linkage disequilibrium with ALDER. We had to run a one population test (modern Armenians as the target with an input from Sardinians), which has limited power, and so we augmented our sample sizes to 99 Armenians and 23 Sardinians with 709,810 SNPs. We were able to detect a significant signal of admixture with a Sardinian-like source in Armenians, with an estimated contribution of 28.0 +/- 3.8%. The timing of the admixture obtained from ALDER suggests a relatively old event (the best signal corresponds to 172.56 +/- 17.23 generations ago), which is closer to the end of the Early Bronze Age in Armenia. However, from our the aDNA data, we know that the admixture might have occurred even later, potentially after the end of the Bronze Age (approximately 3000

ya). Such a scenario is at the limit of the dating estimated by ALDER – the end of the Bronze Age is between 3 to 4 standard deviations from the best estimate.

Discussion

Our study, based on a combined dataset of modern and ancient genomes from the Armenian Highland, revealed a strikingly high level of regional genetic continuity for well over six thousand years, with only one detectable input from a Sardinian-like source during the Late Bronze Age or after. A recent study also suggest a similar level of stability up to the Bronze Age in the South Caucasus (there was no test for later inputs as this study used only aDNA) [13]. This pattern stands in contrast to most other Western Eurasian populations, which have undergone multiple large influxes [10,14]. A high level of continuity sets the Armenian population apart even in comparison to the Sardinians, who have long been considered as a genetic isolate in the region since the Neolithic, but recent studies have shown that the island received numerous genetic inputs after the Bronze Age [12].

We focused on solving a long-standing puzzle regarding Armenians' genetic roots. Although the Balkan hypothesis has long been considered the most plausible narrative on the origin of Armenians, our results strongly reject it, showing that modern Armenians are genetically distinct from both the ancient and present-day populations from the Balkans. On the contrary, we confirmed the pattern of genetic affinity between the modern and ancient inhabitants of the Armenian Highland since the Chalcolithic, which was initially identified in previous studies [15-17].

However, we found that the genetic continuity in the Armenian Highland was disrupted during the second half/after the end of the Bronze Age by an input from a Sardinian-like source. The time scale for this input, as well as its source, coincide with a similar event detected in East Africa [18,19], suggesting large scale population movements from the Middle East going North and South. Our analyses failed to find a good source for this expansion among the available aDNA samples.

Our results on the source population and date for mixture are mostly in line with the studies on Armenians conducted so far. An elevated Sardinian-like ancestry in Armenians was also detected in the previous study [2] based on modern genomes, and the gene flow rate was

estimated as 29%, which is quite comparable with our findings. Our study including aDNA samples enhances the power of admixture tests by providing proper sources for population ancestry. As such, we could not find any evidence for mixtures of multiple populations during the time period of 3,000–2,000 BCE. In contrast, we found a signal for a single admixture event from a Sardinian-like source which happened after the Early Bronze Age, and possibly after the end of the Bronze Age (but the lack of UDG treated samples for the latter part of this period in Armenia warrants some caution).

Conclusions

Thus, we conclude that there was large-scale movement across the Middle East towards the end or after the Bronze Age. From the genetic point of view, this movement has led to an essential input of Neolithic ancestry in the populations inhabiting the region. In particular, as a result of its movement northward, the population of the Armenian Highland has become genetically altered from its regional ancestors. While Sardinians are the best proxy for this gene flow, questions like where it comes from and what was a trigger for such a widespread migration wave are still unanswered. To investigate this further, further studies on the complex demographic processes of the region should be conducted with the incorporation of more ancient and modern data.

Materials and Methods

Sample collection and sequencing

Blood samples were collected from unrelated individuals with all four grandparents (4GP) originating from the western (n=2), central (n=2) and eastern (n=2) parts of the Armenian Highland. This study was approved by the Ethics Committee of the Institute of Molecular Biology NAS RA (IRB #00004079). Armenian subjects were informed about the aim of this study and gave their consent to participate.

DNA samples were extracted at the Institute of Molecular Biology NAS RA using a modification of the salting out procedure [20]. The DNA was normalized (~50 ng/μl) and sent for sequencing (Macrogen Company, South Korea) at high coverage (~30x) using paired-end whole genome sequencing on the Illumina HiSeq X Ten.

Data processing and alignment

Adapter sequences were trimmed from the ends of reads using *trimadap* [21]. Sequences were aligned to the hs37d5 build of the human reference genome using Burrows-Wheeler Aligner (BWA) version 0.7.16 [22] and clonal reads were removed with *samblaster* [23]. Variant calling was performed using Genome Analysis Toolkit (GATK) version 2.5.2 [24].

Merging with ancient and modern reference dataset

We merged our six modern Armenian genomes with the previously published two Armenian (4GP) whole genomes from the Simons project [25]. Fastq data were aligned and genotyped with the same pipeline described above. We used PLINK 1.09 [26] to combine our modern Armenian samples with calls from modern populations in the Human Origins (HO) dataset [10] and ancient samples from Lazaridis et al., 2017 [14] which also includes genotype calls for previously published ancient individuals [15, 16, 27-29]. We downloaded FASTQ files of Iron Age samples from the Armenian Highland [30], aligned them to the hs37d5 build of the human reference genome using BWA and duplicate reads were removed using SAMtools version 0.1.19 [31]. Indels were realigned using GATK RealignerTargetCreator and IndelRealigner [24]. Sequences with a mapping quality of ≥ 30 were retained with SAMtools [31]. Pileupcaller was used to sample alleles from low-coverage sequenced Iron Age Armenian genomes [32]. The merging with other dataset led to 1,054,527 SNPs when modern Armenians and ancient samples were analyzed, and to 591,558 when modern samples from the HO dataset were also included.

Population genomic analyses

Principal component analysis (PCA) was performed with a broad panel of modern and ancient samples using the *smartpca* software from Eigensoft 7.2.0 [33] with the outlier removal option off. All ancient samples were projected onto the principal components by using the options `lsqproject:YES`.

D-statistics on SNP array data were computed using the qpDstat program in the ADMIXTOOLS package [34]. Statistics were considered significant if Z-score was greater than 3 corresponding to a P-value <0.001 . To account for the potential effect of ancient DNA damage, we repeated our analyses both with and without transition substitution sites [35].

A formal continuity test was performed using the method described in Schraiber 2018 [9]. Prior to the analysis, we fitted alpha and beta priors to the discrete reference allele frequencies. Files of ancestral alleles were downloaded from the following source: ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase1/analysis_results/supporting/ancestral_alignments/. We restricted the analyses to sites with high-confidence calls where the ancestral state is supported by all sequence comparisons. We also did not include alleles with the frequency 0 or 1 in the modern population. Thus, for tests of population continuity with capture data, we were able to use 685,797 SNPs. For shotgun data, prior to running the analyses, we performed LD pruning with the option `-indep-pairwise 200 25 0.4` and further random subsampling of half of the remaining SNPs. This resulted in 1,826,787 SNPs available in the test of population continuity.

To date the time of admixture, we used ALDER [36] (version 1.03) with `mindis 0.005`. We processed FASTQ data on 23 Sardinian samples from Human Genome Diversity Project (HGDP)-CEPH panel [37] with the similar pipeline as for modern Armenian genomes. We combined those with data on 99 Armenians from Haber et al [2] genotyped for 710,870 snps.

Data accessibility

Raw reads and BAM files are available for download through the accession number (will be available upon the publication).

Authors' contributions

A.M., A.H. and L.Y. conceived and designed the study. L.Y. and Z.K. collected the data. A.H. performed the bulk of the bioinformatics and statistical analyses under the supervision of A.M. as well as prepared all the figures. E.J., P.M.D., J.S., A.Hak., As.M., H.S., and L.S. contributed to the analyses. A.H. and A.M. wrote the manuscript; all authors contributed to its editing and approved the final version.

Competing interests

We declare that we have competing interests.

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Footnotes

Electronic supplementary material is available online here ()

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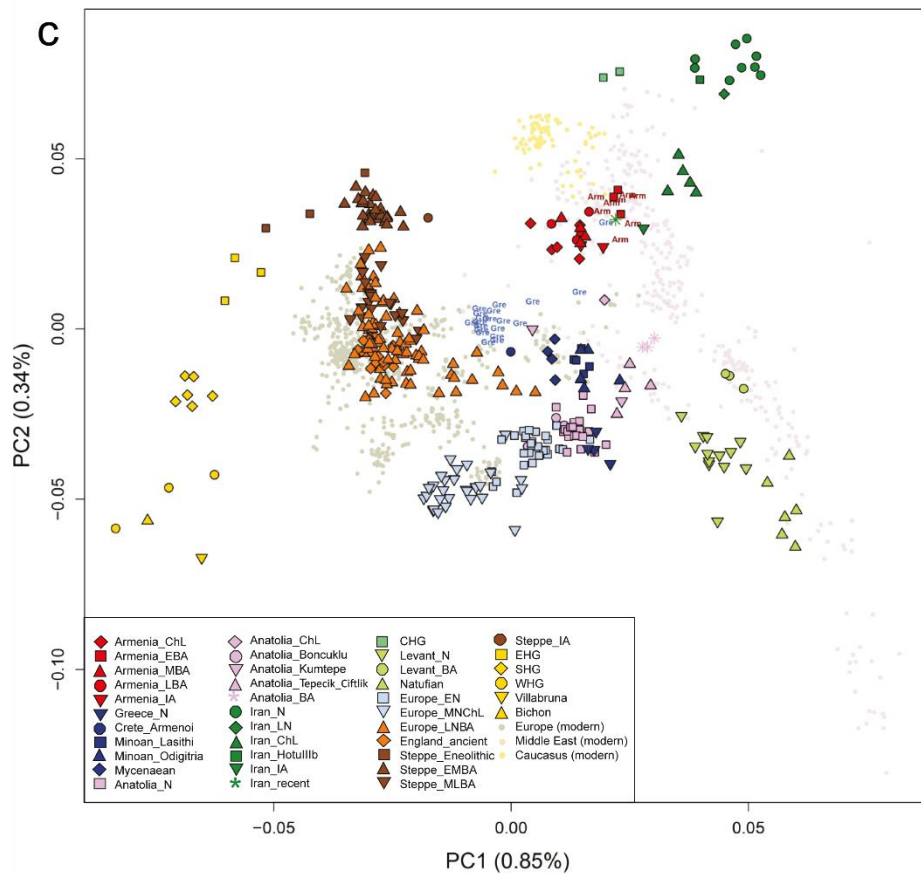
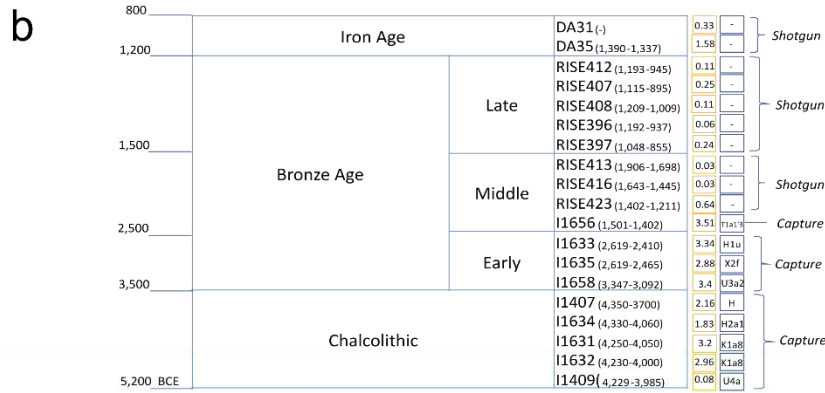


Figure 1. (a) Proposed route of Armenian migration according to the Balkan theory. Symbols represent populations and their shapes are described on the panel c. The numbers next to them indicate a sample size (if there is no number then the sample size is equal to one). WA, CA, EA are the abbreviations for modern Armenian samples from the western, central and eastern parts of the Armenian Highland, respectively. (b) Table with ancient Armenian samples. Radiocarbon dates (in cal BP) are shown under the sample name. Mean genome coverage is shown in yellow squares, mitochondrial haplogroups in blue squares (c) Principal component analysis (PC1 vs PC2). Values in parenthesis represent the percentage of variance explained by a given PC. According to the PCA, ancient samples from the Armenian Highland cluster with contemporary Armenians, while modern and ancient samples from the Balkans show distinctive clustering closer to other European populations.

A	B	X	Y	D	Z score	ABBA	BABA
Modern and ancient Armenian samples form a clade ($Z < 3$) with respect to Balkan samples							
Armenian	Armenia_ChL	Greece_N	Mbuti	0.0015	0.503	9615	9588
Armenian	Armenia_ChL	Minoan_Lasithi	Mbuti	-0.0045	-1.556	8839	8918
Armenian	Armenia_ChL	Minoan_Odigitria	Mbuti	-0.0001	-0.027	2377	2378
Armenian	Armenia_ChL	Mycenaean	Mbuti	-0.0088	-2.026	3535	3597
Armenian	Armenia_ChL	Crete_Armenoi	Mbuti	-0.0079	-0.643	402	408
Armenian	Armenia_EBA	Greece_N	Mbuti	-0.0003	-0.092	9333	9338
Armenian	Armenia_EBA	Minoan_Lasithi	Mbuti	-0.01	-3.106	8581	8754
Armenian	Armenia_EBA	Minoan_Odigitria	Mbuti	0.003	0.503	2341	2327
Armenian	Armenia_EBA	Mycenaean	Mbuti	-0.0077	-1.667	3433	3486
Armenian	Armenia_EBA	Crete_Armenoi	Mbuti	-0.0102	-0.703	400	408
Armenian	Armenia_MBA	Greece_N	Mbuti	0.0056	1.357	8513	8419
Armenian	Armenia_MBA	Minoan_Lasithi	Mbuti	-0.0023	-0.538	7908	7945
Armenian	Armenia_MBA	Minoan_Odigitria	Mbuti	0.0051	0.602	2208	2186
Armenian	Armenia_MBA	Mycenaean	Mbuti	0.0076	1.28	3169	3122
Armenian	Armenia_MBA	Crete_Armenoi	Mbuti	-0.0203	-1.02	380	396
Armenian	Armenia_LBA	Greece_N	Mbuti	-0.0012	-0.254	4848	4860
Armenian	Armenia_LBA	Minoan_Lasithi	Mbuti	0.004	0.731	4503	4467
Armenian	Armenia_LBA	Minoan_Odigitria	Mbuti	0.0061	0.545	1243	1228
Armenian	Armenia_LBA	Mycenaean	Mbuti	-0.0035	-0.433	1826	1838
Armenian	Armenia_LBA	Crete_Armenoi	Mbuti	-0.0004	-0.014	233	234
Armenian	Armenia_IA	Greece_N	Mbuti	-0.0015	-0.374	9433	9463
Armenian	Armenia_IA	Minoan_Lasithi	Mbuti	0.0035	0.801	8368	8310
Armenian	Armenia_IA	Minoan_Odigitria	Mbuti	-0.0079	-0.9	2154	2189
Armenian	Armenia_IA	Mycenaean	Mbuti	-0.0074	-1.183	3355	3405

Armenian	Armenia_IA	Crete_Armenoi	Mbuti	0.0262	1.244	381	362
Armenian	Armenia_ChL	Greek	Mbuti	0.0001	0.044	5052	5051
Armenian	Armenia_EBA	Greek	Mbuti	0.0043	1.542	4936	4893
Armenian	Armenia_MBA	Greek	Mbuti	0.0002	0.068	4494	4492
Armenian	Armenia_LBA	Greek	Mbuti	0.0014	0.311	2629	2622
Armenian	Armenia_IA	Greek	Mbuti	0.0006	0.153	4828	4822

Table 1. The D statistics of the form ($D(\text{Modern_Armenians}, \text{Ancient_Armenian_Highland}; X_{\text{Balkan}}, \text{Mbuti})$) based on SNP-chip data to assess the clustering between the ancient and modern Armenian samples with respect to the ancient and modern samples from the Balkans (transversions only).

A	B	X	Y	D	Z score	ABBA	BABA
There was a genetic influx to the Armenian Highland region after the Late Bronze/ Iron Age							
Armenian	Armenia_ChL	Steppe_Eneolithic	Mbuti	-0.0234	-5.715	6605	6922
Armenian	Armenia_EBA	Steppe_Eneolithic	Mbuti	-0.0104	-2.518	6552	6689
Armenian	Armenia_MBA	Steppe_Eneolithic	Mbuti	-0.0121	-2.237	6044	6192
Armenian	Armenia_LBA	Steppe_Eneolithic	Mbuti	-0.0182	-2.633	3350	3475
Armenian	Armenia_IA	Steppe_Eneolithic	Mbuti	-0.0082	-1.468	6196	6298
Armenian	Armenia_ChL	Steppe_EMBA	Mbuti	-0.0111	-4.567	9578	9794
Armenian	Armenia_EBA	Steppe_EMBA	Mbuti	-0.0115	-4.398	9308	9524
Armenian	Armenia_MBA	Steppe_EMBA	Mbuti	-0.0137	-4.055	8442	8677
Armenian	Armenia_LBA	Steppe_EMBA	Mbuti	-0.0191	-4.73	4811	4998
Armenian	Armenia_IA	Steppe_EMBA	Mbuti	-0.0169	-4.808	9357	9678
Armenian	Armenia_ChL	Steppe_MLBA	Mbuti	-0.0095	-4.117	9595	9778
Armenian	Armenia_EBA	Steppe_MLBA	Mbuti	-0.0086	-3.299	9327	9489
Armenian	Armenia_MBA	Steppe_MLBA	Mbuti	-0.0105	-3.121	8467	8645
Armenian	Armenia_LBA	Steppe_MLBA	Mbuti	-0.0123	-2.884	4845	4966
Armenian	Armenia_IA	Steppe_MLBA	Mbuti	-0.0128	-3.676	9401	9644
Armenian	Armenia_ChL	Steppe_IA	Mbuti	-0.0087	-2.329	7940	8080
Armenian	Armenia_EBA	Steppe_IA	Mbuti	-0.0086	-2.105	7762	7897
Armenian	Armenia_MBA	Steppe_IA	Mbuti	-0.0122	-2.251	7112	7288
Armenian	Armenia_LBA	Steppe_IA	Mbuti	0.0041	0.598	4032	3999
Armenian	Armenia_IA	Steppe_IA	Mbuti	-0.0157	-2.798	7387	7623
Armenian	Armenia_ChL	Anatolia_N	Mbuti	-0.0082	-3.585	9648	9808
Armenian	Armenia_EBA	Anatolia_N	Mbuti	-0.0052	-2.049	9399	9498
Armenian	Armenia_MBA	Anatolia_N	Mbuti	0.0031	0.916	8611	8558
Armenian	Armenia_LBA	Anatolia_N	Mbuti	-0.0002	-0.044	4905	4907
Armenian	Armenia_IA	Anatolia_N	Mbuti	0.0009	0.28	9566	9548

Armenian	Armenia_ChL	Anatolia_ChL	Mbuti	-0.0013	-0.318	5840	5855
Armenian	Armenia_EBA	Anatolia_ChL	Mbuti	-0.0123	-2.731	5701	5843
Armenian	Armenia_MBA	Anatolia_ChL	Mbuti	0.0008	0.125	5448	5440
Armenian	Armenia_LBA	Anatolia_ChL	Mbuti	-0.0048	-0.603	3113	3143
Armenian	Armenia_IA	Anatolia_ChL	Mbuti	-0.0037	-0.602	5301	5340
Armenian	Armenia_ChL	Anatolia_BA	Mbuti	-0.0079	-2.361	7381	7499
Armenian	Armenia_EBA	Anatolia_BA	Mbuti	-0.0119	-3.142	7239	7413
Armenian	Armenia_MBA	Anatolia_BA	Mbuti	-0.0034	-0.693	6800	6847
Armenian	Armenia_LBA	Anatolia_BA	Mbuti	0.0041	0.67	3908	3876
Armenian	Armenia_IA	Anatolia_BA	Mbuti	-0.0001	-0.018	6775	6776
Armenian	Armenia_ChL	Levant_N	Mbuti	-0.0105	-3.242	7414	7571
Armenian	Armenia_EBA	Levant_N	Mbuti	-0.0095	-2.622	7278	7418
Armenian	Armenia_MBA	Levant_N	Mbuti	-0.0054	-1.098	6761	6834
Armenian	Armenia_LBA	Levant_N	Mbuti	-0.0016	-0.271	3915	3928
Armenian	Armenia_IA	Levant_N	Mbuti	-0.0009	-0.188	6895	6908
Armenian	Armenia_ChL	Levant_BA	Mbuti	-0.0084	-2.742	7783	7915
Armenian	Armenia_EBA	Levant_BA	Mbuti	-0.013	-3.894	7601	7802
Armenian	Armenia_MBA	Levant_BA	Mbuti	0.0013	0.276	7144	7126
Armenian	Armenia_LBA	Levant_BA	Mbuti	-0.0008	-0.149	4103	4110
Armenian	Armenia_IA	Levant_BA	Mbuti	-0.0025	-0.552	7199	7235
Armenian	Armenia_ChL	Iran_N	Mbuti	-0.0021	-0.776	9487	9526
Armenian	Armenia_EBA	Iran_N	Mbuti	-0.0097	-3.308	9176	9356
Armenian	Armenia_MBA	Iran_N	Mbuti	-0.0074	-1.904	8354	8478
Armenian	Armenia_LBA	Iran_N	Mbuti	-0.0075	-1.586	4784	4856
Armenian	Armenia_IA	Iran_N	Mbuti	-0.0104	-2.835	9278	9473

Table 2. The D statistics of the form ($D(\text{Modern_Armenians}, \text{Ancient_Armenian_Highland}; X_{\text{ancient_population}}, \text{Mbuti})$) based on SNP-chip data to assess the clustering between the ancient and modern Armenian samples with respect to the ancient Eurasian populations (transversions only). Values with $|Z|$ score > 3 are in bold.

A	B	X	Y	D	Z score	ABBA	BABA
Armenian	Armenia_ChL	Sardinian	Mbuti	0.0015	0.586	5066	5051
Armenian	Armenia_ChL	Sicilian	Mbuti	0.0021	0.84	5052	5031
Armenian	Armenia_ChL	Italian_North	Mbuti	0.0000	0.007	5060	5060
Armenian	Armenia_EBA	Sardinian	Mbuti	0.0066	2.286	4954	4888
Armenian	Armenia_EBA	Sicilian	Mbuti	0.0062	2.178	4933	4872
Armenian	Armenia_EBA	Italian_North	Mbuti	0.0043	1.5	4943	4901
Armenian	Armenia_MBA	Sardinian	Mbuti	0.0021	0.565	4505	4486

Armenian	Armenia_MBA	Sicilian	Mbuti	-0.0001	-0.018	4482	4483
Armenian	Armenia_MBA	Italian_North	Mbuti	0.0003	0.071	4499	4497
Armenian	Armenia_LBA	Sardinian	Mbuti	0.005	1.095	2639	2612
Armenian	Armenia_LBA	Sicilian	Mbuti	0.0036	0.812	2628	2610
Armenian	Armenia_LBA	Italian_North	Mbuti	0.0022	0.502	2634	2623
Armenian	Armenia_IA	Sardinian	Mbuti	0.0033	0.856	4845	4813
Armenian	Armenia_IA	Sicilian	Mbuti	0.0028	0.758	4825	4798
Armenian	Armenia_IA	Italian_North	Mbuti	0	-0.002	4834	4834

Table 3. The D statistics of the form ($D(\text{Modern_Armenians}, \text{Ancient_Armenian_Highland}; X_{\text{modern_population}}, \text{Mbuti})$) based on SNP-chip data. (transversions only).