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Distribution Characteristics and Combined Effect of Polymorphisms Affecting Alcohol Consumption Behaviour in the Hungarian General and Roma Populations

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Abstract

Aims: Harmful alcohol drinking habits, even among Roma children and adolescents, are more common than in the majority population. The aim of the study was to evaluate the genetic susceptibility of Roma to hazardous alcohol consumption compared to the Hungarian general population.

Methods: A total of 1273 samples from the population of segregated Hungarian Roma colonies and 2967 samples from the Hungarian general population were genotyped for 25 polymorphisms. Differences in genotype and allele distributions were investigated. Genetic risk scores (GRS) were generated to estimate the joint effect of individual single-nucleotide polymorphisms (SNPs). After unweighted and weighted GRS were calculated the distribution of scores in study populations was compared.

Results: The allele frequencies differed significantly between the study populations for 17 SNPs ($P < 0.002$), but the genetic alterations that predispose to or protect against harmful alcohol consumption were not overrepresented in the Roma population. The distribution of unweighted GRS in Roma population was left shifted compared to general population ($P = 0.0013$). The median weighted genetic risk score was lower among the subjects of Roma population compared to the subjects of general population (0.53 vs 0.65, $P = 3.33 \times 10^{-27}$) even after adjustment for confounding factors.

Conclusions: Differences in alcohol consumption habits between the Hungarian Roma and Hungarian general populations do not appear to be linked to genetic constitution, this behaviour may occur as a result of different cultural values and environmental exposures. Population-based measures to tackle the fundamental drivers of consumption, which take account of cultural acceptability, are needed to reduce harmful alcohol consumption in the Roma population.

INTRODUCTION

The development of alcohol use disorders is a multifactorial process. It includes both genetic and environmental factors. Thus, the heritability of alcohol dependence is relatively high, ranging from 50 to 60 percent, based on twin and adoption studies, and a number of polymorphisms in genes involved in alcohol metabolism and neurotransmission have been linked to alcohol dependence (Buscemi and Turchi, 2012). However, environmental factors also play an important role, as is apparent from evidence of social patterning of alcohol dependence (Batty *et al.*, 2012) and the observation that rates can change over relatively short periods of time, associated with, for example, changes in the wider economy (Zemore *et al.*, 2013).

Knowledge of the extent to which these factors are important may be useful in situations where two ethnically distinct populations inhabit the same territory. Are any differences in alcohol dependence a consequence of environmental factors, and thus amenable to public policy to tackle demand and/or supply, or are they a reflection of underlying genetic factors? In the USA, Native Americans exhibit higher rates of alcohol dependence than other ethnicities (Ehlers *et al.*, 2012) and the highest alcohol-related deaths of all ethnic groups (Ehlers, 2007). While environmental factors clearly play a role, Native American ancestry is associated with higher frequencies of potentially risky and lower frequencies of protective variants of alcohol dependence genotypes (Wall *et al.*, 2003), although there are exceptions (Wall *et al.*, 2003).

One situation where this may arise is in central Europe, where the Roma population represents the largest minority. They suffer not only from discrimination, social exclusion (Rothenberg and Wang, 2006; Cook *et al.*, 2013), educational and material disadvantage (Colombini *et al.*, 2012) and worse access to health services (Kosa *et al.*, 2007; Colombini *et al.*, 2012), but also differ from the majority population in a number of cultural, social and religious aspects that have a significant influence on health behaviours (Petek *et al.*, 2006).

Several studies have compared levels and patterns of alcohol consumption in Roma and majority populations in Europe. The results of studies on alcohol consumption in Roma populations in different European countries are similar, except for one Hungarian study in which the prevalence of abstainers was significantly higher among Roma than in the general adult population (Kosa *et al.*, 2007). Daily alcohol consumption as well as drunkenness was more common among Roma adolescents, (Sarvary *et al.*, 2012) and a Hungarian study of Roma and non-Roma adolescents found that Roma ethnicity was significantly associated with lifetime prevalence of alcohol intoxication (Gerevich *et al.*, 2010). A study in Moldova found that alcohol and tobacco accounted for 3% of per capita expenditure in Roma families compared to 1% in non-Roma ones (Cace *et al.*, 2007). A Turkish study found that those with Roma ethnicity were at 3.2 times ($P < 0.05$) greater risk than the other ethnicities investigated (Ekuklu *et al.*, 2004). In Spain, a lower proportion (24.8%) of Roma men are teetotallers compared to male individuals of the general population (31.3%) but in women, the opposite holds true (62.1% vs 55.9%, respectively) (Ibáñez, 2009). FundaciónSecretariadoGitano's research found that Roma males start drinking alcohol at earlier ages than non-Roma and high percentages of Roma households have at least one member with an alcohol and/or drug problem, especially in Bulgaria, Greece and the Czech Republic (Fundación, 2006). Roma women were slightly more likely to drink during pregnancy than non-Roma women in a Hungarian study investigating birth outcomes in both populations (Balazs *et al.*, 2010).

Our aim was to compare the genetic susceptibility to harmful alcohol consumption in Hungarian Roma (HR) and the general Hungarian population by analysing the frequencies of alleles and genotypes known to be closely linked to alcohol consumption. Genetic risk scores, unweighted (GRS) and weighted (wGRS), were calculated by summing the total effect of several single-nucleotide polymorphisms (SNPs) utilizing effect size estimates from published genome wide association studies studies. Estimating the extent of genetic susceptibility is important for designing and implementing targeted public health intervention programmes among Roma.

METHODS

Study design

Subjects in this study included 1273 HR individuals living in segregated colonies in North-East Hungary and 2967 individuals in the Hungarian general population (HG). Of the inhabitants living in North-East Hungary 30% are of Roma ethnicity which is the most vulnerable minority population of the country (Adany *et al.*, 2013).

Samples

Sample representative of the HG population

A population-based disease registry, the General Practitioners' Morbidity Sentinel Stations Programme (GPMSSP), provided the Hungarian reference sample. The Hungarian GPMSSP was established in 1998 by the School of Public Health, University of Debrecen and the National Public Health and Medical Officer Service to monitor the prevalence and incidence of chronic noncommunicable diseases (hypertension; diabetes mellitus; liver cirrhosis; ischaemic heart disease; acute myocardial infarction; stroke; and malignancies of the respiratory tract, colon and rectum, breast, cervix and prostate) of great public health importance (Szeles *et al.*, 2005). In the initial phase of the program, individuals from four counties were involved (Hajdú-Bihar, Györ-Moson-Sopron, Szabolcs-Szatmár-Bereg and Zala Counties). However, the programme was extended subsequently to additional regions (two counties from Central Hungary, Komárom-Esztergom and Bács-Kiskun, Baranya from Southern Transdanubia, and Heves from Northern Hungary) of the country (Szigethy *et al.*, 2012).

Samples were drawn from a reference population of males and females aged 20 years of age and over from districts of counties participating in the GPMSSP and registered with health facilities. The sample is representative of the Hungarian adult population in terms of geographic, age and sex distributions. General practitioners (GPs) recorded the medical history and performed standardized physical examinations (weight, height, waist circumference, blood pressure measurements) and collected blood samples for laboratory tests (serum triglyceride, HDL-cholesterol, glucose levels) and DNA isolation. Within the framework of the GPMSSP, 2967 DNA samples were obtained, and used as the reference samples in the present study.

Sample representative of HR living in segregated colonies

Participants were enrolled from Northeast Hungary (Hajdú-Bihar and Szabolcs-Szamár-Bereg counties), an area where the majority of Roma colonies are found, using a stratified multistage sampling method. Segregated Roma colonies in these counties were identified previously by Roma field workers within the framework of a project of the University of Debrecen and the Hungarian Ministry of

Environmental Protection; ethnicity was self-declared (Kosa *et al.*, 2011). Segregated colonies exceeding 100 inhabitants were identified, resulting in 64 eligible colonies, of which 40 were randomly selected (25 colonies from Hajdú-Bihar county and 15 colonies from Szabolcs-Szatmár-Beregcounty). Twenty-five households were randomly chosen in each colony. The final sample consisted of 925 people aged 20 years or older. Full medical histories were taken and medical examinations conducted, involving weight, height, waist circumference and blood pressure measurements. Blood samples were collected for laboratory investigations (serum triglyceride, HDL-cholesterol, glucose levels) and DNA analysis. A total of 757 DNA samples were obtained from Roma individuals for genotyping (Kosa *et al.*, 2015).

An additional source of DNA samples was the recently launched 'Public Health Focused Model Programme for Organising Primary Care Services Backed by a Virtual Care Service Centre' developed in the framework of the Swiss-Hungarian Cooperation Programme. It is an ongoing pilot programme targeting Hungarian primary healthcare reform as a means to reduce social inequalities in health (Adany *et al.*, 2013). The programme is being implemented in the two most-disadvantaged regions of Hungary (North Hungary and the North Great Plain). In these regions, four GPs' clusters were established in the territories of Hajdú-Bihar, Borsod-Abaúj-Zemplén, Jász-Nagykun-Szolnok and Heves counties. As part of the health status assessment within this initiative a further 516 samples, representative of the Roma population living in Northern-East Hungary by age and gender, were collected. In total, 1273 HR DNA samples were used in the study.

The studies were approved by the Ethical Committee of the University of Debrecen, Medical Health Sciences Centre (reference No. 2462-2006) and by the Ethical Committee of the Hungarian Scientific Council on Health (Nos. NKFP/1/0003/2005; 8907-O/2011-EKU and 2213-5-2013/EKU). Written informed consent was gained from all participants in each study population.

SNP selection

A systematic literature search (PubMed) was conducted to identify SNPs most likely to be associated with the characteristics of alcohol consumption in genes coding for enzymes involved in alcohol metabolism (alcohol dehydrogenase: ADH1B, ADH1C, aldehyde dehydrogenase: ALDH1A1, ALDH2) and neurotransmitters in the dopaminergic (SLC6A3, DDC), GABAergic (GABRA2, GABRG1), serotonergic (HTR1B, MAOA, TPH2), cholinergic (CHRM2), glutamatergic (GRIN2A) and opioidergic (POMC, OPRM1, OPRK1) pathways, as well as one SNP in the gene encoding a protein involved in neural development and dendritic growth neurogenesis (BDNF) (Supplementary Table S1).

DNA preparation

DNA isolation was performed from ethylenediaminetetraacetic acid-anticoagulated blood samples using the MagNA Pure LC DNA Isolation Kit – Large Volume (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. A 500-µl aliquot of the initial blood sample was used, and the extracted DNA samples were eluted in 200 µl MagNA Pure LC DNA Isolation Kit-Large Volume Elution Buffer.

Genotype assessment

Genotyping for rs1693482 in ADH1C was performed by real-time polymerase chain reaction with a LightCycler 1.5 System (Roche Diagnostics, Mannheim, Germany), followed by a melting curve analysis. The reaction details are described elsewhere (Toth *et al.*, 2011). The primers and probes were produced by TIB Molbiol, Berlin, Germany.

Genotyping of the other selected 24 SNPs was performed by Mutation Analysis Core Facility (MAF) of Clinical Research Center, Karolinska University Hospital (Stockholm, Sweden) using the Mass Array platform with iPLEX Gold Chemistry. The validation, concordance analysis and quality control were conducted by MAF according to their protocol, resulting in a successful genotyping outcome for 4184 (1267 Roma and 2917 reference) DNA samples.

Statistical analyses

The data were analysed using STATA 9.0 Statistical software (StataCorp LP, College Station, TX, USA). The Mann–Whitney U and χ^2 tests were used to compare the mean age and sex distribution of the two study groups. The existence of Hardy–Weinberg equilibrium (HWE) and significant differences in the allele and genotype frequencies between the two populations were examined with the χ^2 test. After adjusting for multiple comparisons (Bonferroni correction), a p-threshold of 0.002 was applied; otherwise the threshold for significance was 0.05. Haploview software version 4.2 was applied to define the linkage between selected polymorphisms. D' values were calculated and visualized, pairwise linkage disequilibrium (LD) between the selected markers was analysed for both study populations, and haplotype blocks were identified.

Calculation of GRS and wGRS values

Unweighted and weighted genetic scores were calculated. Individuals with any missing SNP genotypes were excluded. In the first score, we assigned to each person a score based simply on the number of risk alleles carried. Thus risk allele homozygotes were coded by genotype '2', the heterozygotes by genotypes '1', while '0' indicated the absence of risk allele. When the effect allele was reported to be protective the coding was '0' for effect allele homozygotes and '2' for other allele homozygotes (Talmud *et al.*, 2010). By using these codes the simple count score (unweighted) was calculated as it is described by equation (1) in which G_i is the number of the risk alleles for the i-th SNP. This model sums up all risk alleles over all loci as a summary score assuming that all alleles have the same effect,

$$\mathrm{GRS} = \sum_{i=1}^{I} G_i \qquad (1)$$

To collect the effect size estimates of the SNPs included in the study a review of literature on large scale alcohol dependence genomic association studies and meta-analyses were done and resulted in the selection of 11 SNPs in eight candidate genes (POMC gene: rs1866146, rs6713532; BDNF gene: rs6265; GABRA2 gene: rs567926, rs279871, rs279858; ALDH1A1 gene: rs610529; ADH1Bgene: rs1229984; ADH7gene: rs1154458; CHRM2 gene: rs324650 and OPRM1 gene: rs1799971). The calculation of weighted genetic risk score is described by equations (2) and (3). In this weighted score, weights (w_{OR_i}) derived from the risk coefficient for each allele based on odds ratios reported in previous association studies were used (Wang *et al.*, 2004; Gratacos *et al.*, 2007; Lind *et al.*, 2008; Racz *et al.*, 2008; Chena *et al.*, 2012; Li *et al.*, 2012; Zintzaras, 2012; Park *et al.*, 2013). The log per-allele odds ratio from meta-analysis or from other independent data is considered

generally as a reasonable weight to apply to each SNP. These weights were (w_{OR_i}) multiplied by 0, 1 or 2 according to the number of effect alleles (X_i) carried by each person (Talmud *et al.*, 2010)

$$\text{wGRS} = \sum_{i=1}^{I} w_{OR\_i} X_i \quad (2)$$

$$w_{OR_i} = \log(OR_i) \quad (3)$$

Where effect estimates were reported for carriage of either one or two copies of each risk allele as a single group (OPRM1), the risk coefficients were multiplied by a score of 0 or 1 (Che and Motsinger-Reif, 2013). Student's *t*-test and Mann–Whitney U test were chosen to compare the distribution of GRS and wGRS, respectively.

To take account of confounding effects of gender and age on differences between study populations in terms of GRS, analysis of variance models were constructed, while for wGRS the study samples were divided into several strata defined by the potential confounding variables (groups of age: 20–29, 30–39, 40–49, 50–59, 60–X).

RESULTS

Allele and genotype comparisons between the study groups

Altogether, 25 SNPs were tested in the DNA samples of 1267 Roma people and 2917 individuals representing the general Hungarian population. The mean age was 40.67 ± 12.44 in the case of Roma and 45.43 ± 14.5 in the case of the general Hungarian population. The mean age of the two study groups was significantly different according to the Mann–Whitney U test ($P < 0.001$). The proportion of male individuals in Roma samples was significantly lower (HR: 0.42 vs HG: 0.49, $P < 0.01$).

The consistency with HWE of the observed genotype frequencies was tested for all SNPs. One SNP (TPH2 rs1386496) deviated from the HWE in both populations, and this polymorphism was excluded from further analysis. MAOA rs979606 deviated from HWE within the Roma group and DDC rs3779084 within the general Hungarian population group. These polymorphisms were retained in the analysis.

The allele frequencies of the study populations, which were calculated on the basis of the obtained genotype distributions, are shown in Table 1. ALDH2 rs671 and SLC6A3 rs6530 were monomorphic in both groups. After multiple test correction, the frequency of alleles, with 17 SNPs, remained significantly different ($P < 0.002$) between the two groups. Concerning the susceptibility alleles, rs7694646 (ADH4), rs1154400 (ADH5), rs279858 and rs567926 (GABRA2) and rs6985606 (OPRK1) were more frequent among the Hungarians (30.77% vs 26.68%, 34.25% vs 29.31%, 38.65% vs 27.17%, 39.77% vs 28.54% and 47.6% vs 33.99%, respectively), whereas higher allele frequencies were observed in the Roma population for rs1799971 (OPRM1), rs1866146 and rs6713532 (POMC) (19.90% vs 13.04%, 52.35% vs 39.37% and 40.17% vs 26.15%, respectively). Three protective SNPs (rs1229984 in ADH1B, rs130058 in HTR1B and rs979606 in MAOA) were more frequent among Roma (12.87% vs 8.0%, 35.59% vs 27.88% and 41.93% vs 31.66%, respectively), and six others (rs1800759 in ADH4B, rs610529 in ALDH1A1, rs6265 in BDNF, rs279871 in GABRA2, rs2221020 in GABRG1 and rs2072450 in GRIN2A) were more frequent among Hungarians (40.79% vs 35.7%,

Table 1. Susceptibility (A) and protective (B) allele frequencies (%) in the general Hungarian and Roma populations

Gene	SNP	Described association	Roma ($N = 1267$)	Hungarians ($N = 2917$)	*P* value
(A)					
ADH1C	rs1693482	Alcoholism, maximum number of drinks/day alcohol dependence	37.37	36.99	0.744
ADH4*	**rs7694646**	Alcohol dependence, drug dependence, cocaine dependence	26.68	**30.77**	<0.001
ADH5	**rs1154400**	Alcohol dependence	29.31	**34.25**	<0.001
ADH7	rs1154458	Alcoholism	39.39	41.29	0.201
GABRA2	**rs279858**	Alcohol dependence	27.17	**38.65**	<0.001
	rs567926	Alcohol dependence	28.54	**39.77**	<0.001
SLC6A3	rs463379	Harmful alcohol consumption	20.97	22.45	0.0536
DDC	rs3779084	Alcohol consumption quantity	18.9	20.14	0.012
OPRK1	**rs6985606**	Alcohol dependence	33.99	**47.6**	<0.001
OPRM1	**rs1799971**	Alcohol dependence, alcohol use disorder	**19.90**	13.04	<0.001
POMC	**rs1866146**	Alcohol dependence	**52.35**	39.37	<0.001
	rs6713532	Alcohol dependence	**40.17**	26.15	<0.001
(B)					
ADH1B	**rs1229984**	Alcoholism, alcohol drinking habits	**12.87**	8.00	<0.001
ADH4	**rs1800759**	Alcohol dependence	35.70	**40.79**	<0.001
ALDH1A1	**rs610529**	Harmful alcohol consumption	31.51	**45.05**	<0.001
ALDH2	rs671	Alcoholism	0	0	
BDNF	**rs6265**	Substance-related disorders	11.34	**19.88**	<0.001
GABRA2	**rs279871**	Alcohol dependence	27.19	**38.67**	<0.001
GABRG1	**rs2221020**	Alcoholism	36.33	**48.23**	<0.001
SLC6A3	rs6530	Harmful alcohol consumption	0	0	
HTR1B	**rs130058**	Alcohol dependence	**35.59**	27.88	<0.001
MAOA	**rs979606**	Alcohol dependence	**41.93**	31.66	<0.001
GRIN2A	**rs2072450**	Alcohol dependence	9.04	**13.72**	<0.001
CHRM2	rs324650	Alcohol dependence	49.51	48.80	0.120

*SNPs with bold character showed highly significantly ($P < 0.0002$) different frequencies between the two populations after multiple test correction. The tests were carried out by using allele frequency data (%) obtained for the study populations.

45.05% vs 31.51%, 19.88% vs 11.34%, 38.67% vs 27.19%, 48.23% vs 36.33% and 13.72% vs 9.04%, respectively).

Comparison of GRS

The GRS for the HR population, based on all SNPs included in the study (Table 1), was calculated for 1090 individuals and ranged from 13 to 28. The GRS for HG subjects, based on all SNPs, was calculated for 2607 individuals and ranged from 12 to 29. The GRS was normally distributed for each study group. The mean gene count score was 20.73 (SD 2.44) in people of the HR group and 21.02 (SD 2.49) in those in the HG group. Distribution of gene count scores showed significant difference between study groups using the Student's *t*-test ($P = 0.0013$).

Thirty per cent of subjects in the HR population were in the bottom fifth (GRS $\leq$ 19) of the gene count score compared with 27.1% of those in HG population. Thirteen per cent of people in HR group were in the top fifth (GRS $\geq$ 24) of the GRS compared with 16.9% of those in HG population ($P = 0.017$), i.e. the distribution of unweighted GRS was found to be left-shifted in the HR population compared to the HG population (Fig. 1).

Since the *POMC* SNPs and *GABRA2* SNPs were in strong LD (see Supplementary Figure S1) only rs1866146 of POMC and rs567926 of GABRA2 were chosen to enter the wGRS, resulting in a wGRS composed of eight SNPs. There were no available effect size estimates for SNPs of ADH4 and ADH5 genes so those were not included in the GRS function. Effect size estimates of the eight SNPs are described in Table 2.

The median wGRS of HR vs HG were 0.53 (interquartile range (IQR): 0.33–0.76) vs 0.66 (IQR: 0.45–0.85) The lower HR median suggests that the HR population have lower weighted genetic risk score on average ($P = 3.33 \times 10^{-27}$). Data in wGRS were non-normally distributed with skewness of −0.18 (SE = 0.07) and kurtosis of −0.13 (SE = 0.14) in HR population and with skewness of −0.12 (SE = 0.05) and kurtosis of −0.17 (SE = 0.1) in HG population. The side-by-side boxplots of the wGRS estimated from study populations are shown in Fig.2.

Analyses adjusted for confounders only where records were complete. Adjustment for age and gender did not change the pattern of GRS ($P = 0.0236$). The stratum specific estimates of wGRS are shown in Table 3. The results are consistent across the strata since the stratum-specific effect estimates of wGRS (median and IQR)

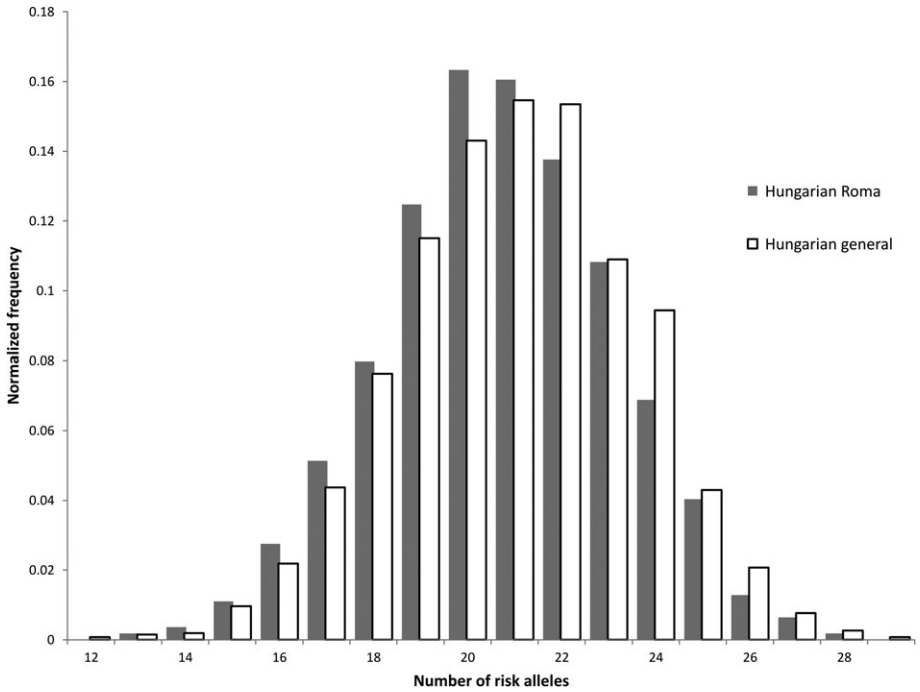

Fig. 1. Distribution of the unweighted genetic risk score for the HG and Roma populations. Distribution of GRS in the Hungarian Roma (HR, grey bars) and Hungarian general (HG, white bars) populations was differed ($P = 0.0013$, Student's *t*-test).

Table 2. All SNPs considered in the wGRS, their genes, and effect alleles being in association with alcohol dependence

SNP	Gene symbol	Effect allele	OR	95% CI	Weight	Publication for effect alleles
rs1866146	POMC	G	0.997	0.77–1.3	−0.001	(Racz *et al.*, 2008)
rs6265	BDNF	T	0.79	0.67–0.94	−0.102	(Gratacos *et al.*, 2007)
rs567926	GABRA2	G	1.35	1.13–1.6	0.13	(Zintzaras, 2012)
rs610529	ALDH1A1	G	0.65	0.43–0.98	−0.187	(Lind *et al.*, 2008)
rs1229984	ADH1B	C	2.06	1.84–2.31	0.314	(Li *et al.*, 2011)
rs1154458	ADH7	C	1.18	0.98–1.42	0.072	(Park *et al.*, 2013)
rs324650	CHRM2	A	1.87	1.05–3.33	0.272	(Wang *et al.*, 2004)
rs1799971	OPRM1	GA+GG vs AA	1.11	0.79–1.55	0.045	(Chena *et al.*, 2012)

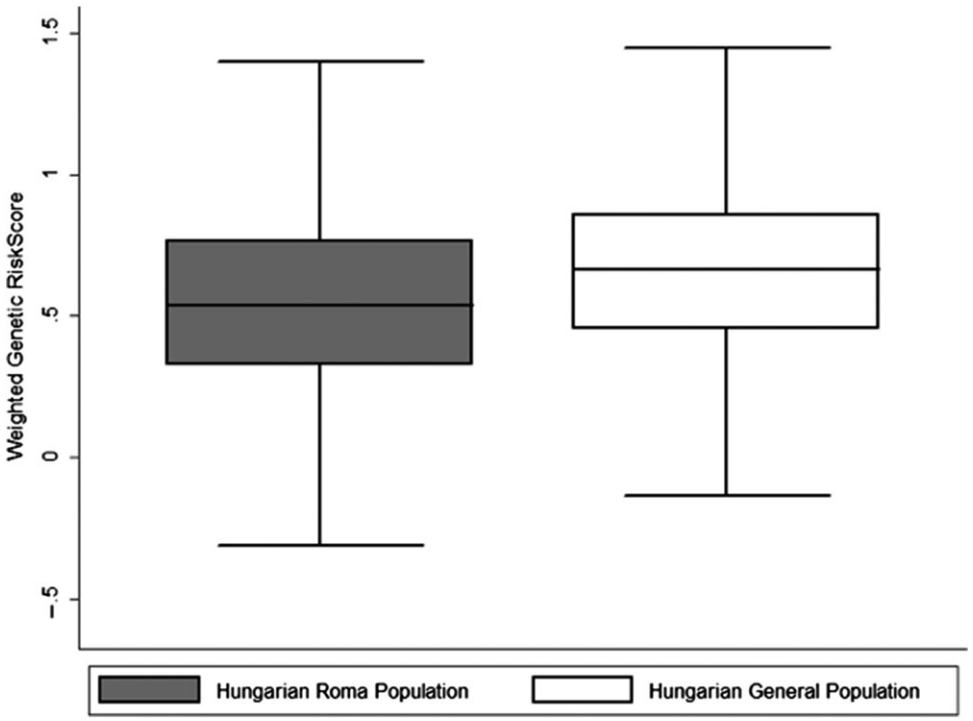

Fig. 2. Boxplots of weighted genetic risk score in HG and Roma populations. GRS analysis was performed using eight SNPs. SNPs were weighted based on the effect size from original publications.

Table 3. Stratum-specific estimates of wGRS in the study populations

Age groups	*N*	Median	IQR	*N*	Median	IQR	*P*
	HR men			HG men			
20–29	96	0.54	0.35–0.69	208	0.62	0.45–0.85	**0.002**
30–39	102	0.53	0.31–0.79	208	0.68	0.46–0.84	**0.007**
40–49	96	0.53	0.31–0.73	251	0.65	0.42–0.88	**0.0005**
50–59	63	0.52	0.35–0.73	253	0.67	0.44–0.87	**0.0126**
60	25	0.51	0.35–0.66	160	0.67	0.4–0.88	**0.0072**
	HR women			HG women			
20–29	128	0.5	0.29–0.72	202	0.68	0.47–0.87	**0.00001**
30–39	157	0.57	0.32–0.78	243	0.68	0.46–0.87	**0.0006**
40–49	144	0.57	0.35–0.77	259	0.68	0.46–0.87	**0.0008**
50–59	102	0.46	0.38–0.82	312	0.63	0.42–0.84	**0.0002**
60	35	0.62	0.38–0.85	238	0.69	0.46–0.86	**0.2627**

HR, Hungarian Roma; HG, Hungarian general; *N*, sample size of subgroups; IQR, interquartile range.

Significant *P*-values are shown in boldfaced ($P < 0.05$).

show that the Roma population have lower weighted genetic risk score on average, independent of age and gender.

DISCUSSION

In the majority of Central and Eastern European countries, Roma represent over 5% of the population (Bernath, 2009). Their health status is significantly worse than that of the majority population. It is widely accepted that a range of factors contribute to this situation, including material deprivation and hazardous behaviours, but attention has also focussed on possible genetic explanations, for alcohol consumption, reviewed in (Buscemi and Turchi, 2012), encouraged by recognition of their high consanguinity rate (Kalaydieva *et al.*, 2001).

The aim of our study was to determine whether a genetic susceptibility does indeed contribute to the higher prevalence of harmful alcohol consumption in the Roma population.This is the first study to investigate several susceptible loci for harmful alcohol consumption among Roma living in segregated colonies and to compare them with data for the majority Hungarian population. The SNPs in the current study encompass genes encoding proteins involved in alcohol metabolism and in several neurotransmission systems linked to alcohol consumption.

Twenty-five polymorphisms associated with alcohol drinking habits were genotyped, and differences in 17 polymorphisms remained significant after multiple test correction. The susceptibility alleles of rs7694646, rs1154400, rs279858, rs567926 and rs6985606 were more frequent among Hungarians, whereas rs1799971, rs1866146 and rs6713532 were more frequent in the Roma population. Among the protective SNPs, the rs1800759, rs610529, rs6265, rs279871, rs2221020 and rs2072450 polymorphisms were found to be more frequent among Hungarians; with rs1229984, rs130058 and rs979606, higher allele frequencies were observed in the Roma population.

Despite these differences, the allele frequency of the ALDH2 polymorphism was concordant, that is, the allele was found to be monomorphic within both study populations. This result is in agreement with dbSNP data for the Central European populations, i.e. the frequency of this protective allele in the HapMap-CEU population is 0%. An SNP of the aldehyde dehydrogenase two gene (rs671) has a strong protective effect against alcohol dependence and other alcohol-induced diseases in Asians (Li *et al.*, 2012), leading to alcohol sensitivity as a result of an enzyme with low—if any—catalytic activity, with consequently strongly delayed acetaldehyde oxidation.

Our results show differences in allele frequencies in the vast majority of the selected polymorphisms between the general Hungarian and Roma populations. In contrast, the allele frequencies of ALDH2 rs671 and SLC6A3 rs6530 were identical in the two study samples.

An association with harmful alcohol consumption was confirmed for five of the polymorphisms investigated by a meta-analysis. Results of a meta-analysis of association studies published in the previous 21 years showed a strong association of ADH1B rs1229984 with alcohol use disorders (dependence and abuse), with an allelic odds ratio (OR) of 2.06 (95% confidence interval (CI):1.84–2.31) (Li *et al.*, 2011). The association of BDNF rs6265 was tested in a meta-analysis of individual case-control studies with different categories of mental disorders (eating disorders, substance-related disorders, mood disorders and schizophrenia), and in the case of substance-related disorders, a 21% protective effect was estimated (Gratacos *et al.*, 2007). Concerning alcoholism, a generalized odds ratio of 1.27 (95% CI: 1.01–1.60) for GABRA2 rs279858 and an allele-specific OR of 1.24 (95% CI:1.06–1.46) for GABRA2 rs567926 were estimated (Zintzaras, 2012). Ethnicity-specific meta-analyses of the variant rs1799971 (OPRM1) revealed a significant association with alcohol dependence in Asians (OR: 1.73) but not in Caucasians (Chena *et al.*, 2012).

In addition, our study introduced the concept of GRS, a means to aggregate information from multiple gene variants into a single score. Our results indicate that both GRS and wGRS were significantly lower in the HR population even after controlling for the effects of possible confounding factors. Although a relatively small set of SNPs was selected for inclusion in GRS, the results imply that it is possible to derive a genetic score that has at least some discriminative ability. The results strongly suggest that no genetic susceptibility underlies the high prevalence of harmful alcohol consumption in the Roma population and, instead, it is environmental, including socio-economic and cultural factors that explain differences in behaviour.

The obvious limitation of the current study is that the Roma study population was not representative of the overall Roma population in Hungary. By design, the analysis excluded Roma who have, to various degrees, assimilated with the Hungarian population. However, because many people are reluctant to self-define their ethnicity as Roma, this constraint would be very difficult address. It is also important to note that the representative sample of the general Hungarian population included some people who are Roma. It is possible that their inclusion resulted in a slight underestimation of the differences between the populations.

The genotyping technology applied in the current study is a high-throughput method for SNP genotyping, but it does not allow the analysis of variable number tandem repeat (VNTR) polymorphisms. However, it would be advantageous to extend our study towards investigation of VNTR polymorphisms, since convincing findings in the literature show that some VNTR polymorphisms may affect alcohol drinking phenotypes (Catanzaro *et al.*, 2012; Vasconcelos *et al.*, 2015).

This descriptive study assumes that the effect size of SNPs studied was similar in our study populations than it was found in the populations previously studied and the effect size described for. Although on the basis of data showing concordance on different populations (Zintzaras, 2012; Li *et al.*, 2014) there is no reason to suppose that the effect size would be different in the populations we studied, a further study may answer the question whether there is any—even minor—difference in the degree of correlation between unweighted and wGRS and alcohol consumption (prevalence of harmful alcohol consumption and amount of alcohol consumed) in the HG and Roma populations.

The harmful alcohol consumption of Roma does not appear to be linked to genetic factors but may occur as a result of different environmental exposures. Highlighting the role of environmental and social factors has great importance in planning of hopefully effective public health interventions among the Roma population. Nevertheless, it is important to emphasize that further genetic research on minority populations is needed.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Alcohol and Alcoholism* online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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