

The Results of Y-DNA and Mitochondrial Haplogroup Testing of an Ancient Burial of XII-XIV Centuries in Old Vladimir Patriarch's Garden Site Near the Klyazma River

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ABSTRACT

The work describes the results of the experiment on Y-DNA and mitochondrial haplogroup defining of an ancient burial in Vladimir Area. The obtained data help to define the Y-haplogroup and mitochondrial haplogroup according to genetic markers studied in the research. The paper outlines the experiment, technology, and the obtained result which defines R1a-M458-L1029 Y-haplogroup and H6c1 mitochondrial haplogroup with high probability.

Keywords: *gene pool, population genetics, paleo-DNA, contamination, SNP and STR markers of Y-chromosome, mt-DNA, haplogroups, sequencing, identity, medieval genome*

1. INTRODUCTION

The paleoDNA testing becomes one of the new challenging and resultative branches of modern biotechnology. Modern technology allows high-quality DNA extracting and analysing it by various number of methods including Y-chromosome and mitochondrial DNA haplogroup defining. It can give a valuable insight to define the affinity of an ancient population to other ancient and modern population groups including living people.

The main aim of the research was to make a haplogroup testing for a Russian medieval burial and to look at its possible analogs in other medieval DNA samples. For the test object a skull excavated in 2017 from the medieval Vladimir cemetery dating back to the 13th–14th centuries was chosen. The first reason for that choice was a serious significance of the Vladimir region in the processes of the Slavic settlement of North-Eastern Russia and the second reason was the good quality of preservation of the skull and the 100% gender determination. The excavations were executed by LLC «Vladimir Region Center for Archaeology» and the leaders of the excavations were Dr. S.V. Ocheretina and Dr. D.A. Kabaev.

The excavations were made from the burial ground on the territory of the Patriarch's Garden in 2016–2017. The burial ground (old cemetery) is located on high promontory formed by the southern slope of the left bank of the river Klyazma and the slope of the relief depression on which the garden is located. There were investigated about 180 burials on an area of more than 1000 m². For the experiment described, the Burial 7 from Excavation 2 was taken. Currently, the materials of the repository are being processed and studied, and the research results of whole cemetery will be presented later.

The experiment of Y-haplogroup and mitochondrial haplogroup testing of the described ancient burial was undertaken in March–July 2020 by the agreement with LLC «DNA-Nasledie» and all the analysis procedure was performed by LLC «DNA-Nasledie». The ancient sample was taken from the collection in Vladimir. The burial contained fragments of skull and teeth. The date of the sample is the end of XIII – beginning of XIV century and the burial mode was of Christian type.

For testing, two sample teeth from the burial were chosen by the research group. These teeth were transferred in 2020 to the office of LLC «DNA-Nasledie». The research was carried out according to the commercial agreement №DNA-LA/01-20 dated by 10 January 2020 with LLC «DNA-Nasledie».

2. METHODOLOGY

DNA analysis was carried out in a module, consisting of four glove boxes. To avoid contamination, each box was strictly separated from others and from the environment, and atmosphere air was replaced by neutral gas of high purity.

The DNA from the teeth was extracted by means of the method with SiO₂ columns, what is used in criminalistics and forensic medicine for the extraction of degraded DNA from complicated objects. The method is described in [1].

Obtained DNA was analyzed by means of the standard molecular genetic techniques, including real-time PCR on AB7500 equipment. Measurement of the concentration of the isolated DNA was carried out on a Qubitv2 fluorimeter on High Sensitivity chemistry.

For the research two independent DNA extractions were made. For Y-DNA analysis the concentration was 15 ng in µl, from powder of 6 gramm. For mitochondrial DNA analysis the concentration of isolated DNA was 7 ng in µl from powder of 2 gramm. The fragment analysis for Y-DNA was carried out on the same AB 3500xl analyzer. STRs were scanned at loci, the commercial Yfiler™ Plus kit (ThermoFisher) was used with 27 STR markers.

Analysis of mitochondrial DNA was carried out using NGS sequencing. Target sequencing (NGS) of hypervariable regions (I-III) of the mtDNA was performed using PowerSeq™CRM (Promega) reagents kit.

2.1. Experimental Design and Procedure

All stages of work with the archaeological sample were carried out in glove boxes, in which the atmospheric air was replaced by a neutral gas of high purity. The procedure included the following stages:

1. Sterilization of all instruments and working space in the boxes with the help of chemicals and hard UV-irradiation within 24 hours.

2. The primary mechanical cleaning of the ancient tooth from the surfacelayer of impurities on specialized equipment with dental milling cutters and secondary cavitation cleaning with ultrasound.

3. Grinding of the purified bones in a ball mill. As a result, a bone powder was obtained.

4. DNA extraction from the bone material was carried out by the method based on silicone columns.

5. The concentration contained DNA fragments from 20 to 180 bp in length. During the PCR reaction, STR fragments of the Y chromosome from 90 to 180 bp in length were successfully amplified. Fragments of greater length did not survive due to DNA degradation.

6. As a result of electrophoresis of PCR products on the fragment sequencer AB3500xl and analysis of foreograms, 27 STR loci of the Y chromosome with the values of the alleles indicated in Table 1 were reliably detected (Phoreogramme in Figure. 1 depicts numerically only part of the values). Detection results «out of machine» in initial representation are given in Figure. 2.

7. Analysis of mitochondrial DNA was carried out using a NGS sequencing method with PowerSeq™CRM (Promega) reagents kit and the results are shown below.

2.2. Data and interpretations

The experiment resulted in following data for Y-chromosomal haplogroup (YFiler mode). The Y-DNA analysis showed the R1a-M458 Y-DNA haplogroup, typical for the Slavic and other Central European populations. The result can be grouped with other still scarce Slavic Y-DNA medieval data from Russia and with results from Western Slavic regions.

Table 1 Obtained Data and Comparison with Russian Analog

Name and alleles of STR loci	Vladimir, Patriarch’s Garden, ex.2, №7, XIII-XIV cent.	Radonezh, Burial №1 (Kv-A-B/1-2) XVI-XVII cent.
393	13	13
390	25	22
19	15	14
391	10	
385 a	11	11
385 b	14	14
439	11	10
389 I	13	13
392	11	11

389 II	29	29
458	17	16
437	14	
448	20	20
449	32	32
460	11	11
GATAH4	12	10
456	16	17
576	18	16
570	18	
438	11	11
481	23	25
533	12	
635	23	23
627	16	22
518	42	
F387S1 a	35	32
F387S1 b	38	37

As the number of data is still small, in this paper we compare the result with a direct DNA analogy of a burial from Radonezh archaeological site [2, 3] in Moscow Region near border with Vladimir Region. At the medieval layers of the Radonezh burial ground, excavations in 1988–1990 uncovered 38 burials, as well as remnants of 107 destroyed burials (according to the number of skulls). The excavations were headed by professor V.I. Vishnevsky. Fragments of white stone tombstones from the graves date back to the first half and middle of the 16th century were found. In burial 13, in the right hand of a skeleton, a silver coin from 1560–1584 was found.

In Radonezh DNA research Burial №1 (Kv-A-B/1-2) was studied. «Burial №1 (Kv-A-B/1-2). No traces of a pit. The burial is filled by light-brown soil with ceramic fragments. It is oriented to the South-West by the head, azimuth 245 degrees. The depth is 0.9 from the surface. The bones are of medium safety, the skeleton lies on the right side. The knees lie 20 cm from each other. The feet and shins were destroyed by later burials. The hands are folded together. The orbits are oriented towards the South-West. The skeleton is attributed to a man of the age about 25 years. In the nose bridge and in the crown area there are small oval holes of around 1 cm diameter. An iron knife was found near the hips, a bronze cross with the Calvary depiction was found below the right shoulder. The cross was dated by M. V. Sedova by 16th–17th centuries. During the

excavating, just below the burial, another burial was found (burial №13) [2, 3].

Burial №1 is an exception in the position of the backbone and the placement of arms. It can be explained either by a casually neglected coffin, in the event that the funeral took place in an extreme situation. The deceased died a violent death as just above the bridge of the nose, bullet holes were found towards the top. Probably, the haste of the funeral is explained by the presence of artefacts with the deceased: a cross on the chest and a knife in a leather sheath on the belt (while all the burials, except for two, are non-inventory). The small depth of the grave is explained by the fact that the gravediggers at the hood came across the skeleton of an earlier burial, and rather than pull it out, they put the deceased on top. Burial №1 was taken out by a monolith and brought to the museum, and while dismantling it directly under burial skeleton 1, one earlier skull (skull №2) was found» [2, 3].

This burial can be mentioned as the direct DNA-analog of the described newly introduced one from Vladimir Patriarch's Garden cemetery.

The definition of Y-haplogroup with 27 loci according to the computer predictor NEVGEN [4] based on actual Y-haplotype data (database of the predictor is dated by 30.08.2020) gives 99% probability of R1a Y-haplogroup, and the most probable subclade is R1a-M458-L1029 (the obtained phoreogramme is given in figure 1). The

haplogroup of Radonezh burial was detected the same with 97% probability of R1a and most probable subclade is the same R1a-M458-L1029. We see the coincidence of many loci values in both samples analysed.

Analysis of NGS sequencing for mitochondrial haplogroup gives the following values of HVRI and HVRII in mitochondrial haplogroup sequence of studied sample.

HVRI: 16400T, 16482G

HVRII: 239T, 263G, 315.1C.

According to FTDNA haplogroup defining mutation list [5], it can be interpreted and assigned as H6c1 subclade by mutations 239T and 16400T occurring simultaneously. The both mutations identify the subclade in unique way. No ambiguity is possible in this case as it happens sometimes in other cases for several different HVRI and HVRII combinations.

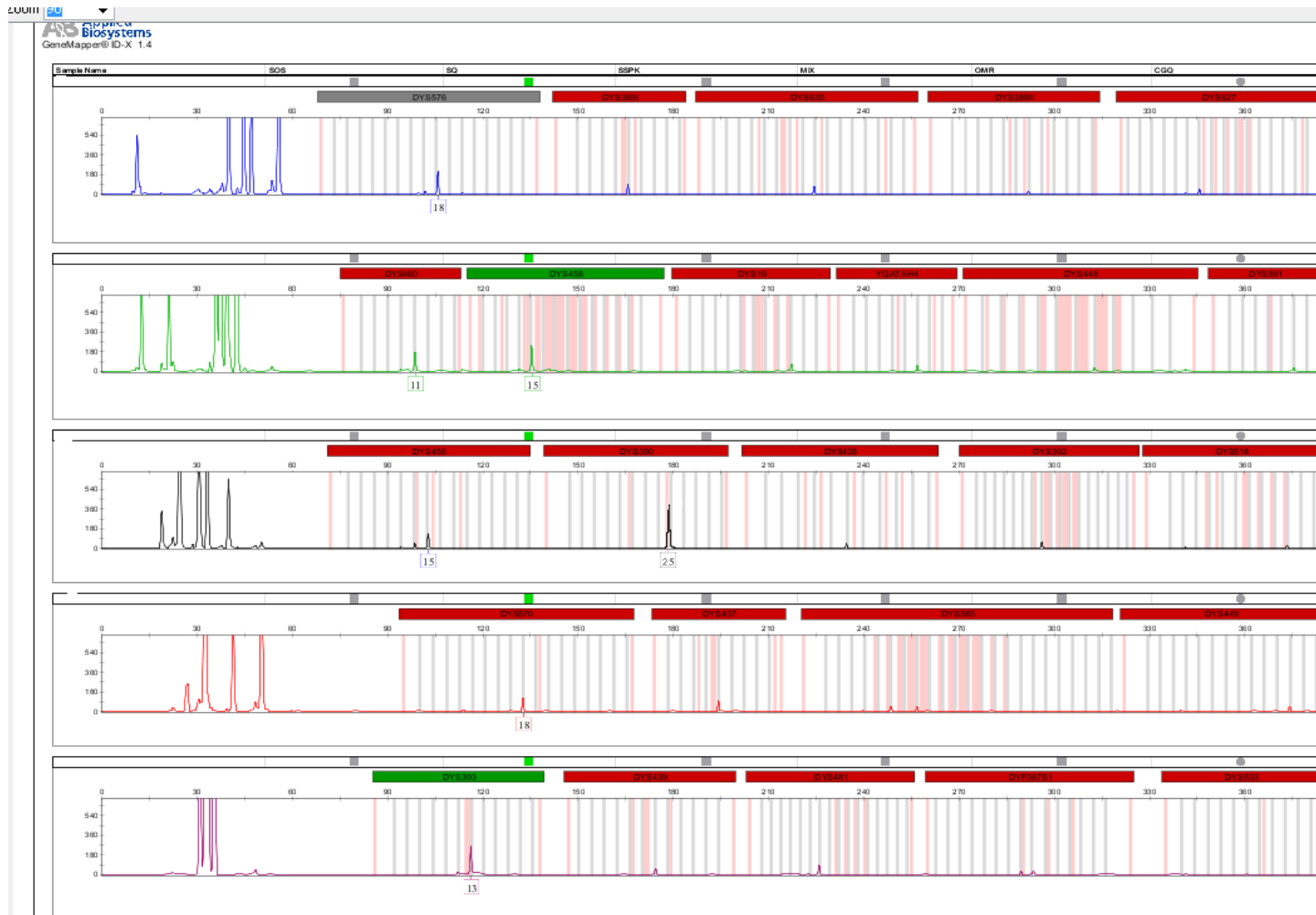


Figure 1 Phoreogramme of Y-haplotype STR loci of the studied sample

Sample	File	Sample Name	Marker	Allele 1	Allele 2		
DR	RUS_H01_2020-20-03-MIX-ANCIENT	18	DNA_19-57-26.fsa	DR	RUS	DYS576	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	13	DNA_19-57-26.fsa	DR	RUS	DYS389I	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	23	DNA_19-57-26.fsa	DR	RUS	DYS635	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	29	DNA_19-57-26.fsa	DR	RUS	DYS389II	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	16	DNA_19-57-26.fsa	DR	RUS	DYS627	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	11	DNA_19-57-26.fsa	DR	RUS	DYS460	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	17	DNA_19-57-26.fsa	DR	RUS	DYS458	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	12	DNA_19-57-26.fsa	DR	RUS	DYS19 15	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	20	DNA_19-57-26.fsa	DR	RUS	YGATAH4	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	10	DNA_19-57-26.fsa	DR	RUS	DYS448	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	16	DNA_19-57-26.fsa	DR	RUS	DYS391	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	25	DNA_19-57-26.fsa	DR	RUS	DYS456	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	11	DNA_19-57-26.fsa	DR	RUS	DYS390	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	11	DNA_19-57-26.fsa	DR	RUS	DYS438	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	42	DNA_19-57-26.fsa	DR	RUS	DYS392	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	18	DNA_19-57-26.fsa	DR	RUS	DYS518	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	14	DNA_19-57-26.fsa	DR	RUS	DYS570	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	11 14	DNA_19-57-26.fsa	DR	RUS	DYS437	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	32	DNA_19-57-26.fsa	DR	RUS	DYS385	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	13	DNA_19-57-26.fsa	DR	RUS	DYS449	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	11	DNA_19-57-26.fsa	DR	RUS	DYS393	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	23	DNA_19-57-26.fsa	DR	RUS	DYS439	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	35 38	DNA_19-57-26.fsa	DR	RUS	DYS481	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	12	DNA_19-57-26.fsa	DR	RUS	DYS481	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	12	DNA_19-57-26.fsa	DR	RUS	DYF387S1	
DR	RUS_H01_2020-20-03-MIX-ANCIENT	12	DNA_19-57-26.fsa	DR	RUS	DYS533	

Figure 2 Result of detection of Y-haplotype STR loci in initial form «out of machine»

3. DISCUSSION AND ANALYSIS

Subclade R1a-M458 is considered as a subhaplogroup inherent to Slavic and Baltic people, with maximal density

in Poland, Lithuania, Czech Republic, Slovakia, Ukraine, and North-Western Russia [3]. The main area is limited to Poland, the Czech Republic, and Slovakia. In central and southern Poland, the frequency of haplogroup reaches its

maximum, about 40 %, while in the north, east, and south-west the range is from 15 to 23 %. In the Czech Republic it is 25 %, and 18–25 % in Slovakia. Subclade M458 is present more to the East as well. In the eastern part of Europe, the frequency of M458 is still high, for example, in Ukraine and Belarus from 7 % to 22 % in the eastern part of Ukraine and Western Russia. Maximum frequencies in Russia are recorded in the Orel region (14 %), and in the Pskov, Belgorod and Kostroma regions (uniformly up to 12 %). Further to the east, in the Tatar populations, the measure is approximately 4–5 %. In the west of Poland and in Germany, the R1a-M458 frequency drops slightly to 10 %. In the Balkans it is also present: 9 % in Croatia, 8 % in Bosnia, 5 % in Albania, 5 % in Romania, 4 % in Greece, 4 % in Macedonia, 3 % in Serbia, and 2 % in Slovenia. In Scandinavia, the frequency of haplogroups is greatly reduced to 2 % in Swedes, although in Denmark it is about 4 %.

The old DNA from East Slavic people is still known and published by a limited amount of data. We know two DNA sets from Western Slavic people. In two works from Germany, a group of results has already been obtained from a burial in Usedom, on the Polish-German border, and in Saxony-Anhalt, near Dresden (burial of Krakauer Berg), which indicate the presence of the West Slavic subclade R1a-M458.

Mitochondrial haplogroup H is the most common mitochondrial haplogroup in Europe, as it includes more than half of the modern female population of North-West Europe. This haplogroup is also common in North Africa and the Middle East. The frequency of the spread of this haplogroup in Europe decreases towards the southeast part of the Eurasia, accounting for only 20 % in the Middle East and the Caucasus, and less than 10 % in the Persian Gulf, North India, and Central Asia. As it is shown further the subclade H6c is inherent to Central and Eastern Europe

Concerning Vladimir area population process, the determination in a burial at the end of the 13th - beginning of the 14th century in the Patriarch's Garden of Vladimir, of haplogroup Y-DNA **R1a-M458** in combination with mtDNA **H6c1** does not contradict the assumption that the ancestors of buried man belonged to the Slavic population that settled in North-Eastern Russia. At the same time, the probability of movement direction from the Western part of Slavic world is not excluded (for example, the process of movement of Slavic people to XII century Klyazma River settlements from the western side was described in [6]).

Two medieval R1a-M458 from Usedom (12th–14th centuries, German-Polish border) [7] and Krakauer Berg (11th–15th centuries) [8] support this view. One R1a-M458 was fixed even westwards in Netherlandish burial Oldenzaal (14th century) [9].

Mitochondrial haplogroup H6, having counterparts in modern Poland, also fits this view. According to [10] haplogroup H6 is very interesting because two its branches, H6a and H6b, are characteristic, correspondingly, for European and Central Asian/Near Eastern populations, thus reflecting a long-time separation of Asian and European H6

mtDNA pools. Mitochondrial haplogroup H6c has its representatives in Central and Eastern Europe, especially in Poland.

The research revealed that the medieval Russian people from the Vladimir and Radonezh of XIII–XVI centuries had the typical subclades of Northern and Eastern Europe and Slavic original is the most probable.

4. CONCLUSION

The first evidence of the presence of R1a-M458 among the population of North-Eastern Russia in the XIII–XVI centuries. (Radonezh, Vladimir) not only supports the view of R1a Y-haplogroup presence for all medieval Russian lands, regardless of principality borders, but also hints that certain genetic unity with the western parts of Slavic areal could exist (as the most serious concentrations of R1a-M458 are noticed in the western part of the Slavic areal). We conclude that the experiment was proper and a positive result is obtained. In conclusion, the experiment of haplogroup estimating on a new and recently introduced burial showed that the method developed at the Moscow Institute of Physics and Technology works well for the medieval samples in Russian soil and can be a good base for future development of historical genetics of Russia.

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