DISTRIBUTION OF *ADH1B* GENOTYPES PREDISPOSED TO ENHANCED ALCOHOL CONSUMPTION IN THE CZECH ROMA/GYPSY POPULATION

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SUMMARY

Objective: The aim of the study was to analyse the frequencies of rs1229984 genotypes within the alcohol dehydrogenase (*ADH1B*) gene in a Gypsies/Roma population and compare them with other populations and with ethanol consumption.

Methods: We analysed the *ADH1B* (rs1229984; Arg47→His; c.143G>A) genotype using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in two ethnically different groups – Gypsies/Roma (N = 301) and Czechs (N = 300) where one day alcohol consumption was recorded.

Results: ADH1B genotype/allelic frequencies did not significantly differ between the populations (p = 0.32). The frequency of minor A allele carriers was slightly higher in Gypsies/Roma (14.7%) than in Czechs (11.9%). The prevalence of subjects reporting alcohol intake on the previous day was non-significantly lower in Gypsies/Roma (10.5% vs. 16.4%), as was the amount of alcohol consumed the day before the examination in ethanol consumers (36.1 ± 18.3 g vs. 43.0 ± 27.2 g).

Conclusions: The frequency of rs1229984 genotypes in the *ADH1B* gene within the Gypsies/Roma population corresponds with frequencies obtained in North India/Central Asia, the putative country of this ethnic origin. Our results suggest that the minority Gypsies/Roma population consume slightly less alcohol than the Czech majority population.

Key words: alcoholic beverages, alcohol dehydrogenase, Czechs, Gypsies/Roma, polymorphism

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INTRODUCTION

Ethanol (consumed as an alcoholic beverage) and nicotine are two the most common legally accepted and widespread drugs. Although it is conjectured that low and regular consumption of ethanol may have some healthy benefits (1), it is almost certain that these potential benefits are negated by the overall negative consequences of ethanol consumption (2–6).

It is acknowledged that the response to alcohol consumption is characterised by significant heterogeneity. Alongside other factors such as gender and ethnicity or religion, genetic factors (polymorphisms, mutations, etc.) are regarded as important predictors of ethanol consumption (7, 8), and account for roughly 50% of predispositions to alcohol dependence.

Alcohol dehydrogenase 1B (*ADH1B*) is the gene that codes for alcohol dehydrogenase 1B (OMIM acc N. 103720; Gene ID: 125; enzyme activity EC 1.1.1.1). *ADH1B* is a member of a family of alcohol dehydrogenase enzymes with a high sequence identity (95%). It is a key enzyme in ethanol catabolism, converting ethanol (CH_3–CH_2–OH) into acetaldehyde (CH_3–CH=O).

The common *ADH1B* polymorphism, arginine 47 → histidine (c.143 guanine > adenine; rs1229984), exerts an extreme effect on enzyme activity, representing a 100-fold ethanol oxidation difference (as V_{max}) between the major and minor allele homozygotes (9). Most Europeans are carriers of the GG (Arg/Arg) genotype, which is associated with lower enzyme activity (10). The presence of the His allele is associated with lower alcohol intake in both men and women of different ethnicities as well as with a lower risk of alcoholism development (11–13), which may be caused by enhanced acetaldehyde production and subsequent unfavourable reactions to ethanol consumption (14).

Gypsies/Roma individuals represent the largest ethnic minority in Central European countries. Epidemiological studies (15–17) have shown that this minority, in comparison with majority populations from the same regions, have a higher prevalence of metabolic diseases (diabetes mellitus, cardiovascular disease, etc.), and a generally worse health status than majority populations*. These trends can be at least partially attributed to typically more hazardous behaviour, which can lead to the adoption of unhealthy lifestyles. But although there are clearly some genetic

*http://ec.europa.eu/justice/discrimination/files/Romani_health_en.pd.

differences between Gypsies/Roma and non-Gypsies/Roma subjects (18–21), to what extent these unhealthy lifestyles might be "inherited" is still unclear.

Our study analysed the prevalence of the *ADH1B* rs1229984 genotypes in Czech Gypsies/Roma, as there is almost no information about the distribution of *ADH1B* alleles or about the associated consumption of alcoholic beverages for this ethnic group.

MATERIALS AND METHODS

Study Populations

We examined two ethnically distinct populations inhabiting one region of South Bohemia. Six hundred and one unrelated adults (at least 18 years old at the time of examination) were included in the study (18). Czech Gypsies/Roma (N = 301) were recruited using snowball sampling (22) and Czech Caucasians/Slavs (N = 300) using quota sampling (23–25). Ethnicity was based on self-reported information.

Written informed consent was provided by all subjects involved in the study. The study protocol was approved by institutional ethics committee and conducted according to the Good Clinical Practice guidelines and in agreement with the Helsinki Declaration of 1975.

Alcohol Consumption

All subjects completed a one-day dietary record, involving a complete summary of all food and drink consumed one day before the examination (breakfast, snack, lunch, snack, dinner, snack).

The questions concerning alcoholic beverages focused on the amount of alcohol consumed on the day before the examination. Previous-day alcohol consumers were defined as "consumers". The total amount of alcohol consumed was calculated based on the assumption that beer contains 5% of ethanol, wine 11%, and spirits 40%. From the available data, we then calculated percentages of alcohol consumption/non-consumption cases and the amount of alcohol consumed in grams per day.

Extraction of DNA and Polymorphism Analysis

DNA was isolated using the Xtreme DNA Isolation Kit and DNA buccal swabs (both Isohelix, Cell Projects Ltd, UK) according to conditions specified by the manufacturer.

Rs1229984 genotypes were analysed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (26). Briefly, oligonucleotides 5' aca atc ttt tct gaa tct gaa cag ctt ctc and 5' ttg cca cta acc acg tgg tca tct gcg were used for DNA amplification. All PCR chemicals were provided by Fermentas International Inc. (Burlington, Ontario, Canada) and PCR reactions were performed on the MJ Research DYAD Disciple PCR device. The PCR product (97 bp) was digested by 5 units of the Hin6I restriction enzyme (Fermentas International Inc., Burlington, Ontario, Canada) at 37°C overnight. The PCR product was cut on 70 bp and 27 bp restriction fragments where the common G allele was present. Restriction fragments were separated using 10% polyacrylamide gel using the MADGE system (27).

Statistical Analysis

The deviance of genotype frequencies among the groups was analysed according to Hardy-Weinberg equilibrium*. Differences in allelic and genotype frequencies were compared using an online chi-squared test**. Non-parametric ANOVA (Kruskal-Wallis test) was used to compare alcohol intake between the ethnic groups. A p-value < 0.05 was considered significant.

RESULTS

General characteristics of the examined subjects are summarised in Table 1. We successfully genotyped 285 (94.7%) out of 301 Czech Gypsies/Roma (144 males and 141 females) and 286 (95.3%) out of 300 Czech Caucasian/Slavs (146 males and 140 males) for the *ADH1B* rs1229984 variant. In both groups, genotype distributions were within Hardy-Weinberg equilibrium (both $p > 0.18$). No gender differences (within ethnic groups) in genotype frequencies were observed.

Among Czech Gypsies/Roma individuals, carriers of the minor A allele were more represented than in the Czech/Slavic sample,

Table 1. *General characteristics of examined subjects*

Parameter	Gypsies/Roma population	Czech/Slavic population
N	301	300
Males/females (%)	50/50	50/50
Age (years)	39.2 ± 12.8	39.5 ± 15.1
BMI (kg/m^2)	29.9 ± 5.6	25.0 ± 6.0
% of consumers*	10.5	16.4
Alcohol intake of consumers (g/day)	36.1 ± 18.3	43.0 ± 27.2

Alcohol intake of consumers is given in grams per day and calculated based on alcohol consumed the day before the examination.
*Defined as positive consumption the day before the examination

*http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm
**www.physics.csbsju.edu/cgi-bin/stats/contingency_form.sh?nrow=2&ncolumn=3

but the prevalence of rs1229984 genotypes was not significantly different between the two ethnic groups under study (Table 2, 3). However, a significant difference has been observed when comparing Gypsies/Roma genotype frequencies with a large group of Czech Caucasian adults (N = 6,497; the Czech branch of the HAPIEE study; minor A allele carriers 10.8% vs. Gypsies/Roma 14.7%; p = 0.035) (11). In contrast, the *ADH1B* genotype frequencies were in our Caucasian/Slavic group very close to the previously analysed Czech Caucasian population from the HAPIEE study (p = 0.54) (11), and to frequencies observed in the Czech post-MONICA study (N = 2,595 subjects; 10.5%; p = 0.48) (Hubáček and Adámková, unpublished results).

Within reported drinkers was the amount of alcohol consumed the day before the examination nonsignificantly lower in Gypsies/Roma than in majority population (36.1 ± 18.3 g vs. 43.0 ± 27.2 g) (Table 1).

DISCUSSION

This is the first report to compare frequencies of the major genetic determinant of alcohol intake – the rs129984 variant within the AHD1B gene – in the Gypsies/Roma community and Caucasian/Slavic population in the region of the Czech Republic.

Individual allele frequencies of the rs1229984 polymorphism exhibit huge interethnic differences (28, 29). The A allele (His47-coding), which is present in a maximum of 15% (mostly far less) of Caucasians, is a very common allele in China/Japan (usually present in more than 85% of inhabitants). The distribution of individual rs1229984 alleles is about 50%/50% in populations around the Red Sea. Our study confirms that the frequency of the minor A *ADH1B* allele in our group of Czech Gypsies/Roma subjects is almost identical to the frequency reported in the putative area of ethnic origin in North India (30, 31).

Our results concerning genotype frequencies are in agreement with a recent report by Diószegi et al. (32), who analysed the identical polymorphism in a large (N = 1,267 Gypsies/Roma and N = 2,917 majority population) sample of Hungarian subjects. They also found a slightly higher frequency of the minor His allele in the Gypsies/Roma minority in comparison to the Hungarian general population.

Analysis of "previous-day alcohol consumption" is definitely less precise than analysis of mean alcohol intake over a longer period of time. If we compare our relatively small group of Czech Caucasians with the ethnically and geographically identical sample from the HAPIEE study (11), it is clear that total alcohol consumption has been overestimated here. It is likely that subjects with generally lower alcohol intake have avoided alcohol consumption before the examination.

In case of alcohol consumption, it is of interest that our results are in agreement with other studies that focus on a similar topic. Our finding that the Gypsies/Roma population consumed less alcohol than the Czech majority population is in agreement with the earlier HepaMeta study, which was performed in the neighbouring country of Slovakia and involved a comparable number of subjects (33). Using an identical question ("alcohol intake one day before"), they found that only 5.5% of Gypsies/Roma subjects consumed alcohol the day before the examination in comparison with 8.7% of non-Gypsies/Roma individuals. Similar results have been described for Slovak patients undergoing coronary angiography (34) – in this study, authors also report lower alcohol intake for Gypsies/Roma subjects in comparison with the majority population. Finally, the identical drinking pattern was observed in the Hungarian Gypsies/Roma population (32).

We detected no significant association between the *ADH1B* rs1229984 genotypes and alcohol consumption across both groups. Here, the relative low number of examined subjects may have accounted for the non-significant results.

Table 2. *Frequencies of ADH1B rs1229984 genotypes in the minority Gypsies/Roma and Czech/Slavic populations*

	Gypsies/Roma population (N = 285)		Czech/Slavic population (N = 286)		p-value
rs1229984	**n**	**%**	**n**	**%**	
GG	243	85.3	252	88.1	0.32
GA	42	14.7	34	11.9	
AA	0	0.0	0	0.0	
G	528	92.6	538	94.1	0.33
A	42	7.4	34	5.9	

A – adenine, G – guanine

Table 3. *Distribution of ADH1B rs1229984 genotypes in "previous-day alcohol consumers" and "non-consumers"*

	Gypsies/Roma population (N = 285)				Czech/Slavic population (N = 286)			
	Consumers		Non-consumers		Consumers		Non-consumers	
rs1229984	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
GG	26	86.7	217	85.1	44	93.6	208	87.0
GA	4	13.3	38	14.9	3	6.4	31	13.0
AA	0	0.0	0	0.0	0	0.0	0	0.0

A – adenine, G – guanine

In summary, in comparison with the majority population, lower alcohol intake in the Gypsies/Roma minority has been reported in the Czech Republic, Slovakia and Hungary, but not in Gypsies/Roma minorities inhabiting Western European regions (32). We can however speculate that the much lower socioeconomic status of this minority (which is also valid for the majority population) in non-Western European regions could be one of the reasons for this difference.

Our results, rather surprisingly, but in agreement with some previously published data, do not support the general assumption of higher alcohol intake within the Gypsies/Roma minority. However, we cannot exclude the higher prevalence of harmful alcohol drinking habits (such as binge-drinking) among the Gypsies/Roma minority.

CONCLUSION

We report increased frequency of the minor allele of the rs1229984 polymorphism within the *ADH1B* gene in Czech Gypsies/Roma in comparison with the Czech/Slavic majority population. Further, it seems that alcohol consumption is lower (although not statistically significant) in Czech Gypsies/Roma than in the majority Czech/Slavic population.

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Conflict of Interests

None declared

REFERENCES

1. Katsiki N, Tziomalos K, Mikhailidis DP. Alcohol and the cardiovascular system: a double-edged sword. Curr Pharm Des. 2014;20(40):6276-88.
2. Graham K, Bernards S, Knibbe R, Kairouz S, Kuntsche S, Wilsnack SC, et al. Alcohol-related negative consequences among drinkers around the world. Addiction. 2011 Aug;106(8):1391-405.
3. Health risks and benefits of alcohol consumption. Alcohol Res Health. 2000;24(1):5-11.
4. Thakker KD. An overview of health risks and benefits of alcohol consumption. Alcohol Clin Exp Res. 1998 Oct;22(7 Suppl):285S-98S.
5. Rehm J, Gmel G, Sempos CT, Trevisan M. Alcohol-related morbidity and mortality. Alcohol Res Health. 2003;27(1):39-51.
6. Grønbaek M. The positive and negative health effects of alcohol- and the public health implications. J Intern Med. 2009 Apr;265(4):407-20.
7. Moore AA, Gould R, Reuben DB, Greendale GA, Carter MK, Zhou K, et al. Longitudinal patterns and predictors of alcohol consumption in the United States. Am J Public Health. 2005 Mar;95(3):458-65.
8. Pautassi RM, Camarini R, Quadros IM, Miczek KA, Israel Y. Genetic and environmental influences on ethanol consumption: perspectives from preclinical research. Alcohol Clin Exp Res. 2010 Jun;34(6):976-87.
9. Matsuo Y, Yokoyama R, Yokoyama S. The genes for human alcohol dehydrogenases beta 1 and beta 2 differ by only one nucleotide. Eur J Biochem. 1989 Aug 1;183(2):317-20.
10. Peng GS, Yin SJ. Effect of the allelic variants of aldehyde dehydrogenase ALDH2*2 and alcohol dehydrogenase ADH1B*2 on blood acetaldehyde concentrations. Hum Genomics. 2009 Jan;3(2):121-7.
11. Hubacek JA, Pikhart H, Peasey A, Kubinova R, Bobak M. ADH1B polymorphism, alcohol consumption, and binge drinking in Slavic Caucasians: results from the Czech HAPIEE study. Alcohol Clin Exp Res. 2012 May;36(5):900-5.
12. Holmes MV, Dale CE, Zuccolo L, Silverwood RJ, Guo Y, Ye Z, et al. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ. 2014 Jul 10;349:g4164. doi: 10.1136/bmj.g4164.
13. Edenberg HJ, Foroud T. Genetics and alcoholism. Nat Rev Gastroenterol Hepatol. 2013 Aug;10(8):487-94.
14. Wall TL. Genetic associations of alcohol and aldehyde dehydrogenase with alcohol dependence and their mechanisms of action. Ther Drug Monit. 2005 Dec;27(6):700-3.
15. Simko V, Ginter E. Short life expectancy and metabolic syndrome in Romanies (gypsies) in Slovakia. Cent Eur J Public Health. 2010 Mar;18(1):16-8.
16. Parekh N, Rose T. Health inequalities of the Roma in Europe: a literature review. Cent Eur J Public Health. 2011 Sep;19(3):139-42.
17. Hajioff S, McKee M. The health of the Roma people: a review of the published literature. J Epidemiol Community Health. 2000 Nov;54(11):864-9.
18. Hubáček JA, Adámková V, Šedová L, Olišarová V, Adámek V, Tóthová V. Frequency of adult type-associated lactase persistence LCT-13910C/T genotypes in the Czech/Slav and Czech Roma/Gypsy populations. Genet Mol Biol. 2017 Apr-Jun;40(2):450-2.
19. Kósa Z, Széles G, Kardos L, Kósa K, Németh R, Országh S, et al. A comparative health survey of the inhabitants of Roma settlements in Hungary. Am J Public Health. 2007 May;97(5):853-9.
20. Sumegi K, Duga B, Melegh BI, Banfai Z, Kovesdi E, Maasz A, et al. Marked differences of haplotype tagging SNP distribution, linkage, and haplotype profile of APOA5 gene in Roma population samples. Pathol Oncol Res. 2017 Oct;23(4):853-61.
21. Szalai R, Hadzsiev K, Melegh B. Cytochrome P450 drug metabolizing enzymes in Roma population samples: systematic review of the literature. Curr Med Chem. 2016;23(31):3632-52.
22. Hughes AO, Fenton S, Hine CE, Pilgrim S, Tibbs N. Strategies for sampling black and ethnic minority populations. J Publ Health Med. 1995 Jun;17(2):187-92.
23. Adámkova V, Hubacek JA, Nováková D, Dolák F, Adámek V, Lánská V, et al. Genetic and biochemical characteristics in the Roma minority in the South Bohemia Region. Neuro Endocrinol Lett. 2015;36 Suppl 2:29-34.
24. Šedová L, Tóthová V, Olišarová V, Adámkova V, Bártlová S, Dolák F, et al. Evaluation of selected indicators of overweight and obesity of Roma minority in the region of South Bohemia. Neuro Endocrinol Lett. 2015;36 Suppl 2:35-42.
25. Tóthová V, Bártlová S, Šedová L, Olišarová V, Prokešová R, Adámkova V, et al. The importance of self-management in the prevention and treatment of excessive weight and obesity. Neuro Endocrinol Lett. 2015;36 Suppl 2:5-10.
26. Hubáček JA, Pikhart H, Peasey A, Kubínová R, Bobák M. Nobody is perfect: Comparison of the accuracy of PCR-RFLP and KASP™ method for genotyping. ADH1B and FTO polymorphisms as examples. Folia Biol (Praha). 2015;61(4):156-60.
27. Day IN, Humphries SE. Electrophoresis for genotyping: microtiter array diagonal gel electrophoresis on horizontal polyacrylamide gels, hydrolink, or agarose. Anal Biochem. 1994 Nov 1;222(2):389-95.
28. Borinskaya S, Kal'ina N, Marusin A, Faskhutdinova G, Morozova I, Kutuev I, et al. Distribution of the alcohol dehydrogenase ADH1B*47His allele in Eurasia. Am J Hum Genet. 2009 Jan;84(1):89-92.
29. Li H, Mukherjee N, Soundararajan U, Tarnok Z, Barta C, Khaliq S, et al. Geographically separate increases in the frequency of the derived ADH1B*47His allele in eastern and western Asia. Am J Hum Genet. 2007 Oct;81(4):842-6.
30. Mendizabal I, Valente C, Gusmão A, Alves C, Gomes V, Goios A, et al. Reconstructing the Indian origin and dispersal of the European Roma: a maternal genetic perspective. PLoS One. 2011 Jan 10;6(1):e15988. doi: 10.1371/journal.pone.0015988.
31. Rao VR, Bhaskar LV, Annapurna C, Reddy AG, Thangaraj K, Rao AP, et al. Single nucleotide polymorphisms in alcohol dehydrogenase genes among some Indian populations. Am. J. Hum. Biol. 2007 May-Jun;19(3):338-44.

32. Diószegi J, Fiatal S, Tóth R, Moravcsik-Kornyicki Á, Kósa Z, Sándor J, et al. Distribution characteristics and combined effect of polymorphisms affecting alcohol consumption behaviour in the Hungarian general and Roma populations. Alcohol Alcohol. 2017 Jan;52(1):104-11.
33. Babinská I, Gecková AM, Jarcuska P, Pella D, Mareková M, Stefková G, et al. Does the population living in Roma settlements differ in physical activity, smoking and alcohol consumption from the majority population in Slovakia? Cent Eur J Public Health. 2014 Mar;22 Suppl:S22-7.
34. Sudzinova A, Nagyova I, Studencan M, Rosenberger J, Skodova Z, Vargova H, et al. Roma coronary heart disease patients have more medical risk factors and greater severity of coronary heart disease than non-Roma. Int J Public Health. 2013 Jun;58(3):409-15.

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