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Short communication

Diversity of Y-chromosomal and mtDNA markers included in Mediscope Chip within two Albanian subpopulations from Croatia and Kosovo: Preliminary data

Miran Čoklo[1], Dubravka Havaš Auguštin[1], Jelena Šarac[1], Natalija Novokmet[1], Elza Khusnutdinova[2], Serghey Litvinov[2], Sara Haydar[3], Corinne Lautier[3], Christophe Normand[3], Redha Attaoua[3], Madalina Vintila[3], Anna Bosch-Comas[4], Helena Suarez[4], Pedro Jares[4], Ramon Gomis[4], Saša Missoni[1,5], Damir Marjanović[1,6] and Florin Grigorescu[3]

[1]Institute for Anthropological Research, Zagreb, Croatia
[2]Institute of Biochemistry and Genetics, Ufa Scientific Center, Russian Academy of Sciences, Ufa, Bashkortostan, Russia
[3]Laboratory of Molecular Endocrinology, IURC, UMR-204 NUTRIPASS (IRD, UM, SuprAgro), University of Montpellier, France
[4]Laboratory of Diabetes and Obesity, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Mallorca 183, Barcelona, Spain
[5]Medical Faculty, Univeristy of Osijek, Osijek, Croatia
[6]International Burch University, Sarajevo, Bosnia and Herzegovina

ABSTRACT

The aim of this preliminary study is to analyze genetic specificity of Kosovo Albanians comparing with neighboring populations using new genetic tool – MEDISCOPE gene chip, to investigate the feasibility of this approach. We collected 37 DNA samples (9 Croats, 17 Albanians from Croatia and 11 Albanians from Kosovo) from unrelated males born in Croatia and Kosovo. Additionally, samples were expanded with female individuals and mtDNA analysis included a total of 61 samples (15 Croats, 23 Albanians from Croatia and 23 Albanians from Kosovo). This pilot study suggests that the usage of the MEDISCOPE chip could be recognized as an efficient tool within recognition of the population genetic specificity even within extremely small sample size.

Key words: *Y-chromosome, mtDNA, haplogroup, MEDISCOPE Chip, Albanian, Croatia, Kosovo*

Introduction

Several previous studies clearly concluded that most of Western Balkans inhabitants are descended of the "old Europeans" who survived the LGM in the several European refugiums[1–3]. The rest of the population are the offspring of people who arrived in this part of Europe following the southeastern route, in the last approximatively 10 000 years, mostly during and after the Neolithization process[1,4].

Albanians, as non-Slavic speakers of the Western Balkan region, compared to the rest of the Western Balkan populations, have a somewhat different cultural, demographic and linguistic background. They are descendants of an ancient Paleo-Balkan people, also known as Illyrians as part of the Hallstatt culture. The Messapian language in Southern Italy is considered an Illlyrian dialect. Albanian immigrants are estimated at 1.5 million and live in Kosovo, the Republic of Macedonia, Serbia, and Montenegro. Historical immigration is known in Turkey, Greece and Italy.

Due to extensive migrations during the second half of 20th and first decades of 21st century, Albanian population is spread out all over European continent, including Croatia (Figure 1). However, traditionally social-grouping of the Albanians was excessively strong[5,6].

The latest studies concluded that linguistic Western Balkans differences seem to have had no significant impact on the present variation of uniparentally inherited or autosomal markers[7]. Nevertheless, the same study, as the

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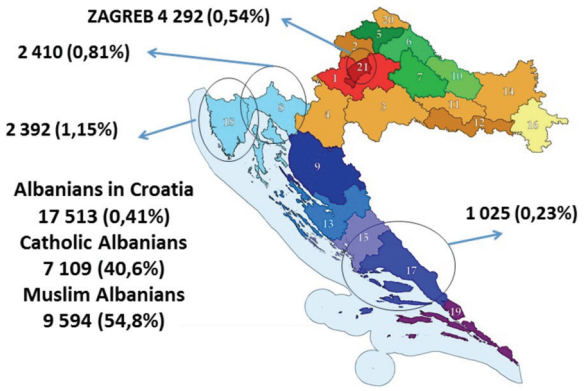

Fig. 1. Albanian minority in Croatia (according to the latest Census data – 2011).

previous ones[8,9] confirmed once again certain level of genetic specificity of Kosovo Albanians comparing with neighboring populations. Since Albanians mostly populated in Albania and Kosovo, therefore we have decided to analyze that specificity within this preliminary study using new genetic tool MEDISCOPE gene chip, to investigate the feasibility of this approach.

MEDIGENE European program (www.medigene-fp7.eu) intended to study genetic and environmental factors of metabolic syndrome (MetS) in Mediterranean populations. The interest in this syndrome was increased since in epidemiological studies, MetS but not its components apart correlated better with Health indices and even longevity in ethnic populations[6,7]. In front of several recent GWAS studies for MetS the goal is challenging, particularly because of still insufficient density in the genome of *single nucleotide polymorphism* (SNP) markers on commercial gene chips. In particular, there are no commercial gene chips containing together autosomal and mtDNA or chr Y anthropological markers to stratify populations during GWAS. While the MEDISCOPE gene chip was already successfully used in characterizing several genes with autosomal SNPs, in this study was assed to investigate the feasibility of the same chip using SNP for mtDNA and Chr Y DNA[10,11].

Material and Methods

Ethical Statement

All collected samples were declared to the Ministry (MESR) in France (CODECOH DC-2014-222). The preliminary collected sample consists of 37 (9 Croats, 17 Albanians from Croatia and 11 Albanians from Kosovo) unrelated males born in Croatia and Kosovo. Additionally, sample is expanded with female volunteers and mtDNA analysis include in total 61 (15 Croats, 23 Albanians from Croatia and 23 Albanians from Kosovo). Blood samples were collected and located in *Institute for Anthropological Researches* (INANTRO). Subjects (volunteers) were recruited in accordance with the *Helsinki Declaration* (as revised in 1983), after approval of national Ethical Committee approval in Croatia; informed consent was obtained from all patients. Approval was obtained for *import/export* of samples, inside and outside European Union (Nr IE 2014-762), while anonymous samples were declared to the CNIL commission in France (Nr 1788839).

Genotyping

DNA samples were genotyped with a customized Affymetrix gene chip called MEDISCOPE. Chip composition will be described elsewhere; Briefly, it contain beside 500,000 SNPs of the commercially available EUR chip, a number of 100,000 additional SNPs for candidate several genes. MEDISCOPE gens chip was then enriched with 161 SNPs for Y chromosome and 129 SNPs for mtDNA. For Chr Y haplogroup names were given according to Karmin et al. 2015, while for mtDNA the SNP were defined acoording to www.phylotree.org. Plates were processed on GenTitan (IDIBAPS, Barcelona) and raw data were transferred through the MEDIGENE server (https://xz.univ-montp1.fr) and introduced in the pipeline of analysis in Montpellier (France). mtDNA and Chr Y SNP analysis was performed as collaboration between Croatian and Russian partners. QC was performed with *Analysis Suite* 1.1 and *SNP Polisher* soft, implemented on Dell Precision T5500 computer. Dish QC and the call rate (CR) were obtained for each sample and plate while output files were obtained as.vcf. From 758,605 SNPs available on the chip 94.94% were recovered as Poly High Resolution (566,170), NoMinorHom (81,151), other (70,002), Mono High Resolution (16,028), OTV (1,465), Hemizygous (836). Below threshold were 19,953 SNP (2.6%). The mean call rate was 97%.

Results and Discussion

Chr Y haplogroups

Y-chromosome haplogroup frequencies in the populations from Albania and Croatia, obtained using MEDISCOPE chip, showed domination of old European markers (I2 and R1a) in Croats, as it was reported previously[1–3], for a representative sample of Croatian population (Figure 2). Albanian immigrants in Croatia, and Kosovo showed several common haplogroups with comparable frequencies: R1b-M269, E-M243, J2b-M241 (J2b2'3).

Domination of old Early Farmer and Greek expansion markers (E+J) and Balkan R1b from some unspecified place in the West Asia, but independently of farmers, was found in both Albanian populations (from Croatia and Kosovo) (Figure 2) as it was expected according to previously published data[4].

However, increased presence of R1a in the Albanians from Croatia could suggest possible mixtures with local Croatian population (Figure 2). It has to be emphasized that, since this is pilot data, sample size is very small, but it could represent a preliminary recognized migrational flow.

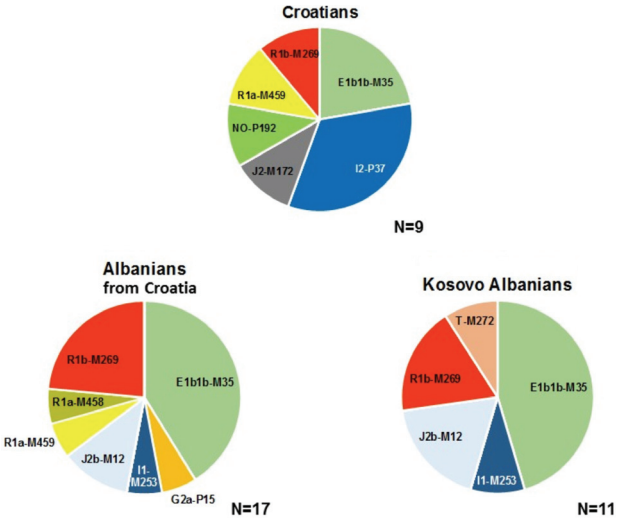

Fig. 2. Y-chromosome haplogroup frequencies in Croatians, Albanians from Croatia and Kosovo Albanians obtained using MEDISCOPE chip.

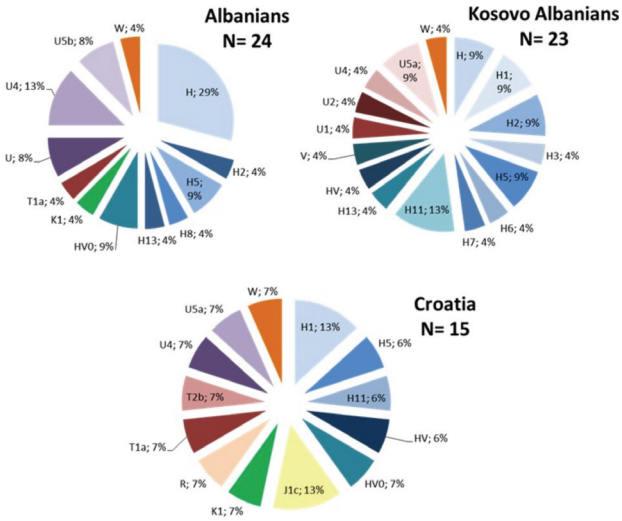

Fig. 3. mtDNA haplogroups in Croatians, Albanians from Croatia and Kosovo Albanians obtained using MEDISCOPE chip.

mtDNA haplogroups

mtDNA haplogroup frequencies in the populations from Albania in Croatia, obtained using MEDISCOPE chip, showed a very pronounced domination of haplogroup H (29% H, 4% H2, 9% H5, 4% H8 and 4% H13), followed by a typical European haplogroups as seen in majority of European populations (Figure 3). However, their frequency distribution differs significantly among Croatians on one side and both Albanian groups on the other (Figure 3).

It could be suggested from the analysis of NRY haplogroup frequencies that the two Albanian subpopulations (from Croatia and from Kosovo) have a similar and expected haplogroup distribution pattern, with the dominant portion of E1b1b-M35 individuals. Albania has previously been pinpointed as the probable place of origin for this lineage, and our results also bolster this view.

Albanian subpopulation from Croatia additionally shows more variation and harbors traces of other NRY haplogroups, which could be interpreted as an input from the local Croatian population.

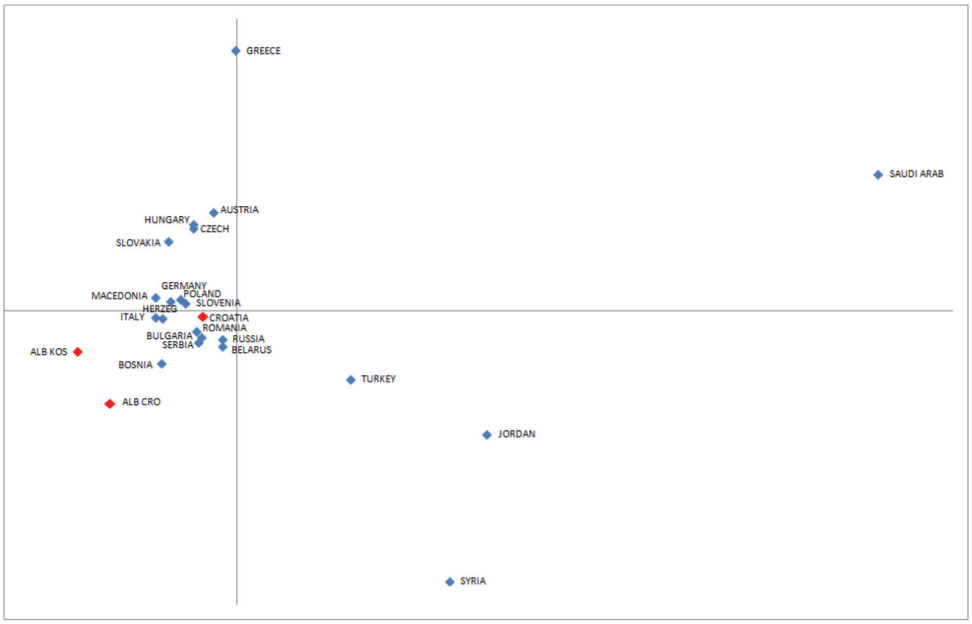

Fig. 4. PCA (principal component analysis) plot of several European and Middle Eastern populations based on mtDNA haplogroup frequencies.

It is evident from the analysis of mtDNA haplogroup frequencies that two Albanian subpopulations (from Croatia and Kosovo) have also very similar and expected hg distribution pattern, with the dominant portion of individuals harboring hg H, the most prominent European haplogroup. Similar results could be recognized within previous study[7].

PCA (*principal component analysis*) plot of several European and Middle Eastern populations based on mtDNA haplogroup frequencies (Figure 4)[12,13], together with population of Albanians from Croatia and Kosovo show clustering of both Albanian subpopulations, while Croatian subpopulation, although represented by a very small number of individuals fits in the center with other European populations.

This pilot study suggests that the usage of the MEDISCOPE chip could be recognized as an efficient tool within recognition of the population genetic specificity even within small sample size. Additional analysis including increased number of samples per population and observation of some other specific populations would provide more information about efficiency of this tool.

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M. Čoklo

Institute for Anthropological Research, Ljudevita Gaja 32, 10 000 Zagreb, Croatia
e-mail: miran.coklo@inantro.hr

RAZLIČITOST MARKERA Y-KROMOSOMA I mtDNA IZMEĐU DVIJE ALBANSKE SUBPOPULACIJE (IZ HRVATSKE I KOSOVA) – PRELIMINARNI PODACI

SAŽETAK

Cilj ove preliminarne studije je analizirati genetičku specifičnost kosovskih Albanaca u usporedbi sa susjednim populacijama koristeći novi genetski alat – MEDISCOPE genski čip, kako bismo utvrdili isplativost navedenog pristupa. Prikupili smo 37 uzoraka DNA (9 Hrvata, 17 Albanaca iz Hrvatske i 11 Albanaca s Kosova) nesrodnih muškaraca rođenih u Hrvatskoj i na Kosovu. Dodatno, uzorak je proširen ženskim osobama i analiza mtDNA je uključila ukupno 61 uzorak (15 Hrvatica, 23 Albanke iz Hrvatske i 23 Albanke s Kosova). Ova pilot studija sugerira da korištenje MEDISCOPE čipa može biti prepoznato kao efikasan alat za raspoznavanje genetičkih specifičnosti čak i kod vrlo male veličine uzoraka.