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DYSTROPHIN DNA DIVERSITY IN THE ARMENIAN HIGHLAND

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We investigated the variability of dys44 segment on Xp21.3 and the distribution of dys44 haplotypes and allele length variance in repeat polymorphism in 414 Armenian chromosomes from eight regions of Armenia. Gene diversity was high in the total Armenian population (h=0.8) and this parameter showed differentiation between regions, which were further confirmed by exact tests of population differentiation. Our studies showed that Europeans could not be clearly distinguished from Armenians and from Middle Easterns. In this study, the lack of noticeable overall pattern of the X-chromosome dys44 haplotype distribution suggests that there was either never any cline of dys44 haplotypes in those regions or that post-Neolithic gene flow may have removed any previously existing signatures of population migration.

Ուսումնասիրվել է Xp21.3 քրոմոսոմի հատվածի փոփոխականությունը, ինչպես նաև *dys44* հապլոտիպերի և երկարությամբ կրկնվող պոլիմորֆիզմի բաշխվածությունը Հայաստանի ութ շրջաններից ընտրված ազգությամբ հայերի 414 քրոմոսոմներում: Հետազոտված հայկական պոպուլյացիայում գենային բազմազանությունը գտնվում էր բարձր մակարդակի վրա (h=0.8), և այդ ցուցանիշը տարբեր էր ուսումնասիրված շրջաններում, որը հաստատվեց պոպուլյացիոն դիֆերենցման ճշգրիտ թեստերի միջոցով: Մեր ուսումնասիրությունները ցույց տվեցին, որ եվրոպական պոպուլյացիան տեսանելի չի տարբերակվում հայկական կամ մերձավոր արևելյան պոպուլյացիաներից. Այս հետազոտության մեջ X քրոմոսոմի *dys44* հապլոտիպերի էական բաշխվածության բացակայությունը թույլ է տալիս ենթադրել, որ ուսումնասիրված շրջանակներում կամ երբևիցե չի նկատվել *dys44* հապլոտիպերի որևէ տարածաշրջանային զրադացիա կամ հետ նեոլիթյան գենների հոսքը հավանաբար վերացրել է պոպուլյացիոն տեղաշարժերի նախորոք գոյություն ունեցած հետքերը

Исследованы изменчивость сегмента *dys44* хромосомы Xp21.3, а также распределение *dys44* - гаплотипов и изменчивого в длину микросателлитного повтора в 414 армянских хромосомах из 8 регионов Армении. Для исследованной армянской популяции был характерен высокий параметр генетического разнообразия (h=0.8), согласно которому наблюдалась дифференциация между регионами, что было в дальнейшем подтверждено с помощью "точных тестов дифференциации популяций". Исследование показало, что европейские популяции не были очевидно отличны от армянских и ближневосточных. Отсутствие патерна распределения *dys44* - гаплотипов Х-хромосомы говорит о том, что в данных регионах, либо никогда не наблюдались определенные структуры по *dys44*-гаплотипам или же пост-неолитический поток генов смыл имеющиеся следы.

Dys44 - dystrophin - polymorphism - SNPs - X-chromosome

It is generally accepted that the Near East was the place through which migrations between Africa and Asia took place. Farming and animal domestication are recent phenomena in human history, which occurred from 10,000 yea

BP onward. Farming arose independently in several parts of the world, including in a region of the Near East known as the "Fertile Crescent". There are data, indicating that late Natufians were probably the earliest farmers in the Levant [1]. Also it is acknowledged that in the Near East agriculture arose and expanded in both western and eastern directions [2]. There exist two contradicting models about the mechanism of dispersal of agriculture: the demic [3-5] and cultural diffusion [6-8] models. The demic diffusion model postulates that extensive migrations of Near Eastern farmers brought agricultural techniques to Europe. Whereas, cultural diffusion suggests that the transfer to food production occurred without significant population movements and the majority of the genetic diversity within Europe should have its roots in the Palaeolithic Europeans.

Armenians belong to one of the oldest Middle Eastern civilizations with a recorded history of about 3000 years, and they inhabit an area of great interest in relation with theories of early human population expansion and Indo-European language development. Archaeological excavations in Armenia reveal that the Armenian plateau in Asia Minor has been one of the earliest cradles of civilization going back as far as the Early Bronze Age [9-12]. The present-day Republic of Armenia is a landlocked country in southwestern Asia; located up in the mountains between the Black and the Caspian Seas, bordered on the north by Georgia, on the east by Azerbaijan, on the south by Iran and on the west by Turkey.

Genetically, the Armenian population has been investigated little. Studies of their genetic diversity were previously carried out at the level of mitochondrial and Y-chromosome DNA [13-16]. In spite of the definite virtues of these systems, their value as markers of population history is limited, for each represents only a single locus, they reveal either maternal or paternal inheritance only, their effective population size is one fourth that of autosomes.

To understand the genetic structure of populations and to trace their genetic past, we need to collect information also from autosomal and/or X-chromosome loci. As a model for Armenian population studies we used 8-kb intronic genomic segment (*dys44*) of human dystrophin gene on chromosome Xp21.3. Variations in these genomic segments are an informative haplotypic system for a reconstruction of recent human population history. *Dys44* variability suggests the existence of at least two separate founder lineages. Sub-Saharan Africans represent these two chromosomal lineages with their distinct genetic histories, reflecting the fragmentation of early human populations during periods of glaciations. Expansion of one of these lineages led to global colonization, while second one remained local to Africa. In addition, previous analysis of worldwide samples revealed a third lineage represented by an ancient haplotype found in Eurasia and the Americas that is virtually absent in sub-Saharan Africa [17].

In this paper we aim (i) to investigate the variability of *dys44* segment and the distribution of *dys44* haplotypes and allele length variance in repeat polymorphism in eight regions of Armenia, (ii) to analyse 416 Armenian chromosomes with previously typed 971 chromosomes from Africa, Middle East, Asia and Europe.

Material and methods. Samples. Blood samples were taken from unrelated Armenians having at least 3 generations of ancestors in the following 8 Armenian population groups: Ararat (town Ararat, in the Ararat valley; n=46), Yerevan (capital of Armenia; n=47), Lori (northern region, town Dilijan; n=48), Shirak (northern region, town Gyumri; n=45), Sevan (region, surrounding Lake Sevan; n=34), Syunik (southern region, town Sisian; n=44), Nagorno-Karabakh (autonomous republic between Armenia and Azerbaijan with Armenian inhabitants; n=60), "historical western Armenia" (in this group we included individuals whose ancestors were from provinces of historical western Armenia, nowadays eastern Turkey; n=43). In total, 414 Armenian chromosomes have been studied; all chromosomes are of males except those in the Yerevan region.

Material for our study is genomic DNA extracted from whole peripheral blood. DNA extracted by using blood and tissue kit "Purgene" ("Gentra Systems", USA).

Procedures of collection of blood samples and extraction of DNA were performed at the Center of Medical Genetics (Yerevan, Armenia) from 2000 to 2003. Further molecular and statistical analyses were done at the laboratory of Population Genetics of the Research Center of Ste-Justine Hospital (Montreal, Canada) in 2004-2005.

In this paper we also analysed 971 previously typed chromosomes from Africa (MButi n=58, Mossi n=30, Biaka n=85, Rimaibe n=31, African American n=86), Asia (Mongolian: Khalkha n=27, Khoton n=30, Uriankhai n=34, Olet n=30, Kazakh n=54), Middle East (Jewish: Iraqi n=27, Iranian n=21, Yemenite n=31, Moroccan n=29, Ashkenazim n=110, Bulgarian n=18; Palestinian n=23, Druze n=33, Bedouin n=26) and Europe (Italian n=26, Polish n=30, German n=73, Cretan n=39, Basque n=20) [17,18] which in total with the Armenian data gave 1387 chromosomes from 32 population groups.

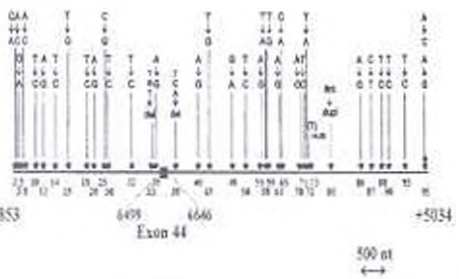


Figure 1. *Dys44* DNA segment: exon 44 (cDNA positions 6499 through 6646) and its flanking introns between positions − 2853 to − 1 upstream and positions 1 to 5034 downstream. Numbers are indicating each of polymorphic sites and corresponding stars are showing position and nucleotide change of polymorphisms of *dys44* region (adopted from E. Zietkiewicz et al., 1998 [19]).

Genotyping. The genomic segment *dys44* consists of exon 44 (148 bp) and its surrounding intronic sequence (positions -2853 to -1 of intron 43 and positions 1 to 5034 of intron 44) of the human dystrophin gene at Xp21.3 (GenBank accession number U94396 [19]).

Polymorphisms of *dys44* intronic region, length of the sequence 7622 bp, were previously detected by single-strand conformational polymorphism (SSCP) combined with heteroduplex analysis in 250 worldwide-distributed chromosomes [20,21]. 35 Simple nucleotide polymorphisms were found within *dys44* intronic DNA segment, including 31 biallelic nucleotide substitutions, 2 three-nucleotide deletions, 1 eight-nucleotide duplication, and 1 three-allelic due to 2 substitutions. The segment consists also a T_n microsatellite (Figure 1).

Standard PCR were performed for amplifying intronic *dys44* segment (8 kb), by 2 kb four times. Allele-specific oligonucleotide (ASO) hybridization was used to find out the allelic position of each polymorphic site [22, 23]. Visualization of Tn polymorphism was performed by standard polyacrylamide gel electrophoresis, using SAGA^{TM} microsatellite software of "LI-COR Biosciences".

Haplotypes. 182 definite haplotypes were previously derived and classified for *dys44* genomic segment, as described by D Labuda et al. [24] and Zietkiewicz et al. [17]. Haplotypes found in more than one continental group of populations called common; those originated in one continental group or typical for one population called specific (24). In derivation and classification of haplotypes, T_n polymorphism was not taken into consideration.

Statistics. ARLEQUIN software [25] was used to calculate nucleotide diversity, gene diversity, genetic distance (pairwise F_{ST}), pairwise exact tests of population differentiation [26]. F_{ST} matrices were computed using frequencies of haplotypes. Comparison of populations was done through a principal component analysis (PC-analysis) using haplotypes frequencies; also through pairwise F_{ST} distance matrix and the results were displayed by multidimensional scaling (MDS) analysis (STATISTICA package, version 6.0). STATISTICA software was used also to estimate mean value, standard deviation and variance (S^2) of T_n microsatellite in the population.

Results and discussion. Distribution of *dys44* - haplotypes in the Armenian population. Thirthy-nine extended *dys44* - haplotypes were observed in the analysis of 416 X-chromosomes from eight different Armenian regions, which are presented in Table 1. B001, B002, B003, B005, B006, B008 are common haplotypes, that represent 83.35% of the Armenian chromosomes studied. Other haplotypes that are common, but found rarely in the Armenian population, formed 12.79%. We also found 15 new Armenian specific haplotypes representing 3.86% of chromosomes, which can be derived from the frequent haplotypes assuming simple recombination event, gene conversion or simple mutation. Most of them are seen one time (B196, B197, B198, B199, B201, B202, B205, b206, b208, b209), except B200 and b203 (each 0.72%). None of the African specific haplotypes with African-specific mutations have been observed in regions studied.

Table 1. **Frequencies of *dys44* - haplotypes, nucleotide positions relative to exon 44 and 36 polymorphic sites in the studied Armenian population: empty cells indicate that haplotypes have the ancestral position at the allele. Abbreviations are the following: NP (Nucleotide Position), PS (Polymorphic Sites), HT (Haplotypes), Anc (Ancestral)**

NP		-2782	-2781	-2777	-2676	-2441	-2291	-2033	-1788	-1430	-1313	-1058	-1056	-593	-182	-115	116	556	880	1241	1485	1613	1651	1654	2149	2150	2599	2659	2663	2684	3144	3729	4093	4128	4264	4586	4587
PS		2	3	5	8	10	12	14	15	18	20	25	30	32	33	35	38	40	45	48	50	55	58	59	64	65	70	71	72	T_A	85	86	87	88	90	93	95
HT	%																																				
Anc		C	A	G	A	T	A	T	T	T	A	C	T	T	TOT	A	TCA	A	T	G	T	A	T	T	A	C	A	T	T	14-24	IN	A	C	T	T	T	A
B001	39.42		C					C					C				G			G		C		A	G			G			14,15,19,21,22					C	C	G
B002	7.71	A			C			C					C											A					C		15							
B003	16.91		C					C					C				G			G		C		A	G		A	G			14,15					C	C	G
B005	5.50							C			G		C				G		G			C		A	G		A				15,22					C	C	G
B006	6.51						C						C																		15,16	DUP						G
B008	8.50							C			G		C				G					C	G	A	G			G			14,15					C	C	G
B004	0.24	A			C			C					C											A					C		15					C	C	G
B011	2.41		C					C			G		C				G		G	G		C		A	G		A				21,22					C	C	G
B016	2.90		C					C			G		C						G			C		A	G		A	G			15					C	C	G
B019	0.24		C					C					C				G		G			C		A	G			G	C		15					C	C	G
B024	0.73							C					C				G		G			C		A	G			G			15,16					C	C	G
B028	0.24	A			C			C					C						G			C		A	G			G			15					C	C	G
B030	0.24	A			C			C					C				G		G			C		A	G			G			15					C	C	G
B032	0.48		C					C			G		C				G		G			C		A	G			G			15					C	C	G
B033	0.73		C					C					C				G		G			C	G	A	G			G			15					C	C	G
B042	0.24							C			G		C				G		G			C		A	G						22					C	C	G
B043	0.24							C			G		C				G		G			C		A	G		A	G			14					C	C	G
B051	0.48							C			G		C				G		G	G		C		A	G		A				22					C	C	G
B067	0.24		C					C			G		C				G		G	G		C		A	G						22					C	C	G
B134	0.24							C			G		C				G		G			C	G	A	G						22					C	C	G
B142	0.24												C																		16							G
B144	0.24		C					C			G		C				G			G		C	G	A	G			G			14					C	C	G
B145	0.24	A			C			C					C											A							15							
b071	0.97		C					C			G		C				G					C	G	A	G			G			15,16					C	C	G
b128	0.73							C			G		C				G					C		A	G			G			14,15					C	C	G
b148	0.48				C			C					C											A							15							
b191	0.24	A				C							C																		15	DUP						G
B196	0.24							C					C				G		G			C		A	G		A				15	DUP				C	C	G
B197	0.24							C			G		C				G		G	G		C	G	A	G			G			21					C	C	G
B198	0.24		C			C		C					C						G			C		A	G			G			16	DUP				C	C	G
B199	0.24		C					C			G		C						G			C		A	G			G			15					C	C	G
B200	0.73		C					C					C				G		G			C		A	G		A	G			15							
B201	0.24		C					C			G		C				G		G			C		A	G						22					C	C	G
B202	0.24		C					C					C				G					C		A	G		A	G			15							G
b203	0.73		C			C							C																		15,16							G
B205	0.24	A			C			C					C				G		G	G		C		A	G		A				22					C	C	G
b206	0.24		C					C			G		C				G		G			C	G	A	G						22					C	C	G
b208	0.24						C						C											A	G		A				16							G
b209	0.24						C						C														A				16	DUP						G

The distribution of frequent common haplotypes in eight Armenian population groups is presented in Table 2. B001 is the most frequent haplotype (39.4%) spread in all eight regions studied, with relatively the same frequencies. This haplotype appears to be the best marker of the recently expanded lineage [17]. B003 is the second most frequent haplotype (15.9%) found in our population, with highest frequency in the western Ararat region (0.239) and lowest in the northern Lori region (0.042) (Table 2). The third frequent haplotype is B008 (8.5%), previously found at high frequency in Middle Eastern populations [18]. Relatively high frequencies of this haplotype were observed in historical western Armenian chromosomes (0.14), southern Syunik region (0.136) and northern Lori region (0.146). The other three frequent common haplotypes, B002, B005 and B006, were found in the total Armenian population with frequencies 7.71%, 5.30% and 6.51%, respectively.

Table 2. The frequencies of common frequent haplotypes in eight Armenian population groups

Haplotype	N of chrom.	B001	B002	B003	B005	B006	B008
Ararat	46	0.457	0.087	0.239	0.022	0.044	0.065
Historical Western Armenia	43	0.326	0.140	0.163	0.069	0.069	0.140
Karabakh	60	0.433	0.067	0.117	0.067	0.083	0.083
Lori	48	0.354	0.146	0.042	0.083	0.104	0.146
Sevan	34	0.382	0.147	0.176	0.029	0.059	0.059
Shirak	45	0.489	0.067	0.133	0.067	0.089	0.044
Syunik	44	0.386	0.068	0.205	0.046	0.046	0.136
Yerevan	96	0.333	-	0.188	0.042	0.042	0.042

In our study, haplotype B002 is chiefly associated with allele T15 (Table 1) and it is not present in the Yerevan region (Table 2). B002 associated only with allele 15 were common for non-African populations [17], but in the study of A. Lovell et al. [27] B002 were found in association with alleles 16, 17, 22 in Ethiopian, Yemenite and Iraqi Jews, non-Jewish Ethiopians and Saharawi populations, and were hypothesized that these alleles are identical by descent and therefore present through gene flow. Haplotype B006, found in all eight Armenian population groups, considered as a marker of Eurasian influence and is virtually absent in Sub-Saharan and low in East Asian populations [17, 27].

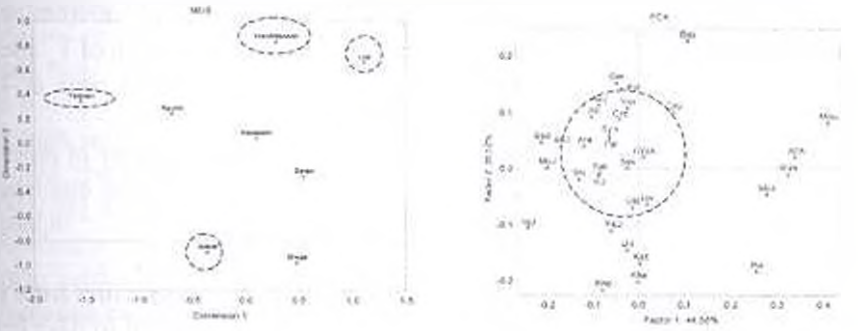
Summary statistics, Gene diversity (h), nucleotide diversity, expected K (number of alleles or haplotypes) values were obtained for all chromosomes studied. Gene diversity (based on *dys44*-haplotype frequencies) in total Armenian population were h=0.8, lower values than this in Syunik, Karabakh, Shirak and Ararat regions. In Yerevan, historical western Armenia and Lori regions, values of gene diversity were particularly higher (h=0.83, 0.84, 0.83, respectively). This parameter shows genetic differentiation within Armenia, which were not significantly seen from frequencies of frequent haplotypes (Table 2). The value of gene diversity of analysed Armenian chromosmes (h=0.8) occurred to be close to European and Arabian diversity (0.82 and 0.78, respectively), while for the Afri-

can population it is higher (0.9) and lower for Mongolian (0.74) and Jewish (0.72) populations.

The average nucleotide diversity in Armenian population groups was 6.56 x 10^{-4}. The highest value of nucleotide diversity (8.4 x 10^{-4}) was observed in northern Lori region, the lowest one in Ararat region (5.1 x 10^{-4}). Nucleotide diversity in the total Armenian population (6.56 x 10^{-4}) is relatively close to that of the Arabian Peninsula (6.58 x 10^{-4}) and Europe (6.68 x 10^{-4}, without Basque). Comparatively high nucleotide diversity was seen in African chromosomes and was low in Jewish ones.

F_{st} analysis indicates that only 0.63% of genetic variations are among the Armenian population groups and 99.37% – within the groups. A pairwise F_{st} can measure the magnitude of the population variance that is due to the difference among subpopulations; if two subpopulations diverge and remain genetically isolated, F_{st} is expected to grow with time [28]. F_{st} value for the total Armenian population was 0.0063. Population comparison was done by PC-analysis (based on extended haplotype frequencies) and pairwise F_{st} value. Both analyses showed the similar separation of population groups and we illustrate here the results of F_{st} - analysis displayed by MDS (Diagram 1a).

Circled populations, Yerevan, historical western Armenia, Lori and Ararat showed statistically significant differentiation by pairwise exact test: Yerevan was statistically different from historical western Armenia and Lori. Lori – from Ararat (Diagram 1a).



a) b)

Diagram 1. a) Comparison of different Armenian regions based on pairwise F_{st} analysis, displayed by MDS. Circled regions showed significant differences (significance level = 0.05); b) Population's comparison through PC-analysis based on the haplotype frequencies. The dotted circle encompasses the Armenian regions. Population abbreviations are: Ara (Ararat), HWA (historical western Armenia), Kar (Karabakh), Lor (Lori), Sev (Sevan), Shi (Shirak), Syu (Syunik), Yer (Yerevan); MBu (MButi), Mos (Mossi), Bia (Biaka), Rim (Rimaibe), AAf (African American); Kha (Khalkha), Kho (Khoton), Uri (Uriankha), Ole (Olet), Kaz (Kazakh); IqJ (Iraqi Jews), InJ (Iranian Jews), YeJ (Yemenite Jews), MoJ (Moroccan Jews), AsJ (Ashkenazim Jews), BuJ (Bulgarian Jews), PaJ (Palestinian), Dru (Druze), Bed (Bedouin); Ita (Italian), Pol (Polish), Ger (German), Cre (Cretan), Bas (Basque).

The comparison of all thirty-two-population groups was done by principal component analysis, which allows us to observe the combined effects of extended haplotype frequency gradients. The first two components of the PC-analysis showed 65% of observed variation (Diagram 1b). The first component is only determined by variation of haplotype B001, second component is focussed by

variation at haplotype B002.

The dotted circle encompasses the Armenian regions (Diagram 1b). Majority of populations (European, Armenian, Middle Eastern) concentrated in the upper central part of the plot. African populations are located on the right and Mongolians – in the top left parts of the plot; exact tests of population differentiation (based on haplotype frequencies) showed that they are statistically differentiated from other populations and from each other. Inside of African populations, Biaka and MButi also showed statistically significant separation from each other and the rest of African populations. From Armenian regions, Ararat were statistically different from Basques, Khalkha, Kazakh and African populations; historical western Armenian region, Karabakh and Syunik were statistically separated from Khalkha, Kazakh, and African populations, also from Ashkenazim Jewish population; Sevan were statistically differentiated just from African populations, and Shirak also from Khalkha and Kazakh populations. Exact tests of population differentiation demonstrated that Yerevan region additionally statistically separated from German, Yemenite and Ashkenazim Jewish populations.

In the main, Jewish populations were not grouped together; exact tests showed that they are not statistically differentiated from each other, except that Yemenite, Iranian and Iraqi Jews were differentiated from Ashkenazim. Of the remaining Middle Eastern populations, exact tests showed that Bedouins are statistically distinct from Palestinians; and Druze and Palestinians – from Ashkenazim Jews.

T_n – microsatellite variance. *Dys44* haplotypes could be associated with different T_n alleles (T_{14-24}). The association of B-haplotypes with T_n - microsatellite is presented in Table 1. In Armenian chromosomes, the distribution of T_n alleles is bimodal, with a larger peak at T_{15} and a comparatively smaller peak at T_{22}; alleles T_{17}, T_{18}, T_{19}, T_{20} and T_{24} were not observed.

T_n diversity can be described by the variance S^2 in the number of repeats. We grouped flanking alleles T_{14} and T_{16} (differ from the major T_{15} by one length unit only) in length mode T_{15}, and chromosomes with alleles T_{21}, T_{23} – in length mode T_{22}.

In the T_n distribution in our population, the flanking alleles differ from the major alleles (T_{15} and T_{22}) by one length unit only and thus appear to have been derived from the major allele by a simple addition or deletion of 1 unit at a time. Only in Shirak, Lori and historical western Armenian regions the variance in both length modes T_{15} and T_{22} were observed, while in others – the variance in length mode T_{22} was not present. Compared with previous studies done on Middle Eastern and European chromosomes [17, 27], the variance in T_n microsatellite of Armenian samples is closer to European variance, which were 0.20 (mode $T_{(15)}$) and 0.12 (mode $T_{(22)}$), whereas Middle Eastern variance in length mode $T_{(22)}$ were higher (0.47), which could be explained by African gene flow.

The greater variance in T_n allele repeats length and also high gene diversity of African populations is not a new phenomenon, this was described on other population models [31-33]. Similar to other systems *dys44* segment also reveals greater diversity in Africa and supports "Out of Africa" model of colonization of

the World [11, 17, 19, 31, 35, 36, 37].

Among non-African populations, in Armenian population was observed high genetic diversity by the dystrophin segment studied. 39 *dys44* haplotypes were found in the analysis of 414 X-chromosomes. Gene diversity was high in the total Armenian population (0.8), and this parameter showed differentiation between regions; particularly high genetic diversity were seen in Yerevan, historical western Armenia and Lori regions. Further analysis (F_{st} - analysis, exact test) showed statistically significant differentiation between historical western Armenian and Yerevan, Yerevan and Lori, Lori and Ararat regions. Observed statistical distinction of the Yerevan region could be explained by the number of chromosomes studied (N=94), which is higher compared to Lori (N=48) and historical western Armenian (N=43) regions, also in the region of country's capital a more diverse population is concentrated. Additionally, region of historical western Armenia is geographically separate from Yerevan. Results of exact tests concerning Ararat and Lori regions could be explained by their geographic separation. On the other hand, Ararat (western) and Shirak (northern) regions were grouped close on the MDS diagram and did not illustrate statistically significant separation. Of the remaining population groups, which localized at the center of the MDS plot, exact tests show no statistically significant differentiation; this could be explained by the common origin of the studied populations, high percentage of admixture, movements within regions and across the population.

It is known that B001 is the marker of recently expanded lineage out of Africa, and that both African and non-African populations are characterized with low diversity of this haplotype associated with T_n-microsatellite. In our study, we observed relatively high variance of B001_T_n. It has been suggested, that B001 could have been recently derived from B003 haplotype by removal of allele A in the position 2150 (A>C) (Table 1). If such an event happened, it could explain the high variance of the length mode T_{12}. On the other hand, in our population studied, the relatively high variance of T_n - microsatellite (B001_16, B001_22, B001_21) have been observed compared with previous data [17,27], which is providing good evidence for the African origin of this lineage. By presence of the length mode T_{12} in the Armenian population we are questioning (i) is this a mark of separate expansion events, or (ii) does it is a result of genetic drift, or else, (iii) 'expanding "chromosomes of Africa were already diluted within the existing gene pool and did not achieve the high frequencies observed outside of Africa. The further study of recombination within the haplotype lineages (B001, B002, B003, B004, B005) can also aid in the search of their origin.

Likewise, comparable gene diversity of Armenian population to Europe and Middle East also suggests gene flow rather than isolation as well as a possibility of admixture, consistent with the geography of Armenia, situated at the borders of Europe and Asia. These conclusions are also in agreement with the PC-analysis

Our studies showed that Europeans could not be clearly distinguished from Armenians (Near Easterners) and from Middle Easterners. In this study, the lack of noticeable overall pattern of the X-chromosome *dys44* haplotype distribution suggests that there was either never any cline of *dys44* haplotypes in those regions

or that post-Neolithic gene flow may have removed any previously existing signatures of population migration.

Further statistical tests need to be performed for seeing if they're any genetic, geographic, language correlations that may affect on population differentiation. Furthermore, studies of Y-chromosome marker systems in Europe and Near East showed a significant correlation between genetic-geographic distances and less of a correlation between genetic-language association.

Also, selection is often a reason for differences in allele distribution across loci [34]. Alternatively, the *dys44* segment is located in the intronic region of the large dystrophin gene, and has a genetic diversity typical of neutral variation [19]. *Dys44* found in a region of X-chromosome with high recombination rate, and is unlikely being affected by selection on the adjacent loci. It is definite to perform tests of selective neutrality (Ewens-Watterson test, exact test based on Ewens' sampling theory, etc.) for predictions about the magnitude and pattern of genetic variation, and to determine weather molecular variation is consistent with neutral theory.

Further studies of the Near Eastern populations by *dys44* DNA segment in comparison with Armenian chromosomes will provide additional information to the history of Middle East, Europe and Asia, will give more clues to understand the ancient routes and migrations of modern humans within Eurasia.

REFERENCES

1. *Bar-Yosef O.*, Evolutionary Anthropology, 159 – 177, 1998.
2. *Quintana-Murci L. et al.* Am. J. Hum. Genet. 68:537–542, 2001.
3. *Ammerman A. and Cavalli-Sforza L.L.* Princeton University Press, Princeton, 1984.
4. *Piazza A. et al.* Proc. Natl. Acad. Sci. USA, 92, 5836-5840, June 1995.
5. *Barbujani G. et al.* Am. J. Hum. Genet. 62:488–491, 1998.
6. *Dennell R.* Academic Press, London, 1983.
7. *Zvelebil M.* Cambridge University Press, Cambridge, 5-15, 1986.
8. *Zvelebil M.* Cambridge University Press, Cambridge, 57-59, 2000.
9. *Aghayan, TC. et al.* Yerevan, Academy of Sciences. Institute of History, I, 250-276, 1971.
10. Lang, David Marshall Armenia: Cradle of civilization, 1978, 2nd edition, George Allen & Uniwin publishers.
11. *Cavalli-Sforza L.L., Menozzi P., Piazza A.*, 1994, Princeton Univ. Press, Princeton.
12. *Samuelian T.* 2000, Yerevan, 2nd edition 2004.
13. *Weale M., Yepiskoposyan L. et al.* Hum Genet. 109: 659-674, 2001.
14. *Nasidze I. and Stoneking M.* Proc R Soc Lond B, 268, 1197–1206, 2001.
15. *Nasidze I., Sarkisian T., Kerimov A., Stoneking M.* Hum Genet 112, 255–261, 2003.
16. *Nasidze I. et al.* Annals of Human Genetics 68, 205–221, 2004.
17. *Zietkiewicz, E. et al.* Am. J. Hum. Genet. 73: 994-1015, 2003.
18. *Feng-Xia Xiao et al.* Eur. J. Hum. Genet. 12: 301-311, 2004.
19. *Zietkiewicz, E., et al.* J. Mol. Evol. 47: 146-155, 1998.
20. *Zietkiewicz, E., Sinnett, D., Labuda, D., et al.* Hum. Genet. 89: 453-456,

1992.
21. *Orita, M.*, Proc. Natl. Acad. Sci. USA 86: 2766-2770, 1989.
22. *Zietkiewicz, E., et al.* Gene 205: 161-171, 1997.
23. *Bourgeois S., Labuda D.* Anal Biochem. 2004 Jan 15: 324(2): 309-11.
24. *Labuda, D., Zietkiewicz, E., Yotova, V.* Genetics 156: 799-808. 2000.
25. A software for population genetics data analysis, web site: http://anthro.unige.ch/arlequin
26. *Raymond M. and Rousset F.* Evolution, 49: 1280-1283, 1995
27. *Lovell A. et al.* Annals of Human Genetics, 69, 1-13, 2005.
28. *Wright S.* The Theory of Gene Frequencies, University of Chicago Press, Chicago, 1969, volume 2.
29. *Wells RS, Yuldasheva N., et al.* Proc. Natl. Acad. Sci. USA 98:10244–10249, 2001.
30. *Zerjal T. et al.* Am. J. Hum. Genet. 71:466–482, 2002.
31. *Cann R. et al.* Nature 325: 31-36, 1987.
32. *Stoneking M. et al.* Genome Res 7:1061-1071, 1997.
33. *Hammer M. et al.* Mol. Biol. Evol. 15: 427-441, 1998.
34. *Cavalli-Sforza L.L.* Proc. R. Soc. Lond. B. Biol. Sci. 164 (995): 362-79, 1966 Mar 22.
35. *Wainscoat J. et al.* Hum Gen 74:90-92, 1986.
36. *Stringer C. and Andrews P.* Science 239:1263-1268, 1988.
37. *Tishkoff S. et al.* Science 271:1380-1387, 1996.

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