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Frequency of *ADH1B* *RsaI* (rs2066701) single nucleotide polymorphism in a population of Bosnia and Herzegovina

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Abstract

Apart from its physiological role in the cellular oxidation of ethanol an interesting feature of *ADH1B* gene is its characteristic geographical distribution where certain variants of *ADH1B* peak in different parts of the world. Therefore, *ADH1B* rs2066701 polymorphism is used as a genetic marker in tracing the evolutionary processes and human migrations over time. Taking into consideration the complexity of population genetic structure and a number of migration events in the history of the Balkan populations this study aimed to estimate the frequency of *ADH1B* rs2066701 polymorphism in the population of Bosnia and Herzegovina. The total of 101 randomly sampled individuals were genotyped for rs2066701 polymorphism in *ADH1B* gene using PCR-RFLP method. The obtained frequencies were used to calculate heterozygosity, fixation indices and Hardy-Weinberg equilibrium. The observed population-structure parameters were compared with other population values available in ALFRED database. Dimensional relations between the investigated populations were visualised with the NM-MDS (non metric multidimensional scaling) analysis using PAST. The minor allele frequency for rs2066701 was 0,257. Inter-population analysis including other European and non-European populations from ALFRED database proved the above-mentioned European genetic background of the B&H population.

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Keywords

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Introduction

ADH1B gene is the first class gene in the alcohol dehydrogenase (*ADH*) gene family which, together with the other two members of the aforementioned class (*ADH1A* and *ADH1C*), represents the liver's greatest asset in the oxidation of ethanol (Hurley et al.,

2002). *ADH1B* contains many polymorphic sites in the coding region and it has been suggested that some of them, like the one positioned in exon 3, provide differential protection against alcoholism (Thomasson et al., 1994). Another interesting feature of *ADH1B* gene is its characteristic geographical distribution where certain variants of *ADH1B* peak in different parts of the world. There are 23 variants

of *ADH1B* gene listed in the ALFRED database which were the subject of previous studies. For instance, the derived *ADH1B*47His* variant can be found at high frequencies in Far Eastern populations (Li et al., 2007), but with extremely low frequencies in other regions (Goedde et al., 1992; Li et al., 2007; Neumark et al., 1998). In order to better understand the evolutionary context of *ADH1B*, a comprehensive intron polymorphism studies, such as the analysis of the *ADH1B* *RsaI* SNP, have been carried out worldwide (Osier et al., 1999).

The non-coding *ADH1B* *RsaI* SNP (GenBank accession number: rs2066702) was discovered via *RsaI* restriction digest of genomic DNA followed by Southern blott and hybridization using pADH 36 clone as a probe (Smith, 1986). *ADH1B* *RsaI* SNP is located 906 bp downstream from the functional polymorphic nucleotide position in exon 3 (position 956) in the pADH 36 clone. The sequence of the exon 3 in the pADH 36 clone has been proved to be authentic to the published *ADH1A* and *ADH1C* sequences (Osier et al., 1999). The *RsaI* SNP is represented as a transition of the ancestral C into a T in intron 3. As outlined by Osier et al. (2002), all loci of the ADH gene family are clustered tightly on the long arm of chromosome 4 spanning the distance of approximately 380 kb. For this reason, PCR genotyping is quite challenging.

ADH1B *RsaI* SNP shows specific geographical patterning just as its functional counterpart in exon 3. The derived variant of the intron SNP, designated as *ADH1B*A2* in further text (Table 1), is common in populations of Southeast Asia reaching frequencies well above 70%. In almost all other parts of the world the ancestral variant of the SNP is dominant, especially

in African and Native American populations (Osier et al., 1999).

The presence of the derived *ADH1B*A2* variant in populations of the Far East is due to events of recombination with haplotypes carrying the exon *ADH1B*47His* (Li et al., 2011; Osier et al., 2002). The recombination event successfully explains the presence of both SNPs in populations of the Far East. However, the event alone does not explain their high frequencies in the populations of the Far East, particularly in some Southeast Asian populations. It is now known that *ADH1B*47His* functional SNP was subject to positive natural selection (Han et al., 2007). Because of its proximity to *ADH1B*47His* functional SNP in exon 3, the intron *ADH1B* *RsaI* SNP was also affected.

The aim of this study was to estimate the frequency of *ADH1B* *RsaI* polymorphism in the population of Bosnia and Herzegovina (B&H) and by doing so, to complement the ALFRED frequency database with the data on an additional European population. Samples from this analysis can be genotyped for other exon and intron SNPs thus providing haplotype frequency data for future ADH gene loci studies of the B&H population.

Materials and methods

The sample included 101 unrelated individuals inhabiting wider city area of Sarajevo. Among them are settlers from other parts of Bosnia and Herzegovina. After informed consent had been obtained from all participants, the DNA samples were collected by swabbing the buccal mucosa. The swabs were labelled, air-dried and stored in paper

Table 1. Designations for the two *ADH1B* *RsaI* allele variants used in this paper. Earlier designations, codon sequences and the official SNP designation

Allele designation used in this paper	Earlier designation ¹	Difference in codon sequence ²	Official designation of the SNP ³
<i>ADH1B*A1</i>	<i>ADH2*A1</i>	<u>G</u> C <u>A</u>	rs2066701
<i>ADH1B*A2</i>	<i>ADH2*A2</i>	<u>G</u> T <u>A</u>	

¹Designation used in Osier et al. (1999).

²Codon sequence obtained from ALFRED database (*The Allele Frequency Database*) (<http://alfred.med.yale.edu/>).

³Official designation of the SNP in ALFRED database (*The Allele Frequency Database*) (<http://alfred.med.yale.edu/>), and dbSNP (*Database of Single Nucleotide Polymorphisms*) (www.ncbi.nlm.nih.gov/SNP).

envelopes until processing.

Genomic DNA was extracted from buccal cells using the salting out method (Miller et al., 1988). Genotyping via PCR-RFLP was carried out in 15 µl PCR reaction volume as described by Osier et al. (1999). Cleaved DNA was separated in 1.5 % agarose gel and stained with ethidium bromide. DNA fragments were exposed using UV-transilluminator and analyzed with Kodak EDAS software. As shown in Figure 1, three RFLP patterns were observed: uncut PCR product of 236 bp (CC), cut PCR product of 176 bp (TT) and heterozygote (CT) which exhibits both bands.

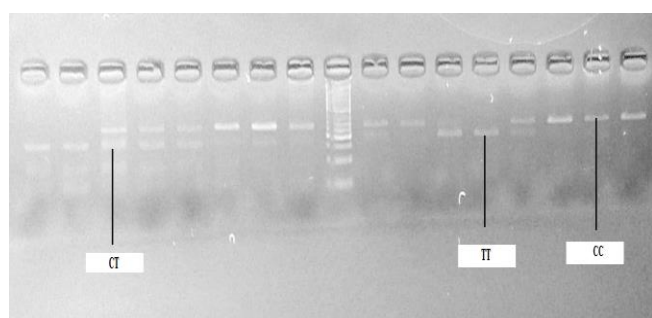


Figure 1. PCR amplicons digested with *RsaI*. The two letter designations stand for the three possible genotypes. The ancestral homozygote CC (*RsaI* absent), TT and CT genotypes were observed in a random sample of B&H population

Parameters such as number of alleles and genotypes, allele frequencies, expected and the observed heterozygosity, fixation indices and Hardy-Weinberg equilibrium were calculated for the total B&H sample using the GenAIEx (version 6.501) computer software (Peakall & Smouse, 2012). The PIC (polymorphism information content) value was estimated with the *PICcalc* online program (Nagy et al., 2012).

Inter-population analysis including the total B&H sample and 41 European and Global populations was also carried out. For this purpose forty-one populations from the ALFRED database (Rajeevan et al., 2003) were used (supplemental data).

We have calculated the Nei's gene diversity (including their respective standard deviations) (Nei, 1987) using the Arlequin (version 3.5) (Excoffier & Lischer, 2010). The same software was used for pairwise F_{ST} estimation (pF_{ST}) (Weir & Cockerham, 1984) implementing 1000 permutations ($P < 0.05$). Genetic distance (D_A) between populations was calculated according to Nei et al. (1983) using the POPTREEW software program (Takezaki et al., 2014). Dimensional relations between the investigated populations were visualised with the NM-MDS (Non metric multidimensional scaling) analysis using PAST (version 3) (Hammer & Harper, 2006). The analysis is based on Euclidian distance in two-dimensional space.

Results and Discussion

As evident from Table 2, the sample analysed in this study showed an allele ratio in favour of the ancestral allele (>0.74). Both heterozygosity indices (H_e and H_o) are in expected range for moderate diverse human population range. The quite low value of fixation index score ($F=0.068$) confirms the previous statement as it indicates not significantly inbred population. Polymorphism information content value (PIC) of 0.3107 is representative of a mildly informative gene marker. The Chi squared test value ($\chi^2 = 0.463$), and its probability ($P=0.496$), showed no statistically significant deviations from the Hardy-Weinberg equilibrium.

The Nei's gene diversity parameter (including its standard deviation) (Nei, 1987) was calculated for forty-four populations retrieved from ALFRED database (Rajeevan et al., 2003) and for the population sample that was subject of this study (table not shown). The gene diversity value of the B&H sample (0.3861 ± 0.0426) was similar to those of other European populations (Russians 0.3844 ± 0.0444 , Adygei 0.4061 ± 0.0393 , Irish 0.3342 ± 0.0343 etc.).

Table 2. Genotype and allele count, allele frequency, expected heterozygosity (H_e), observed heterozygosity (H_o), fixation index (F), polymorphism information content (PIC), Chi squared test (χ^2) and its probability value (P) for the sample of Bosnians and Herezgovinians (B&H)

B&H sample									
No. of genotypes	No. of alleles	Freq. of <i>ADH1B*AI</i>	Freq. of <i>ADH1B*A2</i>	H_e	H_o	F	PIC	χ^2	P
3	2	.743	.257	.382	.356	.068	.3107	.463	.496

The sample of this study also shared similar diversity values with some Asian populations (Druze 0.3896 \pm 0.0332, Samaritans 0.3986 \pm 0.0469 and Yakut 0.3737 \pm 0.0446). Highest gene diversity scores were recorded in South and Central Asian populations and the lowest ones were observed in African and Native American populations. The results of genetic distance (Nei et al., 1983) and pF_{ST} (Weir & Cockerham, 1984) (Table 3) show that there are no statistically significant differences between the B&H sample and any other comparator from the European population. The same absence of differences can be observed when B&H sample is compared with African Somali

population, the Middle Eastern Druze, Palestinian and Samaritan populations, and some Asian populations such as the Mongolians, Manchus, Nasioi, Micronesians and Yakut people. Non-parametric multidimensional scaling analysis shows weak clustering for European populations (Figure 2). The B&H sample clusters with the populations of Adygei, Yakut, Druze but also with the Samaritans, Hungarians and Russians. Stronger clustering is observable for specific African and Native American populations due to high ancestral allele frequency. The allele frequencies of the sample analysed in this study are concordant with the general allele ratio

Table 3. Pairwise F_{ST} (pF_{ST}) value (including respective P value) and the Nei's (1983) genetic distance (D_A) between the total B&H sample and other samples of populations from the ALFRED database

Sample name	pF_{ST} (p value)	D_A	Sample name	pF_{ST} (p value)	D_A
Kung San	.09798 (.00195 \pm -.0014)	.031	Mongolian	.01701 (.18066 \pm -.0128)	.008
Bantu speakers	.11882 (.00293 \pm -.0016)	.041	Hazzara	.06511 (.02148 \pm -.0049)	.019
Biaka	.11759 (.00000 \pm -.0000)	.036	Ami	.29189 (.00000 \pm -.0000)	.088
Hausa	.08815 (.00391 \pm -.0019)	.031	Atayal	.47626 (.00000 \pm -.0000)	.173
Ibo	.19775 (.00000 \pm -.0000)	.076	Chinese	.46941 (.00000 \pm -.0000)	.166
Mbuti	.17514 (.00000 \pm -.0000)	.064	Japanese	.5144 (.00000 \pm -.0000)	.197
Yoruba	.24825 (.00000 \pm -.0000)	.085	Kachari	.1733 (.00195 \pm -.0014)	.051
Jews Ethiopian	.08192 (.03613 \pm -.0047)	.036	Koreans	.50433 (.00000 \pm -.0000)	.189
Somali	.00137 (.41309 \pm -.0097)	.005	Manchu	.03771 (.07227 \pm -.0075)	.012
African Americans	.06619 (.00293 \pm -.0016)	.019	Cambodians	.21363 (.00000 \pm -.0000)	.061
Druze	-.00807 (.99902 \pm -.0002)	.000	Khmer	.45405 (.00000 \pm -.0000)	.163
Jews Yemenite	.0788 (.00391 \pm -.0019)	.026	Hakka	-.01043 (.68359 \pm -.0120)	.001
Palestinian	.00766 (.20312 \pm -.0112)	.004	Nasioi	.01968 (.12598 \pm -.0100)	.008
Samaritans	-.01113 (.87012 \pm -.0105)	.000	Micronesian	-.00973 (.87207 \pm -.0140)	.000
Adygei	-.00867 (.76758 \pm -.0166)	.000	Yakut	.21583 (.00000 \pm -.0000)	.076
Danes	-.00276 (.44434 \pm -.0197)	.002	Cheyene	.2249 (.00000 \pm -.0000)	.093
European Mixed	-.00159 (.41016 \pm -.0154)	.002	Maya	.15448 (.00195 \pm -.0014)	.074
Hungarians	-.00263 (.48926 \pm -.0136)	.001	Quechua	.28053 (.00000 \pm -.0000)	.093
Irish	-.00134 (.35840 \pm -.0160)	.002	Pima Mexico	.24901 (.00000 \pm -.0000)	.140
Russians	-.01036 (.99902 \pm -.0002)	.000	Pima Arizona	.20609 (.00000 \pm -.0000)	.140
Kazakh	.04795 (.04102 \pm -.0064)	.015	Surui		

found in other European populations included in ALFRED database (Rajeevan et al., 2003). This implies European character of the population of Bosnia and Herzegovina and reaffirms the findings of previous studies of B&H population which were based on different marker systems (Marjanović et al., 2005; 2006; Pojskić et al., 2013; Kovačević et al., 2014; Kapur-Pojskić et al., 2014). *ADH1B* *RsaI* population frequencies listed in ALFRED database (Rajeevan et al., 2003) show that European populations have conservative allele frequencies in favour of the ancestral variant (>0.69). Even if the ALFRED database, lacks data on specific European populations, it is presumable that additional studies for the *ADH1B* *RsaI* SNP polymorphism would produce similar results. Even particular Middle Eastern populations share the same allele frequency ratio common to European people. Indeed certain authors, while analysing ADH gene haplotypes regard European and Middle Eastern samples as one population group (Osier et al., 2002).

Inter-population analysis including other European and non-European populations from the ALFRED database (Rajeevan et al., 2003) proved the above-

mentioned European genetic background of the B&H populations. Besides the European and Middle Eastern, comparison with other populations also showed absence of significant genetic differences. Some of those are various clustered with the B&H sample, as shown by the NM-MDS analysis (Figure 2). The similarity with the sample of Somalis could be due to Arab mediaeval colonisation of Balkan Peninsula, which introduced haplotypes carrying the *ADH1B**A2 allele. The low level of differences between the B&H sample and some Far-Eastern populations like the Nasioi, Micronesians, Yakut people, Mongolians and Manchus could be attributed to the effects of genetic drift or stochastic effects. The latter could be especially probable in the case of Mongolians and Manchus. The ALFRED database (Rajeevan et al., 2003) contains more than one sample of Mongolians and Manchus and some of them have significantly different allele frequencies when compared to each other respectively. Of course, it is possible that none of the mentioned causes account for the lack of difference, and that the absence is merely due to evolutionary events and it reflects the genuine genetic structure of

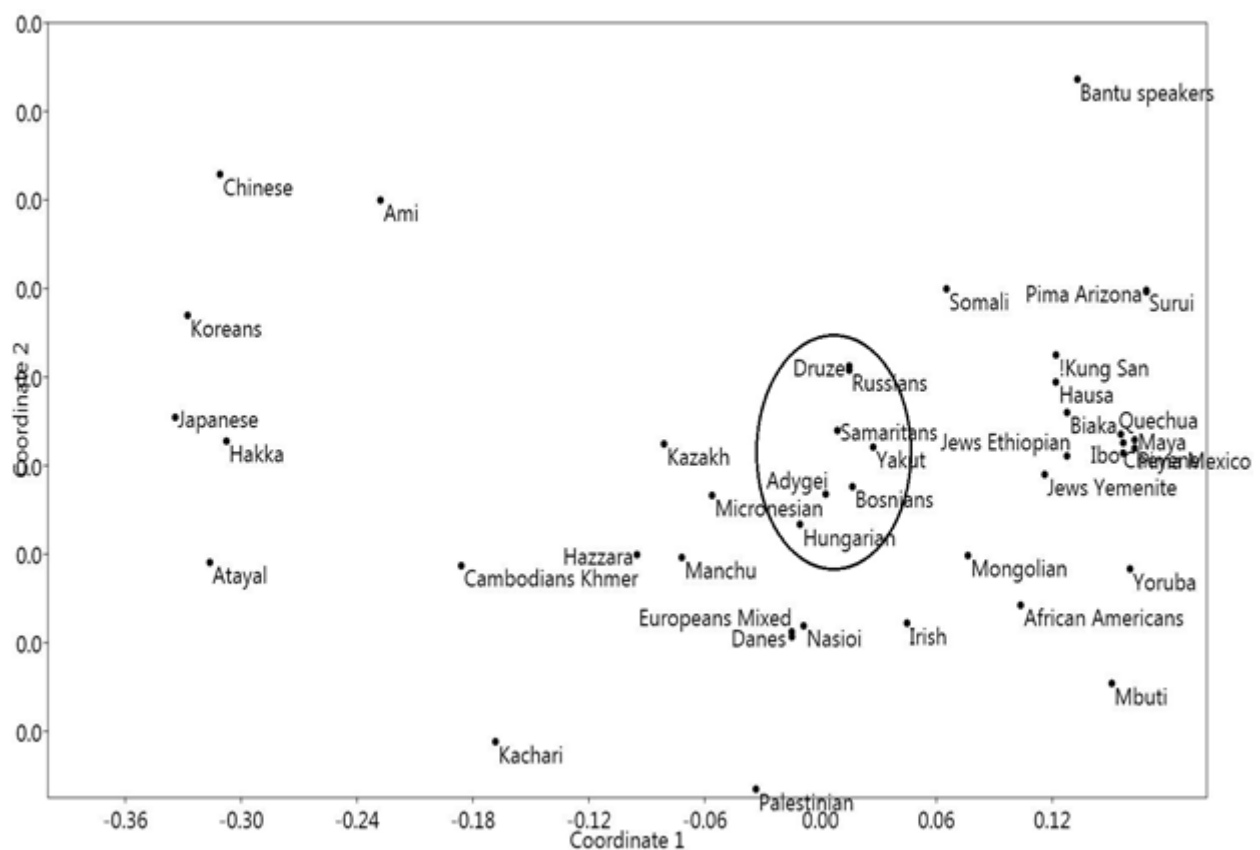


Figure 2. NM-MDS analysis of 42 populations including the BH sample based on Euclidian distance

the aforementioned populations. However, according to NM-MDS B&H population has close position with other European and Middle East populations.

Intron SNPs may lack the physiological importance of their exon counterparts, but one thing clearly sets them aside. Even if tight linkage disequilibrium exists between the *ADIHB* SNP in exon 3 and the *ADH1B* *RsaI* SNP (Osier et al., 1999), the global average heterozygosity of the latter (0.329) is greater compared to the global average heterozygosity of the exon counterpart (0.199) (Rajeevan et al., 2003). This is especially true if we look at the almost fixed frequencies of the *ADH1B*47His* in European population (Rajeevan et al., 2003) compared to their more diverse *ADH1B* *RsaI* frequencies. It is evident that intron sequences experience less constraint caused by effects of natural selection, which itself represents a favourable attribute in studying population genetic differences that arose from recent evolutionary pathways.

Conclusions

The results of our study are in concordance with the results of previous studies of B&H population based on different molecular-genetic markers (STR, *mtDNA*, Y-STR, X-STR, *Alu* polymorphism). The allele frequencies obtained for analysed sample of B&H population are in accord with the allele frequencies of other European populations. Our results confirm the findings of earlier genetic population studies which indicate that B&H population belongs to the European gene pool.

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Supplemental data

Sample name	ALFRED UID	2N	<i>ADH1B</i> *A1	<i>ADH1B</i> *A2
!Kung San	SA000094N	80	0.92	0.08
Bantu speakers	SA000041F	94	0.94	0.06
Biaka	SA000005F	138	0.93	0.07
Hausa	SA000100B	74	0.92	0.08
Ibo	SA000099S	96	0.98	0.02
Mbuti	SA000006G	78	0.97	0.03
Yoruba	SA000036J	144	0.986	0.014
Jews	SA000015G	30	0.93	0.07
Ethiopian	SA002138O	40	0.825	0.175
Somali	SA000101C	178	0.89	0.11
African Americans	SA000008I	160	0.74	0.26
Druze	SA000016H	78	0.91	0.09
Jews	SA002766V	138	0.659	0.341
Yemenite	SA000098R	78	0.73	0.27
Palestinian	SA000017I	104	0.72	0.28
Samaritans	SA000046K	96	0.69	0.31
Adygei	SA000020C	182	0.69	0.31
Danes	SA002023H	178	0.697	0.303
Europeans	SA000057M	190	0.79	0.21
Mixed	SA000019K	94	0.74	0.26
Hungarian	SA002009L	64	0.578	0.422
Irish	SA002013G	64	0.844	0.156
Russians	SA002140H	56	0.554	0.446
Kazakh	SA000002C	80	0.33	0.67
Mongolian	SA000021D	84	0.18	0.82
Hazzara	SA000009J	102	0.19	0.81
Ami	SA000010B	98	0.15	0.85
Atayal	SA000040E	30	0.43	0.57
Chinese	SA000936S	94	0.16	0.84
Japanese	SA002011E	64	0.594	0.406
Kachari	SA000022E	48	0.4	0.6
Koreans	SA000003D	82	0.195	0.805
Manchu	SA000012D	46	0.7	0.3
Cambodians	SA000063J	64	0.62	0.38
Khmer	SA000011C	98	0.76	0.24
Hakka	SA000023F	112	0.98	0.02
Nasioi	SA000013E	100	0.99	0.01
Micronesian	SA000069P	46	0.978	0.022
Yakut	SA000034H	180	0.99	0.01
Cheyene	SA000025H	100	1	0
Maya	SA000014F	60	1	0
Quechua				
Pima				
Mexico				
Pima				
Arizona				
Surui				