PHENOTYPING THE ANCIENT WORLD: THE PHYSICAL APPEARANCE AND ANCESTRY OF VERY DEGRADED SAMPLES FROM A CHALCOLITHIC HUMAN REMAINS

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Abstract

In many cases, the genetic study of ancient samples is quite similar to a forensic critical sample analysis with an unknown origin. In both cases, it is not possible to compare the genetic information with other family members, being almost impossible to achieve the individual identification. In such situations, the prediction of externally visible characteristics (EVC) of an individual and his biogeographical ancestry could definitely be a crucial contribution in a forensic casework.

Therefore, the aim of the present work was the molecular study of a very critical sample, a Chalcolithic (3480±30 YBP) individual found in Asturias, Northern Iberia, intending to

discover a possible geographical ancestry for these remains, and the inference of a group of feasible EVCs (hair, skin and iris pigmentation).
Given that ancient DNA is often highly damaged, two different methodologies were used in order to determine the biogeographical ancestry of the individual: mitochondrial DNA (HVR-I and -II) and Single Nucleotide Polymorphisms typing, since only short fragments of target DNA are required for a successful amplification.
Despite the antiquity of the samples, the genetic information recovered proved of great value. We could determine that the samples donor had a European ancestry, blond hair, light skin color and brown eyes. Such outcome reveal that it is possible to obtain not only biogeographical but also phenotypic information from a very critical sample.

Keywords
Critical samples, EVC, ancestry, Chalcolithic period

1. Introduction

The genetic study of ancient samples is quite similar to a forensic critical sample analysis with an unknown origin. In both cases, it is not possible to compare the genetic information with other family members, being almost impossible to achieve the individual identification. In such situations, the prediction of externally visible characteristics (EVC) of an individual and his biogeographical ancestry could definitely be a crucial contribution in a forensic/archaeological investigation.
In this case, we intended to study the EVC (hair, skin and iris pigmentation) of a skeleton found in Asturias, Northern Iberia that, according to the anthropological information, belonged to a Chalcolithic (3480±30 YBP) 16-18 years old man.

2. Materials and methods

DNA extraction

Two dental pieces (1GV1 and 1GV2) were selected from the skeleton, according to the authenticity criteria [1]. After the external cleaning, one of the samples was pulverized, and the extraction protocol was followed according to Rohland et al. [2]. The DNA of the other sample was directly extracted according to Gomes and Palomo-Díez et al. [3].

DNA amplification

Results were obtained by two different techniques in two independent Spanish laboratories: Laboratory of Forensic and Population Genetics of the Legal Medicine School, Complutense

University of Madrid (UCM) – Lab1- and Forensic Sciences Institute (INCIFOR), of the Santiago de Compostela University (USC) - Lab2.

In Lab1, two short overlapping sequences (175 base pairs – bp - and 170 bp) from mtDNA Hypervariable Region 1 (HVR1) were studied and other two overlapping sequences (119 bp and 100 bp) were also analyzed from the HVR2 [4]. To estimate the most probable biogeographical ancestry of the studied individual, the EMPOP [5] mtDNA database was consulted. In Lab2, 34 SNPs were analyzed to predict the biogeographical ancestry, according to Phillips et al., (2007) [6]. The data achieved from the obtained genetic profiles was employed to calculate a Likelihood Ratio (LR), taking into account the data set for the five major populations: Europe, Sub-Saharan, African, East-Asia, America, and Oceania.

Concerning the EVCs, 35 SNPs were analysed in Lab 2, to predict the most probable hair, skin and eye color pigmentation [7]. For each EVC, the hypotheses considered were: a) iris pigmentation (brown, hazel-green, and blue); b) hair pigmentation (fair versus dark, and for the colour red, blond, brown and black); and, c) Skin pigmentation (white, intermediate and black).

Calculations were performed with the aid of the online snipper app suite v2.0 software [6], despite the lack of a specific present-day Asturian database, and the absence of an ancient DNA database from the studied period (Chalcolithic) population. Therefore, modern DNA population data were used.

3. Results

In Lab.1 the mtDNA haplotype was determined, considering HVR1 (100-389 positions) and HVR2 (16126-16369 positions) regions: 199C 202G 263G 315.1C. Finally, EMPOP mtDNA database also specified three likely haplogroups, considering the referred haplotype (and 202G as a private mutation): haplogroups H3, H10 and H45. SNPs genotypes from Lab2 are shown in in **Table 1** and considered hypothesis and the respective LRs are shown in **Table 2.**

Table 1. Results of the 34 autosomal SNPs analysis for the ancestry determination, and the 35 autosomal SNPs concerning the EVC “Eye pigmentation”, “Hair pigmentation” “Skin pigmentation”. “NN”- without results.

Ancestry						Eye pigmentation		Hair pigmentation		Skin pigmentation	
SNP	Result	SNP	Result	SNP	Result	SNP	Result	SNP	Result	SNP	Result
rs5997008	C	rs773658	C	rs2026721	A	rs12913832	G	rs1129038	NN	rs10777129	NN
rs2304925	T	rs10141763	A	rs4540055	AT	rs1129038	NN	rs11547464	NN	rs13289	C
rs917118	G	rs182549	C	rs1335873	A	rs11636232	C	rs12913832	G	rs1408799	G

rs1321333	T	rs1573020	A	rs16891982	CG	rs12203592	C	rs12931267	C	rs1426654	A
rs2814778	C	rs896788	C	rs730570	T	rs12896399	AC	rs1805006	C	rs1448484	A
rs1024116	A	rs2065160	A	rs1886510	NN	rs1393350	G	rs1805007	NN	rs16891982	GC
rs7897550	C	rs2572307	G	rs5030240	C	rs1667394	C	rs1805008	G	rs2402130	NN
rs722098	NN	rs2303798	C	rs3827760	A	rs16891982	GC	rs1805009	G	rs3829241	C
rs10843344	T	rs2065982	A			rs1800407	C	rs28777	A	rs6058017	NN
rs12913832	G	rs3785181	C			rs4778232	A	rs35264875	A	rs6119471	G
rs239031	NN	rs881929	T			rs4778241	T	rs4778138	T		
rs2040411	A	rs1498444	C			rs7183877	AC	rs7495174	CT		
rs1978806	T	rs1426654	T			rs8024968	AG				

Table 2. Likelihood ratio results for ancestry and for the EVC - Eye, hair and skin pigmentation study.

	Hypothesis	**Likelihood Ratio**
Ancestry	H_1: European ; H_2: American	$9{,}56 \times 10^{14} : 1$
	H_1: European ; H_2: East-Asian	$3{,}18 \times 10^{17} : 1$
	H_1: European ; H_2: North-African	1627,5 : 1
	H_1: European ; H_2: Sub-Saharan African	$3{,}05 \times 10^{30} : 1$
Eye pigmentation	H_1: Brown ; H_2: Hazel - green	143,3 : 1
	H_1: Brown ; H_2: Blue	$1{,}33 \times 10^{8} : 1$
Hair pigmentation	H_1: Light ; H_2: Dark	6,60 : 1
	H_1: Blond ; H_2: Brown	2,86 : 1
	H_1: Blond ; H_2: Redhead	11,69 : 1
Skin pigmentation	H_1: White ; H_2: Intermediate	1,05 : 1
	H_1: White ; H_2:Black	5684,45 : 1

4. Discussion

Obtained results for the ancestry investigation are coincident with the previous published data from this period [8] and supported by the mtDNA pool of the modern population in this geographical area [9]. On the other hand, additional published results concerning the same period of time, from Burgos - Atapuerca site, North of Spain, indicate that 29% of the Chalcolithic individuals had H haplogroups, while it was previously estimated that almost 50% of the Bell Beaker population would have an H [10]. Given the possibility of fine-scale structuring in the ancient Iberian population [4,9] it would be extremely important to find other individuals from the same region and period of time. When observing our LR results, and comparing "Europe" with "North Africa" hypotheses, the obtained LR was the lowest (1627,5 : 1), while all other LR calculation results were always above the 10^{14} magnitude order. This

result is usual within the modern Iberian population, which exhibits a close relatedness to that of North Africa, as was shown on previous studies treating unknown samples with disputed origin between those populations [4,9].

The obtained results also allow us to predict that the most probable EVCs of the studied individual were brown eyes, light blond hair and white skin. The obtained phenotype is not uncommon in the modern northern-Spanish population [12]. Our sample exhibits characteristics that are similar to both the Blond and Brown training set "populations". In the same fashion, skin pigmentation prediction displays lower LR values than usually expected due to loss of three key informative SNPs that could have raised the obtained predictive values.

Our study reveals that it is possible to obtain not only biogeographical but also phenotypic information from a very critical sample.

5. Conflict of interest statement:

None

6. Acknowledgments

This work was supported by G/6401400/8000 research project (Santander-Universidad Complutense de Madrid, Spain) for C.Gomes.

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