Distinct penetrance of obesity associated susceptibility alleles in the Hungarian general and Roma populations.

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TITLE PAGE

Distinct penetrance of obesity associated susceptibility alleles in the Hungarian general and Roma populations.

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Short title

Penetrance of obesity-related polymorphisms

Keywords

body mass index, FTO, gene variants, obesity, Roma, Gypsy, genetic risk score

ABSTRACT

Aims: The aim of our study was to explore differences in genetic predisposition to obesity between the Hungarian general and Roma populations.

Methods: A total of 1152 samples from the Hungarian Roma population and 1743 samples from the Hungarian general population were genotyped for 20 single nucleotide polymorphisms (SNPs) associated with the risk of obesity. Two types of multilocus genetic risk scores were constructed to estimate the combined effect of selected SNPs.

Results: Risk allele frequencies differed significantly between the two populations for 11 SNPs, with no enrichment in any of the two study groups. Variants (rs1558902, rs1121980, rs9939609 and rs9941349) in the fat mass and obesity-associated (FTO) gene exhibited strong but ethnicity-independent association with obesity. Genetic risk scores showed stronger associations with obesity in the Roma population compared with the Hungarian general population; however, without significant gene-population interaction.

Conclusion: Differences in obesity prevalence between the Hungarian general and Hungarian Roma populations could not be explained by their distinct genetic susceptibility, rather by ethnicity-related environmental and behavioural factors. Nonetheless, particular gene-environment interactions might contribute to the distinct penetrance of the obesity-associated genetic factors in populations of different ethnic backgrounds.

INTRODUCTION

Obesity, one of the strongest cardiovascular risk factors, is a serious public health challenge for the 21st century. Recently, a health examination survey revealed 26.2% and 30.4% prevalence of obesity in the Hungarian adult male and female populations, respectively [1]. These values ranked Hungary as the number one “most obese member state” in the European Union [2], which clearly indicates the need of collecting more information and conducting studies about the potential factors influencing obesity on the Hungarian population.

The population of Hungary is reasonably diverse with many ethnic minorities, among them the Roma (Gypsy), who account for approximately 7% of the population [3]. Hungarians originate from the eastern side of the Ural Mountains, whereas the Roma people are from the Indian subcontinent. Genetic ancestry studies have provided sufficient evidence that the Roma’s ancestors migrated to Europe about 850 years ago, with a severe founder event and a very little genetic admixture occurring with populations encountered on the way. After settled in the Balkans, this initial population fragmented and dispersed throughout Europe resulting in the mixture of their genomes with those of various European ethnic groups, including Hungarians. Despite the fact that today’s Hungarian Gypsies have genetic variants typical of India and Europe, the two populations fundamentally differ from each other in their genetic architectures [4,5]. Recent genetic epidemiological studies also indicated significant genetic differences between the Hungarian general (HG) and Hungarian Roma (HR) populations [6-9].

Generally, the Roma minority is considered to possess unfavourable health, and live in a less healthy environment (colonies) than the majority of the population, not only in Hungary but also in other European countries [10]. The low educational status, high unemployment and poverty rates may further contribute to ill health among the Roma [11]. Because of the generally high prevalence of adverse risk factors such as obesity, metabolic syndrome, hypertension, smoking and alcohol consumption, this population is at an increased risk of diabetes, cardiovascular diseases and consequently, early mortality [12-14]. Studies investigating anthropometric features in Roma populations in Slovakia, Spain and Serbia have suggested that the prevalence of obesity and BMI are higher among the Roma than in the general population [15-17].

Environmental factors play a crucial role in the development of obesity; however, there is no doubt that it is also influenced by genetics [18]. In the past few years, genome-wide association studies (GWAS) identified dozens of polymorphisms in genes or gene regulatory regions involved in energy homeostasis, appetite regulation, lipid and carbohydrate metabolism that have cumulative effects on body weight [19]. However, most of these studies were performed in populations of European descent. The results of genetic replication studies investigating the relationship between SNPs identified by GWAS and obesity related traits were not always consistent. The reasons could be that these studies were conducted in populations with different environmental characteristics and/or were performed on samples of relatively small size. Furthermore, there is a considerable genetic heterogeneity across populations in terms of allele frequency, linkage disequilibrium (LD) and haplotype structure that can arise as a result of multiple factors such as genetic drift, mutations, and natural selection. If a true functional SNP is in strong LD with the lead SNP in the population in which the GWAS was performed, but not in a different population, then the lead SNP will not be associated with the phenotype in this second population. Thus, inter-ethnic differences in LD block patterns limit the generalizability of GWAS association signals across populations [20]. Consequently, differences within and among human communities in terms of susceptibility to obesity could be explained by the different genetic background of a population and/or its interaction with various environmental exposures [21]. Moreover, it has not been clarified how much these SNPs contribute to obesity risk and related quantitative factors if combined and whether they can be considered as possible predictors of obesity, which might have implications for early prevention and intervention.

The present study was designed to explore differences in genetic predisposition to obesity between the HG and HR populations by investigating the distribution of 20 recently identified obesity-susceptibility loci, as well as their association, individually and in combination, with the prevalence of obesity.

SUBJECTS AND METHODS

Study sample of the Hungarian general population

In 2006, a cross-sectional study was conducted based on the General Practitioners' Morbidity Sentinel Stations Programme to define the prevalence of metabolic syndrome in the Hungarian population [22]. The source population of the study included all individuals aged 20-69 years who were registered by the 59 participating general practitioners (GPs) of eight Hungarian counties. The study population was randomly selected proportional to the size of the practices to represent the Hungarian adult population based on geographic, age and sex distributions. GPs performed physical examinations (weight, height, blood pressure measurements) and collected blood samples for laboratory investigations and DNA isolation. Information on the socio-demographic status, family history and lifestyle were also collected using self-administered questionnaires. In the framework of this study, 1783 DNA samples were acquired.

Study sample of the Hungarian Roma population

The study population was enrolled from counties in Northeast Hungary, the area where the Roma are most prevalent and the majority of segregated Roma colonies are located. The participants were interviewed by Roma field workers in the framework of two surveys conducted recently [23,24]. The ethnicity of the participants was assessed by self-declaration. Ninety-two segregated colonies with more than 100 inhabitants were considered in the surveys, of which 65 were randomly selected using the GPs' validated household lists. Afterwards, 25 households in each colony were randomly chosen, and one person from each household was invited to the GPs. As a part of the health examination survey, physical examinations (weight, height, blood pressure measurements) were performed, and whole blood samples were taken for routine laboratory tests and DNA extraction. Data on socio-demographic factors, lifestyle and self-assessed health status were obtained using interviewer assisted questionnaires. A total of 1170 DNA samples from 20 to 69-year-old Roma individuals were used for genotyping.

Genotyping

Genomic DNA was extracted from EDTA-coagulated whole blood samples using the MagNa Pure LC DNA Isolation Kit – Large Volume (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Genotyping was performed in the Mutation Analysis Core Facility (MAF) of the Karolinska University Hospital (Stockholm, Sweden) using the Sequenom MassARRAY platform (Sequenom Inc., San Diego, CA, USA) with iPLEX Gold chemistry [25]. Validation, concordance analysis and quality control were conducted by the MAF according to their protocols, resulting in a successful genotyping outcome for 2895 (1743 Hungarian and 1152 Roma) DNA samples.

SNP selection and computation of genetic risk score

A systematic literature review was undertaken in the PubMed database with an emphasis on GWAS to identify SNPs that were found to play a significant role in the development of obesity. Given that most GWAS have described common obesity-associated gene loci in European-descent populations, multi-ancestral meta-analyses were also examined to discover gene polymorphisms showing association with obesity in populations with different ethnic background. SNP selection was based on HapMap data for a European ancestry population sample (CEU) with a minor-allele frequency >5% (http://www.hapmap.org) and previously published results showing significant associations between these polymorphisms and obesity related traits. The top 20 SNPs, identified by the magnitude of the described association (odds ratio or beta value), were selected for further genotyping (table 1).

Two types of multilocus genetic risk score (GRS) were constructed for each individual in the study populations. GRS1 incorporated all independent SNPs, while GRS2 included only SNPs associated with obesity with a $p<0.05$ in the present study. Only one representative SNP was selected from a linkage disequilibrium block if there were absolute LDs with $r^2 \geq 0.2$. GRS was defined by a simple count method (i.e., by summation of the number of risk alleles) assuming additive genetic model in which each risk allele contributes equally to the risk for obesity, and summed over all the SNPs in the set.

Statistical analysis

Data were analysed using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink) [37], Stata v12.0 (StataCorp LP, College

Station, TX, USA) and CaTs [38] software. Departure from Hardy-Weinberg equilibrium (HWE), as well as differences in obesity prevalence and the allele frequencies of individual SNPs were calculated with Pearson's chi-squared ($\chi 2$) test. Distributions of risk allele frequencies and genetic risk scores in the two study populations were compared by binomial distribution and Kolmogorov–Smirnov tests, respectively. To estimate the risk of obesity ($18.5 \leq \text{BMI} < 25$ kg/m^2 versus $\text{BMI} \geq 30$ kg/m^2) for each SNP, we calculated the odds ratio (OR) and 95% confidence intervals (95% CI) using multivariate logistic regression. To ensure the highest statistical power, data of the two study groups were analysed jointly. In the regression analyses, the additive model was used, and it was adjusted for covariates including age, gender and ethnicity. The possible interethnic differences in the association patterns were assessed by introducing a multiplicative interaction term in the model assuming additive allelic effect and dichotomus ethnicity. The threshold for statistical significance was 0.05. The power to detect association was calculated using the minor allele frequency of each SNP in our case-control samples and the effect size calculated in the allelic association analysis.

In a further analysis, we evaluated the association of genetic risk scores with obesity risk ($18.5 \leq \text{BMI} < 25$ kg/m^2 versus $\text{BMI} \geq 30$ kg/m^2) using multivariate logistic regression models. Interactions between genetic effects and ethnicity were analysed by introducing the corresponding interaction term into the models as well.

RESULTS

Demographic characteristics and distribution of obesity prevalence in the study populations

Differences in demographic characteristics, as well as in the distribution of obesity prevalence were observed between the HG and HR population samples (table 2 and fig. 1). The female proportion was higher in the HR (59.6%) than in the HG (53.1%) population, although women were the majority in both study samples in all age groups except the 20-29 year old HG group. The age distribution of the HR sample was slightly shifted towards the younger age groups compared to the HG sample. The average ages in the HG and HR populations were 46 and 41.4 years, respectively.

The prevalence of obesity in both men and women were higher in the HG than in the HR population with significant difference only between HG and HR males. Obesity was less frequently observed among men than women in both study groups. The age- and sex-specific prevalence of obesity followed a similar pattern in younger age groups in both samples; however, in age groups older than 40 – 49 years, the proportion of HR people with obesity, especially of males, was strongly reduced.

Frequency and impact of SNPs in the study populations

All SNPs were in Hardy-Weinberg equilibrium in both study groups. Eleven obesity-predisposing polymorphisms showed significant difference in the risk allele frequencies between the two study groups (table 3). Six of them had significantly higher risk allele frequency in the HR population. Nonetheless, when compared the risk allele distribution of the entire SNP panel between the two study populations, none of them were significantly enriched by obesity associated genetic markers.

The *FTO* gene variants, namely rs1558902, rs1121980, rs9939609 and rs9941349, showed a strong association with obesity in the joint analysis, indicated by the odds ratios of 1.34 (95% CI=1.17-1.54, $p<0.001$), 1.35 (95% CI=1.18-1.56, $p<0.001$), 1.35 (95% CI=1.17-1.55, $p<0.001$) and 1.41 (95% CI=1.22-1.62, $p<0.001$). Further association signals could be replicated for SNPs in the *GNPDA2* (rs10938397), *NPY* (rs16139), *FTO* (rs6499640) and *MC4R* (rs17782313 and rs12970134) genes. The sample size of the joint analysis permitted relatively high power (86 to 100%) for these SNPs to detect association when allowing a false-positive rate of 0.05; however, the sample size showed rather low power for the other SNPs (5 to 64%) suggesting that they have almost no or a rather weak contribution to the development of obesity in the study populations. In addition, significant interaction between genetic variants and ethnicity could be observed only for rs1801282 in the *PPARG* gene, indicating virtually the absence of ethnicity-related genetic predisposition to obesity in our study sample (table 4).

Obesity according to genetic risk score

A set of fourteen independent variants was used to calculate GRS1, while GRS2 were constructed by using four independent SNPs that were associated significantly with obesity in the target samples. SNPs were considered as independent if the LD between them had $r^2<0.2$. Individual GRS1 and GRS2 ranged from 5 to 20 and 0 to 6 among our study subjects, respectively, with higher scores indicating a higher genetic predisposition to obesity. Individuals with less than seven or more than nineteen GRS1 were aggregated because of the small number of observations in those groups. GRSs followed normal distributions which were not significantly different (GRS1 p=0.3; GRS2 p=0.872) between the two study groups (fig. 2).

Significant positive linear relationships were observed between both GRSs and the studied phenotype (fig. 3). GRS2 displayed higher predictive value than GRS1, indicated by higher odds ratios. Each additional GRS1 and GRS2 unit, corresponding to one risk allele, was significantly associated with a 3% (95% CI=1.01-1.05, p=0.003) and 19% (95% CI=1.1-1.28, p<0.001) increase in risk of obesity if the two populations were analysed together. Although significant interaction between the genetic risk and ethnicity could not be observed, both GRSs exhibited different association patterns between the HG and HR populations (table 5). Stratification by ethnicity revealed that a one-unit increment in GRS1 and GRS2 in the HR individuals was significantly associated with a 12% (95% CI=1.05-1.18, p=0.001) and 27% (95% CI=1.12-1.45, p<0.001) higher risk of obesity. The correlation between GRSs and obesity was less pronounced in the HG study sample.

DISCUSSION

There is general agreement that in a genetically isolated population with low environmental variability, such as the Roma, the founder effect and genetic drift can cause an increase in the frequency of particular alleles [39]. In this study, we reported significantly higher risk allele frequencies for SNPs in *NEGR1* (rs2815752), *TMEM18* (rs2867125 and rs6548238), *BDNF* (rs925946 and rs6265) and *UCP* (rs660339) genes but significantly lower risk allele frequencies for variants in *GNPDA2* (rs10938397), *NPY* (rs16139), *PPARG* (rs1801282) and *FTO* (rs6499640 and rs1558902) genes in HR compared with the HG population. By comparing the distribution of investigated risk allele variants, increased genetic predisposition to obesity in the Roma could not be confirmed in this study.

The SNPs with the most robust effect on obesity in our target populations were found in the intronic region of the *FTO* gene, which is mainly involved in the regulation of energy homeostasis and body composition; however, despite intensive research, its exact pathomechanism is still not completely understood [40]. The impacts of *FTO* gene polymorphisms found in this study were consistent with previous reports not only in European-descent populations [28,35,36], but also in the Slovakian Roma [41], indicating that the effect of these susceptibility loci for obesity persists across populations with various ancestral origins. Nonetheless, common variants in the *FTO* gene did not show an obvious association with obesity-related phenotypes in Spanish Roma populations [42].

In the analysis of the combined effect of the selected variants, GRS1 and GRS2 yielded similar results; both of them displayed significant positive correlation with the obesity. The inclusion of only a subset of SNPs that were found to have significant effect in this study has allowed for creating a genetic risk score profile (GRS2) with increased predictive validity, reflected by the association measures. The analysis of GRSs stratified by ethnic groups demonstrated differences between the two study populations. Whereas both GRSs revealed a stronger relationship with obesity risk in the HR population, these associations were weakened in the HG sample, indicating difference in the penetrance of obesity-related variants in our study populations. This finding is an interesting observation because significant interactions of individual SNPs or GRSs with ethnicity could not be established if the two populations were analysed jointly.

Despite the lack of marked heterogeneity of genetic risk to develop obesity in the study populations, excess body weight, surprisingly, was less frequently observed among Roma than in the HG population, consistent with a previous report [43]. This observation may be explained in part by the Roma's generally deprived socio-economic status [44], which imposes limits on the transition from their traditional lifestyle to a westernised one. Although their nutritional characteristics (consumption of fruits and vegetables and type of fat used for cooking) were reported to be generally unhealthier than that of HG population [43], their overall energy intake does not appear to reach the degree with which the HG population can be characterised. Regardless of dietary intake, it is important to consider that other ethnic-specific lifestyle components (alcohol consumption, physical activity levels, or smoking habits) could also act as modifiers of the effect of a given genetic variant on body weight [18]. Our findings suggest that the high environmental variability associated with more favourable socioeconomic conditions, and the cultural habits resulting in abundant energy intake of the Hungarian general population highlight the importance of environmental and lifestyle factors in the attenuation of penetrance and expression of obesity predisposing genetic variants. These facts may be possible explanations for the phenotypic differences observed between the two populations.

Several limitations of the present investigation need to be considered. An important point for discussion is that the HR study population is not fully representative of the overall Roma population in Hungary. Due to the sampling design, those Roma who have assimilated within the Hungarian general population or declared themselves as Hungarians were not included in the HR population survey. Moreover, it is also necessary to note that the representative sample of the Hungarian general population included some people who are Roma because the data collection of HG sample did not involve the ascertainment of ethnicity. Similarly, the presence of participants with mixed Roma/non-Roma ancestry could not be excluded in the recruitment of both study populations. Their inclusion may result in a slight underestimation of the differences between the populations. Furthermore, the lack of information both on environmental factors, including physical activity and dietary habits, and gene-environment interactions is a limitation of our work. Lastly, we acknowledge that the impact of SNPs selected from meta-analyses has not been confirmed by GWAS and the possible absence of their association signals may decrease the predictive value of GRS computed in our study. On the other hand, a series of GWAS-established loci were not included in our analysis but could also contribute to the phenotypic differences between the two populations.

In conclusion, this study is the first to investigate the joint effect of obesity-related gene variants among Roma living in segregated colonies and to compare them with data of the majority of the population. Our findings suggest that the differences in obesity prevalence between the Hungarian general and Hungarian Roma populations could be primarily explained by ethnicity-related environmental and behavioural factors, not by genetics. Nevertheless, particular gene-environment interactions might contribute to the distinct penetrance of the obesity-associated genetic factors in populations of different ethnic backgrounds.

ACKNOWLEDGEMENT

This research was supported by the TÁMOP 4.2.2.A-11/1/KONV-2012-0031 ‘Social Renewal Operational Programme – IGEN-HUNGARIAN’ and the GINOP-2.3.2-15-2016-00005 project. The projects were co-financed by the European Union and the European Regional Development Fund.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethical Committee of the University of Debrecen (reference No. 2462-2006), the Ethical Committee of the Hungarian Scientific Council on Health (reference No. 8907-O/2011-EKU, 285/PI/11) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.

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Table 1. Obesity predisposing SNPs selected for the study.

SNP	*Gene*		*Described association*		*Reference*
	Name	*Abbr.*	*Odds ratio of obesity*	*Mean difference in BMI (β)*	
rs1137101	leptin receptor	*LEPR*	1.13	-	Paracchini *et al.* [26]
rs2815752	neuronal growth regulator	*NEGR1*	-	0.13	Speliotes *et al.* [27]
rs2867125	transmembrane protein 18	*TMEM18*	-	0.31	Speliotes *et al.* [27]
rs6548238			-	0.26	Willer *et al.* [28]
rs1801282	peroxisome proliferator-activated receptor gamma	*PPARG*	-	0.06	Galbete *et al.* [29]
rs2241766	adiponectin, C1Q and collagen domain containing	*ADIPOQ*	1.39	-	Wu *et al.* [30]
rs1501299			0.89	-	Lu *et al.* [31]
rs10938397	glucosamine-6-phosphate deaminase 2	*GNPDA2*	-	0.18	Speliotes *et al.* [27]
rs16139	neuropeptide Y	*NPY*	-	0.58	Yeung *et al.* [32]
rs925946	brain-derived neurotrophic factor	*BDNF*	1.11	-	Thorleifsson *et al.* [33]
rs6265			1.12	-	Thorleifsson *et al.* [33]
rs660339	uncoupling protein 2 (mitochondrial, proton carrier)	*UCP2*	-	0.39	Brondani *et al.* [34]
rs659366			1.06	-	Brondani *et al.* [34]
rs6499640	fat mass and obesity-associated protein	*FTO*	1.16	-	Thorleifsson *et al.* [33]
rs1558902			-	0.39	Speliotes *et al.* [27]
rs1121980			-	0.31	Vimaleswaran *et al.* [35]
rs9939609			-	0.33	Willer *et al.* [28]
rs9941349			-	0.56	Cronin *et al.* [36]
rs17782313	melanocortin 4 receptor	*MC4R*	-	0.2	Willer *et al.* [28]
rs12970134			1.12	-	Thorleifsson *et al.* [30]

Table 2. Demographic characteristics of the study populations.

		Hungarian General Population							*Hungarian Roma Population*						
		Male		*Female*		*Total*		*Female / Male Ratio*	*Male*		*Female*		*Total*		*Female / Male Ratio*
Number of subjects (%)		818	(100)	925	(100)	1743	(100)	1.13	465	(100)	687	(100)	1152	(100)	1.48
Age groups (years)	*20 - 29*	147	(17.9)	137	(14.8)	284	(16.2)	0.93	106	(22.8)	138	(20.1)	244	(21.1)	1.3
	30 - 39	155	(18.9)	160	(17.3)	315	(18.0)	1.03	111	(23.8)	174	(25.3)	285	(24.7)	1.57
	40 - 49	167	(20.4)	202	(21.8)	369	(21.1)	1.21	119	(25.5)	191	(27.8)	310	(26.9)	1.61
	50 - 59	203	(24.8)	236	(25.5)	439	(25.1)	1.16	91	(19.5)	140	(20.3)	231	(20.1)	1.54
	60 - 69	146	(17.8)	190	(20.5)	336	(19.2)	1.3	38	(8.2)	44	(6.4)	82	(7.1)	1.16

Table 3. Allele frequencies of individual SNPs in the Hungarian general and Roma populations.

Gene / SNP	*Risk Allele*	*Risk Allele Frequency*		
		HG	*HR*	$P_{\text{for difference}}$
LEPR				
rs1137101	G	0.46	0.44	0.267
NEGR1				
rs2815752	A	0.66	0.73	<0.001
TMEM18				
rs2867125	C	0.81	0.86	<0.001
rs6548238	C	0.81	0.89	<0.001
PPARG				
rs1801282	C	0.13	0.05	<0.001
ADIPOQ				
rs2241766	G	0.11	0.11	0.916
rs1501299	T	0.29	0.28	0.32
GNPDA2				
rs10938397	G	0.46	0.41	0.001
NPY				
rs16139	C	0.04	0.02	<0.001
BDNF				
rs925946	T	0.26	0.37	<0.001
rs6265	C	0.8	0.89	<0.001
UCP2				
rs660339	G	0.59	0.63	0.001
rs659366	C	0.63	0.65	0.146
FTO				
rs6499640	A	0.59	0.51	<0.001
rs1558902	A	0.45	0.43	0.037
rs1121980	A	0.47	0.46	0.571
rs9939609	A	0.44	0.43	0.373
rs9941349	T	0.45	0.43	0.246
MC4R				
rs17782313	C	0.22	0.22	0.629
rs12970134	A	0.24	0.22	0.063

Abbreviations: HG, Hungarian General population; HR, Hungarian Roma population

Table 4. Associations of individual SNPs with obesity in the study populations (joint analysis) and interactions between individual SNPs and ethnicity.

Gene / SNP	*Risk Allele*	*OR[a] [95% CI]*	*P*	*Power (%)[b] (P=0.05)*	*Expected sample size[c]*		*Interaction analysis[d]*	
					Case	*Control*	*OR[a] [95% CI]*	*P*
LEPR								
rs1137101	G	1.01 [0.87 - 1.16]	0.941	5	>100000	>100000	0.88 [0.66 - 1.75]	0.391
NEGR1								
rs2815752	A	1.13 [0.97 - 1.31]	0.119	64	1313	1313	0.95 [0.69 - 1.31]	0.772
TMEM18								
rs2867125	C	1.08 [0.89 - 1.30]	0.416	23	4826	4826	1.09 [0.74 - 1.63]	0.641
rs6548238	C	1.15 [0.95 - 1.39]	0.159	58	1447	1447	1.33 [0.87 - 2.04]	0.188
PPARG								
rs1801282	C	1.08 [0.85 - 1.37]	0.519	16	7595	7595	0.57 [0.33 - 0.98]	<0.05
ADIPOQ								
rs2241766	G	1.08 [0.86 - 1.34]	0.639	17	6905	6905	1.11 [0.71 - 1.74]	0.635
rs1501299	T	1.13 [0.97 - 1.32]	0.129	63	1370	1370	0.91 [0.66 - 1.24]	0.556
GNPDA2								
rs10938397	G	1.17 [1.01 - 1.34]	<0.05	87	749	749	1.21 [0.91 - 1.61]	0.19
NPY								
rs16139	C	0.56 [0.38 - 0.81]	<0.01	100	239	239	0.88 [0.37 - 2.11]	0.778
BDNF								
rs925946	T	1.13 [0.97 - 1.32]	0.114	64	1332	1332	1.03 [0.76 - 1.39]	0.859
rs6265	C	1.16 [0.95 - 1.39]	0.139	64	1311	1311	0.85 [0.56 - 1.28]	0.434
UCP2								
rs660339	G	1.09 [0.95 - 1.26]	0.235	41	2398	2398	1.21 [0.9 - 1.61]	0.206
rs659366	C	1.09 [0.94 - 1.26]	0.245	40	2454	2454	1.12 [0.84 - 1.49]	0.054
FTO								
rs6499640	A	1.12 [0.98 - 1.29]	0.103	62	1389	1389	1.07 [0.81 - 1.42]	0.631
rs1558902	A	1.34 [1.17 - 1.54]	<0.001	100	241	241	1.21 [0.91 - 1.61]	0.184
rs1121980	A	1.35 [1.18 - 1.56]	<0.001	100	232	232	1.21 [0.91 - 1.61]	0.184
rs9939609	A	1.35 [1.17 - 1.55]	<0.001	100	229	229	1.19 [0.89 - 1.58]	0.224
rs9941349	T	1.41 [1.22 - 1.62]	<0.001	100	181	181	1.24 [0.93 - 1.65]	0.142
MC4R								
rs17782313	C	1.19 [1.01 - 1.42]	<0.05	86	782	782	0.9 [0.64 - 1.23]	0.563
rs12970134	A	1.19 [1.04 - 1.41]	<0.05	87	754	754	0.87 [0.62 - 1.22]	0.431

[a] Increase in the odds of being obese (BMI ≥ 30 kg/m^2) versus being normal weight (18.5 ≤ BMI < 25 kg/m^2) for each additional risk allele.

[b] CaTs software [38] were used for the power calculation under additive genetic model and the prevalence of obesity was assumed 28% according to the literature [1].

[c] Minimum sample size that permits to detect significant (p<0.05) association with the power of 80%.

[d] Based on model assuming additive allelic effect and dichotomus ethnicity in a multiplicative interaction term.

Regression models were adjusted for age, gender and ethnicity.

Table 5 Associations of the GRS1 combining 14 SNPs and GRS2 combining 4 SNPs with risk of obesity in the Hungarian general and Hungarian Roma populations.

	Hungarian general population		*Hungarian Roma population*	
	OR[a] [95% CI]	$P_{for\ trend}$	*OR[a] [95% CI]*	$P_{for\ trend}$
GRS1	1.11 [1.04-1.17]	<0.001	1.12 [1.05-1.18]	0.001
GRS2	1.14 [1.02-1.27]	0.018	1.27 [1.12-1.45]	<0.001

[a] Increase in the odds of being obese (BMI ≥ 30 kg/m^2) versus being normal weight (18.5 ≤ BMI < 25 kg/m^2) for each additional genetic risk score.

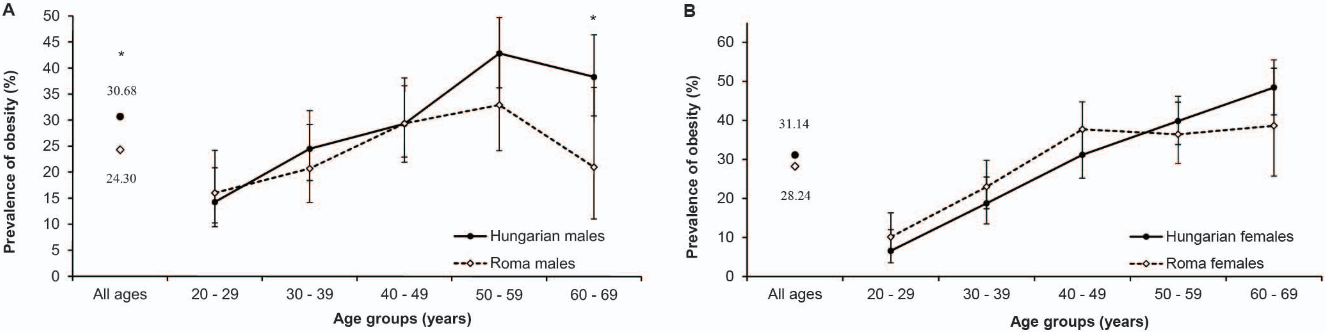
$P_{for\ trend}$ values were adjusted for age and gender.

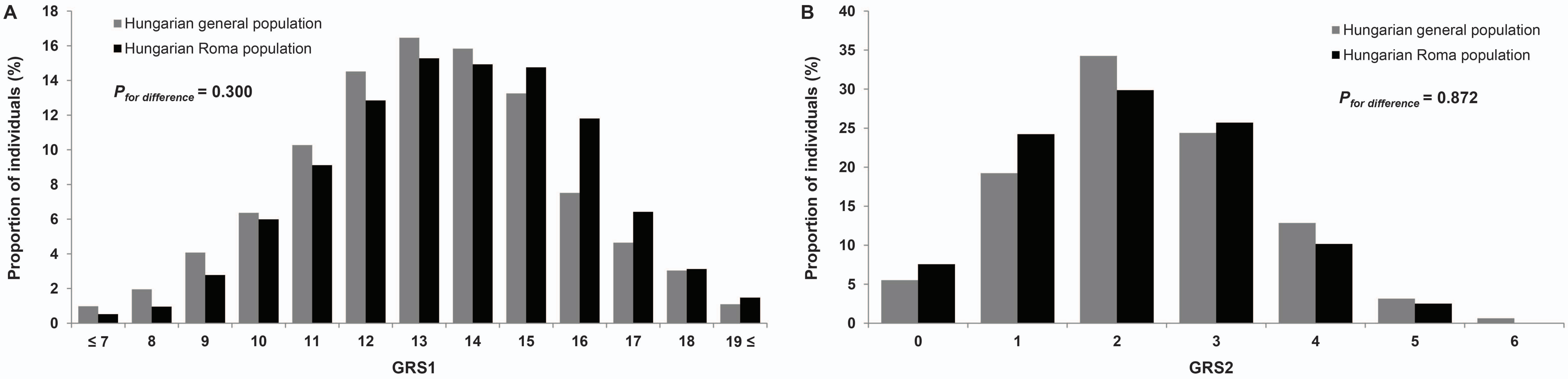
FIGURE LEGENDS

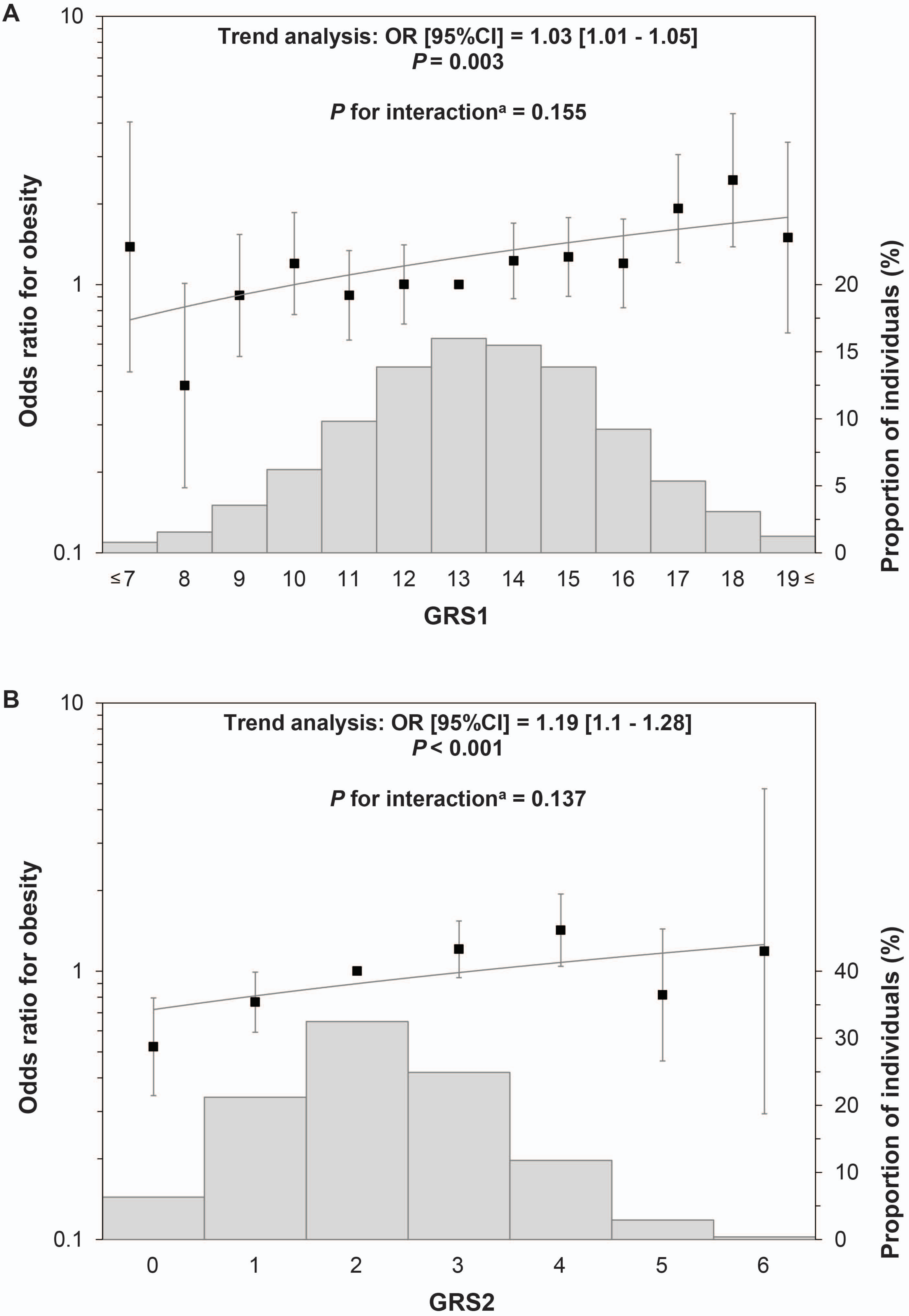
Figure 1. Age-specific prevalence (%; 95% CI) of obesity in the Hungarian general and Roma populations among males (A) and females (B). Statistically significant differences are indicated by asterisks (*p<0.05).

Figure 2. Distribution of the GRS1 combining 14 SNPs (A) and GRS2 combining 4 SNPs (B) in the Hungarian general (grey) and Hungarian Roma (black) populations.

Figure 3. Association of GRS1 combining 14 SNPs (A) and GRS2 combining 4 SNPs (B) with obesity in the study populations (joint analyses). Histograms (Y axis on right, grey bars) represent the percentage of individuals in each risk score category. Odds ratios and mean BMI (Y axis on left) are plotted. Error bars represent 95% confidence intervals.


A
Prevalence of obesity (%)
50
45
40
35
30
25
20
15
10
5
0
*
30.68
24.30
*
All ages
20 - 29
30 - 39
40 - 49
50 - 59
60 - 69
Age groups (years)
Hungarian males
Roma males
B
Prevalence of obesity (%)
60
50
40
30
20
10
0
31.14
28.24
All ages
20 - 29
30 - 39
40 - 49
50 - 59
60 - 69
Age groups (years)
Hungarian females
Roma females


A
Hungarian general population
Hungarian Roma population
$P_{for\ difference}$ = 0.300
Proportion of individuals (%)
18
16
14
12
10
8
6
4
2
0
≤ 7
8
9
10
11
12
13
14
15
16
17
18
19 ≤
GRS1
B
Hungarian general population
Hungarian Roma population
$P_{for\ difference}$ = 0.872
Proportion of individuals (%)
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
GRS2


A
Trend analysis: OR [95%CI] = 1.03 [1.01 - 1.05]
P = 0.003
P for interaction[a] = 0.155
Odds ratio for obesity
10
1
0.1
Proportion of individuals (%)
20
15
10
5
0
≤7
8
9
10
11
12
13
14
15
16
17
18
19 ≤
GRS1
B
Trend analysis: OR [95%CI] = 1.19 [1.1 - 1.28]
P < 0.001
P for interaction[a] = 0.137
Odds ratio for obesity
10
1
0.1
Proportion of individuals (%)
40
30
20
10
0
0
1
2
3
4
5
6
GRS2