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ACE Alu Insertion Polymorphism in Croatia and its Isolates

Maja Barbalić, Marijana Peričić, Tatjana Škarić-Jurić and Nina Smolej Narančić

Institute for Anthropological Research, Zagreb, Croatia

ABSTRACT

Alu elements are a family of interspersed repeats in the genome propagating by retroposition into new chromosomal locations. Alu insertion in Ace gene is known to be polymorphic (presence/absence of Alu element) in worldwide populations and as such serves as marker for population structure analyses. In this study we examined the distribution of genotypes and allele frequencies of this polymorphism in general Croatian population and its two isolates (the island of Hvar and the coastal region of the Middle Dalmatia) and related them to the level of endogamy as an indicator of inbreeding in these populations. Results showed that these three population groups are different with respect to Ace Alu polymorphism. The endogamy was highest on the island of Hvar. With the increase of endogamy a decrease in heterozigosity was observed. The same trend was observed for the frequency of insertion allele. Its frequencies in the village subpopulations of two studied isolates are subject to genetic drift due to small population sizes and high levels of endogamy. This in turn causes genetic differentiation among villages that is observed to be higher on the island of Hvar than in the coastal region. In the worldwide perspective, the Ace Alu insertion allele frequency of 50.6 % in the general Croatian population falls within the range of other European populations.

Key words: *Ace Alu polymorphism, insertion frequency, isolation, Croatia*

Introduction

Alu insertion elements are the most abundant class of short interspersed elements (SINEs) in the human genome, numbering more than million per haploid genome[1]. They are 300 bp long dimeric sequences that are thought to be ancestrally derived from the 7SL RNA gene[2] and to propagate by retroposition into new chromosomal locations[3]. The amplification of *Alu* elements in the primate genome is estimated to last 65 million years[4] and is still an ongoing process. Some *Alu*

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elements have retroposed so recently that they are not yet fixed i.e. their insertion in specific location of genome is polymorphic which make them useful as markers for the population structure analysis. The advantage of *Alu* insertions as markers is that the ancestral state of polymorphism is always known – the absence of the element since there is no mechanism for their removal after the insertion. The other advantage is uniqueness of insertion at specific location since there is no sequence specificity for insertion sites meaning that all loci carrying a particular *Alu* insertion derived from unique event and hence they are identical by descent[5]. These properties are reason that polymorphic *Alu* insertions were used as markers in numerous population structure analyses even at a micro-geographical scale[1,6–10].

Alu insertion studied in this research is situated in angiotensin-converting enzyme gene (*Ace* gene)[11]. Ace plays important role in the blood pressure regulation and electrolyte balance. As a consequence *Ace Alu* insertion polymorphism, precisely absence of insertion, seems to be associated with several disease phenotypes[12–14]. So *Ace Alu* insertion, beside its usefulness as a marker in population structure analyses, is often used in genetic studies of disease phenotypes.

Population structure analyses benefit considerably from the studies carried out in the isolated populations. The low level of new gene influx makes the investigations of other evolutionary forces such as genetic drift and selection in isolated populations easier. Furthermore, level of inbreeding is high in such populations and hence it is possible to study its consequences on genetic structure of population. Croatian isolate populations are well characterized ethnohistorically, anthropologically and genetically through the studies that have been performed since 1971[15–19]. Geographic isolation and socio-cultural reasons such as political privileges in the past resulted in a range of inbreeding coefficients present at both individual and population level[15,20].

In this study we used data from Croatian general population and its two isolates – coastal region and the island of Hvar – both situated in Middle Dalmatia to examine the difference among these populations with respect to *Ace Alu* insertion frequencies and to relate them to the level of endogamy as an indicator of isolation in these populations.

Materials and Methods

A total of 407 adult individuals coming from three different population groups were examined. The first group represented general population of Croatia and consisted of 172 randomly chosen persons from electoral lists from all parts of Croatia. The second group consisted of 131 randomly chosen persons from four villages in the coastal region of Middle Dalmatia. The third group consisted of 104 randomly chosen persons from six villages of the Middle Dalmatian island of Hvar. Informed consent was obtained from all subjects included in the study. The names of villages and their sample sizes (approximately 10 % of total population) are shown in Table 2. Ethnohistorical, demographic and current migrational characteristics of these village populations have been shown in details in previous publications[17–18,21–22].

Genomic DNA was extracted from blood by method of Ponz et al[23]. Amplification of genomic DNA samples by PCR analysis was carried out in the total reaction volume of 20 μl containing 100 ng of genomic DNA sample, 100 ng of each primer (5'CTGGAGACCACTCCATCCTTTCT3', 5'GATGTGGCCATCACATTCGTCAGAT3'), 1.5 U Taq polymerase and 2mM of each deoxyribonucleotide triphosphate. The conditions of PCR reaction were: 94 °C 1 min,

TABLE 1
DISTRIBUTIONS OF *ACE ALU* INSERTION GENOTYPES AND ALLELE FREQUENCIES IN GENERAL POPULATION OF CROATIA AND ITS TWO ISOLATED REGIONS

		II	*ID*	*DD*	Total	*I*	*D*	Total
Croatia – general population	*N*	40	94	38	172	174	170	344
	%	23.3	54.7	22.1	100	50.6	49.4	100
Coast	*N*	24	69	38	131	117	145	262
	%	18.3	52.7	29.0	100	44.7	55.3	100
Hvar	*N*	15	48	41	104	78	130	208
	%	14.4	46.2	39.4	100	37.5	62.5	100
		$\chi^2 = 10.4$, d.f. = 4, $p < 0.03$				$c^2 = 9.02$, d.f. = 2, $p < 0.02$		

58 °C 2 min, 72 °C 2 min in 30 cycles. PCR products were detected on a 2 % agarose gel.

The agreement with Hardy-Weinberg equilibrium was evaluated by χ^2-testing of the difference between observed and expected genotype frequencies. χ^2-test was also used to examine the difference in *Ace Alu* insertion genotype and allele frequencies between three studied population groups. Endogamy was calculated as the product of the fraction of mothers and the fraction of fathers born in the same village as the participant. For this calculation we used the migration matrices that were based on larger sample sizes (ranging up to approximately 30 % of the total population size) given in the previously published papers[17,24]. G_{st} value was calculated according to equation of Nei[25]. The analysis and plots were performed in statistical programs Statistica version 6 and SPSS version 11 for Windows. Graphical presentation of the worldwide distribution of insertion allele was obtained by program Surfer 8 (Golden Software).

Results

Distributions of *Ace Alu* insertion genotypes and allele frequencies in general population of Croatia and its two isolated regions – coastal region and the island of Hvar – are shown in Table 1. Test of Hardy-Weinberg equilibrium did not show the departure from the equilibrium in any of the three studied populations. These populations differed significantly in *Ace Alu* insertion genotype distributions ($p < 0.03$) as well as in *Ace Alu* allele frequencies ($p < 0.02$). The genotype distributions are shown graphically in Figure 1. It is evident that heterozigosity declines gradual-

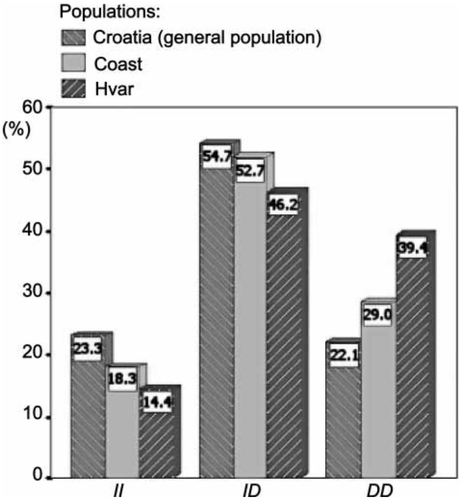

Fig. 1. Graphical presentation of Ace Alu insertion genotypes (II, ID and DD) distributions in general population of Croatia and its two isolated regions.

ly from general population of Croatia through coastal region and is lowest on the island of Hvar.

To examine the pattern of decline of heterozigosity in relation to the extent of isolation, we calculated the average endogamy within the regions. It amounted 72.4 % on Hvar and 32.0 % on the coast, while it was assumed to be 0 in the general Croatian population. Heterozigosity plotted against endogamy clearly shows their negative relation, i. e. the decline of heterozigosity with the increase of isolation (Figure 2).

Lower level of heterozigosity means that the alleles are becoming fixed or lost from a population. As our sample of coastal region and the island of Hvar consisted of four and six isolated villages respectively we expected that in these villages allele frequencies would fluctuate around the average of the region with tendency towards allele fixation or loss from the village populations. In Table 2 the frequencies of *Alu* insertion allele observed in the villages are shown as well as the endogamy levels in these villages. The results are presented graphically in Figure 3. It can be seen that overall *Alu* insertion allele frequency decrease from general Croatian population (50.6 %) through coastal region (44.7 %) to that on the island of Hvar (37.5 %). At the same time allele frequencies of village subpopulation fluctuate around the mean allele frequency of a region and range from 37.0 % to 57.8 % for the coastal region and from 21.1 % to 47.1 % for the island of Hvar. It can be noticed, although statistically not proven due to small number of villages for the appropriate analysis, that this fluctuation is greater for the Hvar villages where higher levels of endogamy are observed. To further investigate this observation, the G_{st} value was calculated for these two regions. G_{st} value estimates the proportion of the total variance that is due to differences among subpopulations: the higher the G_{st} value, the greater the magnitude of genetic differentiation among subpopulations. From results shown in Table 2 it is evident that genetic differentiation is larger on the island of Hvar ($G_{st} = 0.044$) than in the coastal region ($G_{st} = 0.023$) supporting our expectation.

Figure 4 shows the worldwide distribution of insertion allele frequency obtained by entering in Surfer Program the

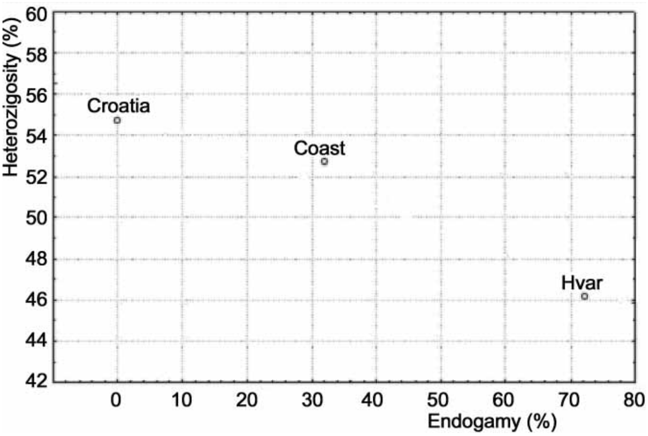

Fig. 2. Plot of heterozigosity of Ace Alu polymorphism with respect to endogamy in general population of Croatia and its two isolated regions.

TABLE 2
ACE ALU INSERTION FREQUENCIES AND ENDOGAMY IN SEPARATE VILLAGES OF TWO ISOLATED REGIONS OF CROATIA

	Village	*N*	*Alu*-insertion frequency (%)	Endogamy (%)	Difference in village's frequency from frequency in general Croatian population	G_{st}
Coast	Jesenice	37	41.9	50.9	–8.7	0.023
	Mimice	23	37.0	24.3	–13.6	
	Zivogošće	39	41.0	23.9	–9.6	
	Zaostrog	32	57.8	24.5	7.2	
Hvar	Vrbanj	19	21.1	70.1	–29.5	0.044
	Svirče	15	46.7	53.0	–3.9	
	Dol	20	32.5	63.8	–18.1	
	Gdinj	15	30.0	82.4	–20.6	
	Zastrazičće	18	50.0	89.5	–0.6	
	Bogomolje	17	47.1	80.5	–3.5	

data adopted from several published papers[6–7,26–27]. Data from all populations listed in these papers were taken into account except for the Americas and Australia where only data of native populations were used. It is evident from Figure 4 that the frequency of insertion allele is lowest in Africa and is getting higher toward Asia and Australia on one side and Americas on the other. The frequency of insertion allele in general Croatian population (50.6 %) is in accordance with the frequencies of neighboring European populations.

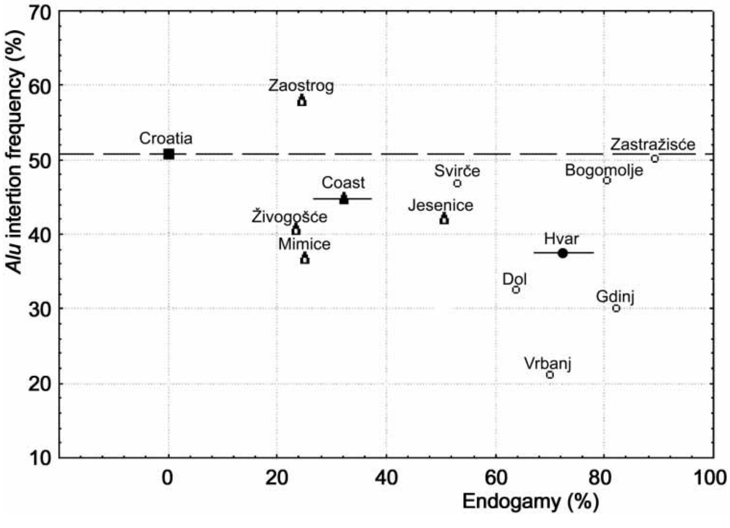

Fig. 3. Plot of Ace Alu insertion allele frequencies with respect to endogamy in separate villages of two isolated regions of Croatia (also are indicated the values of general population of Croatia and average values of its two isolated regions).

Fig. 4. Distribution of Ace Alu insertion allele in the world populations.

Discussion

Alu insertion polymorphisms have several properties that make them very suitable for studying genetic variation in human populations. Firstly, they could be very easily detected and genotyped due to simple evaluation of their alleles by means of PCR amplification and gel electrophoresis. Secondly, they are stable markers that represent a unique evolutionary event, namely insertion of an *Alu* element into a new chromosomal location. Therefore, the ancestral state is always known i.e. the absence of insertion and, as there is no parallel gain or loss of *Alu* elements at a particular chromosomal position, all loci carrying an *Alu* element on that position are identical by descent[5].

In this paper we used one of the *Alu* insertion polymorphisms, namely *Ace Alu* insertion polymorphism to examine its distribution and variation between general population of Croatia and its two isolated regions – coastal region and the island of Hvar – which were the subjects of extended population structure analyses since 1971[15–22,24]. As expected, variation in the distribution of *Ace Alu* insertion genotypes among these three populations was found indicating that evolutionary forces created different genetic picture in isolated populations. Isolation often produces high levels of inbreeding that increases the homozigosity of the population. Inbreeding is also related to population effective size and it is more pronounced in small isolated populations. These are exactly the characteristics of the studied villages in the coastal region of Croatia and the island of Hvar. We assessed inbreeding through the level of endogamy that is known to be correlated with inbreeding in small villages, as most (if not all) pairs of individuals will eventually be related at some point in their ancestry[28]. The obtained levels of endogamy were relatively high for the coastal region and very high for the island of Hvar. The effect of such distribution of endogamy may be seen in the heterozigosity levels of three studied populations with the decline of heterozigosity as endogamy increases.

The effect of isolation, probably through genetic drift, could also be seen in the insertion allele frequency which differs among studied populations with decrease of the frequency in isolated populations, especially on the island of Hvar where it is only 37.5 %. The fact that this frequency is 50.6 % in Croatian general population and falls in range of other European populations (between 45.0 % and 55.0 %) (Figure 4) confirms that the isolation influenced the genetic structure of studied isolates.

When analyzing the village *Alu* insertion frequencies within the isolated regions separately, the effect of genetic drift in these small subpopulations can be supposed. It causes fluctuation of the villages' allele frequencies in a way that the fluctuation is greater on the island of Hvar i.e. in the region with higher endogamy than in the coastal region. Calculated G_{st} value for the island of Hvar was twice-greater that G_{st} value of the coastal region that implies greater magnitude of genetic differentiation among subpopulations on the island of Hvar than in the coastal region. So, as a consequence of genetic drift in the studied small isolated villages, insertion allele is becoming fixed or lost which in turn causes genetic differentiation of the subpopulations that is more pronounced as the endogamy increases.

Graphical presentation of *Ace Alu* insertion worldwide frequency distribution shows that the general Croatian population frequency of 50.06 % falls within the range of European populations. The lowest frequencies which are the closest to the ancestral state i.e. the absence of *Alu* insertion are observed in African populations. That is in agreement with African origin of human populations and subsequent genetic drift in first people left Africa as a consequence of bottleneck[1,6–7]. Differences in allele frequencies are often found between African and non-African populations at genetic loci that are likely to be selectively neutral[1,7,29]. However, the fact that *Ace Alu* insertion is located within *Ace* gene and its deletion variant i.e. absence of insertion was associated with several disease phenotypes implies that selection may also be responsible for current distribution of *Ace Alu* insertion in the world. From this aspect, it would be worthwhile to compare the distributions of different *Alu* insertions i.e. those located in functional regions with those located in silent regions of the genome in order to make assumptions about the selection impact on variants situated in functional regions.

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M. Barbalić

Institute for Anthropological Research, Amruševa 8, 10000 Zagreb, Croatia
e-mail: majab@inantro.hr

ACE ALU INSERCIJSKI POLIMORFIZAM U HRVATSKOJ I NJENIM IZOLATIMA

SAŽETAK

Alu elementi čine porodicu raspršenih ponavljanja u genomu koji se šire retropozicijom na nove kromosomske pozicije. *Alu* insercija unutar *Ace* gena je polimorfna (prisustvo/odsustvo *Alu* elementa) u populacijama širom svijeta te služi kao biljeg u analizama populacijske strukture. U ovoj studiji izučili smo distribuciju genotipova i alelnih frekvencija tog polimorfizma u općoj populaciji Hrvatske i u relativno izoliranim ruralnim populacijama priobalja Srednje Dalmacije i otoka Hvara te ih usporedili s veličinom endogamije kao pokazatelja srođivanja u ovim populacijama. Rezultati su pokazali razliku između izučavane tri populacije s obzirom na *Ace Alu* polimorfizam. Utvrđeno je da je endogamija najviša na otoku Hvaru. S porastom endogamije opažen je pad heterozigotnosti populacija. Isti trend pokazan je za učestalost insercijskog alela. Na učestalosti tog alela u seoskim subpopulacijama dviju izoliranih područja utječe genetski drift zbog male veličine tih subpopulacija i visoke endogamije. On uzrokuje genetsku diferencijaciju među selima, koja je veća na otoku Hvaru nego u priobalju. U kontekstu svjetske distribucije *Ace Alu* insercijskog alela, njegova učestalost od 50.06 % u općoj populaciji Hrvatske unutar je raspona učestalosti drugih europskih populacija.