Neuroendocrinology Letters Volume 36 Suppl. 2 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Genetic and biochemical characteristics in the Roma minority in the South Bohemia Region

Věra Adámková [1], Jaroslav A. Hubáček [2], Dita Nováková [3], František Dolák [3], Václav Adámek [4], Věra Lánská [1], Valérie Tóthová [3], Lenka Šedová [3]

1 Institute for Clinical and Experimental Medicine, Dpt. Preventive Cardiology, Prague, Czech Republic
2 Institute for Clinical and Experimental Medicine, Centre of Experimental Medicine, Prague, Czech Republic
3 University of South Bohemia in České Budějovice, Faculty of Health and Social Studies, České Budějovice, Czech Republic
4 Czech Technical University, Faculty of Biomedical Engineering, Kladno, Czech Republic

Correspondence to: Prof. MUDr. Věra Adámková, CSc.
Institute for Clinical and Experimental Medicine,
Department of Preventive Cardiology,
Vídeňská 1958/9, 140 21 Prague 4, Czech Republic
e-mail: vead@ikem.cz

Submitted: 2015-08-19 Accepted: 2015-10-22 Published online: 2015-12-12

Key words: **Roma; polymorphism; MC4R, TMEM18, lipids; blood sugar; Body Mass Index**

Neuroendocrinol Lett 2015; **36**(Suppl. 2):29–34 **PMID:** 26748524 NEL361015A05

Abstract

OBJECTIVE: At present, health characteristics of Roma minority within the Czech Republic are generally unknown. Therefore we examined a Roma population for some anthropometrical, biochemical and genetic parameters.

DESIGN: Groups include Roma aged above 18 years (men, women), with permanent residence in the South Bohemia Region. The Roma group (302 individuals) was selected using the snowball sampling method – the same method was used to select the non-Roma control group (78 individuals) for total cholesterol and blood sugar reference values. The main non-Roma control group was selected using the quota selection method (298 individuals). All participants completed a life style questionnaire, had their body measurements taken, were weighed, had their blood pressure and heart rates measured, had a capillary blood sample taken.

RESULTS: The non-Roma population had a lower Body Mass Index (BMI) (25.86 ± 4.23 vs. 27.45 ± 6.48 kg/m^2, $p = 0.0004$). The difference in BMI and weight was found to be associated with the *MC4R* gene. The values of systolic and diastolic blood pressure, total cholesterol, blood sugar did not differ with regard to the tested polymorphisms. We did not find any difference in the prevalence of *MC4R* or *TMEM18* between groups.

CONCLUSION: Despite educational efforts to introduce healthier life styles into the Roma population, there has been only limited success; as a result, Roma are very likely to suffer from premature complications of atherosclerotic processes, mostly due to theirs life style. Intensive further research is needed to improve the health conditions of the Roma minority, while still respecting their cultural differences.

ORIGINAL ARTICLE

Abbreviations, units:

MC4R	- melanocortin 4 receptor
TMEM18	- transmembrane protein 18
mmol	- milimol
l	- litre
kg	- kilogram
m	- meter
mm	- millimeter
Hg	- mercury
STR	- short tandem repeat
Gly	- glycine
Ser	- serine
CD	- a leucocyte type
DNA	- deoxyribonucleic acid

INTRODUCTION

The original homeland of the Roma is thought to be Northern India, from where they (according to most historians) migrated to the Balkans in the 14th century. The Roma ethnic group maintains its traditions and customs and, despite efforts, the Roma seldom integrate into the local majority population (e.g., they very rarely marry outside their own community). The Roma constitute the largest minority living in Central and Eastern Europe. Estimates suggest (accurate numbers are impossible to estimate since the Roma themselves often conceal their nationality) that there are 8–12 million Roma living in Europe, including 5 million in Central and Eastern Europe (Vozarova *et al.* 2003). Approximately 500,000 – 1.5 million Roma (Chohaney 2014) live in North America. The reason for such unequal settlement is multifactorial and is the subject of extensive social research. Different life styles are likely important factors, as are negative attitudes between majority and Roma populations (Arsene 2012).

The absence of literature regarding diseases in the Roma population makes it difficult to fully assess health risk factors for the Roma. Also genetics studies can help us partially clarify some questions with regard to (not only) public health care for this minority group. The analyses of the genetic background of different neuropathies and muscular dystrophy in Roma individuals helped confirmed, for example, the timing of their main migration out of India (Morar *et al*. 2004).

The receptor for melanocortin 4 has been intensively researched. Melanocortin 4 receptor is expressed primarily in the hypothalamus, specifically in the satiety and hunger centers. It is encoded by a one-exon gene, located at 18q22, with a highly variable expression. Mutations of the *MC4R* gene rank among the most common causes of serious obesity (in up to 60% of cases). For example, mutations in *MC4R* have been described in Turkey (Turkish Obesity Genome Study) in 40 extremely obese patients (Mergen *et al*. 2001).

We cannot recognize the mutation carriers of the above mentioned gene by phenotype, although homozygous carriers are characterized by early emergence of very serious obesity (although, not in 100% of homozygotes). The incidence of mutations of the *MC4R* gene reported in Czech children with early onset obesity was found to be 2.4%. A mutation of the *MC4R* receptor gene can be found in up to 5.8% of all cases of serious obesity that starts in early childhood. Mutations of *MC4R* have been described in almost all ethnic groups with a prevalence of between 0.5–5.8%, although some reports are inconsistent (Hainerová *et al.* 2007). Further, it was described, that MC4R variant is associated with decrease of BMI after life style intervention (Zlatohlávek *et al.,* in press).

Increased BMI, visceral fat, and insulin resistance has also been described for carriers of *TMEM18*. The *TMEM18* gene is ubiquitously expressed in both rodent and fly tissues, which suggests that it might play a fundamental role in cell function (Prats-Puig *et al*. 2013).

In view of the relatively closed Roma life style, it has been difficult to acquire enough data on health conditions (morbidity, spectrum of diseases, therapy, or mortality) of the Roma to formulate suggestions for prevention of diseases, particularly those of the cardio-vascular system. Especially if it is required those suggestions will be enough culturally sensitive and acceptable. On the other hand, the closed life style and self-isolation of the Roma community could help to analyze the potential genetic predispositions for obesity in the Roma ethnic group. Based on recent studies, we decided to analyze the polymorphisms of the *MC4R* and *TMEM18* genes.

MATERIALS AND METHODS

All participants were above 18 years of age and had permanent residence in the South Bohemia Region of the Czech Republic. The group of **Roma minority** participants was built using snowball sampling, in which new cases were obtained based on the process of gradual nomination of additional persons based on previously known individuals. Gender structure was derived from the general population, and it was assumed that the Roma minority had approximately a 50:50 representation of men and women.

Roma group and non-Roma **control groups:**

The Roma group consisted of 302 individuals selected through snowball sampling.

For the purposed of this study, two non-Roma controls groups were created and used in the study.

Control Group A – non-Roma, selected using a 50:50 gender quota selection method (n = 298).

Control Group B – non-Roma individuals used as reference values for blood sugar and total cholesterol, also selected using snowball sampling (n = 78).

After signing an informed consent, the participants completed a life style questionnaire. A capillary blood sample was taken to determine total cholesterol and blood sugar values. Both control groups (A and B) provide a buccal mucosa smear that was used to obtain DNA for genetic examination of polymorphism of

the following genes: *MC4R* (rs17782313) and *TMEM* (rs4854344). Genetic tests were carried out using polymerase chain reactions and restriction analyses as described in details elsewhere (Zlatohlavek *et al.* 2013). In addition, participants had their weight, height, heart rate, and blood pressure (BP) measured. BPs were measured using a digital aneroid Fazzini device, while seated, on the right forearm, and with a BP cuff appropriate for arm circumference. Blood sugar (One Touch device) and total cholesterol (Accutrend plus device) were determined from capillary blood samples (puncture to the distal part of the 5th finger). Body Mass Index was calculated as weight divided by height square (kg/m^2).

We evaluate 302 Roma, 298 non-Roma from control group A and 78 non-Roma from control group B. The project was approved by the Ethics Board of the University of South Bohemia. Only complete data were included for analysis.

Statistical evaluation used the ANOVA method, chi-square (χ^2) method, analysis of contingency tables, and by one-way analysis.

RESULTS

The prevalence of individual polymorphisms of the genes under study did not differ between groups (Table 1). The Roma were found to have higher BMI compared to the non-Roma participants (25.86 ± 4.23 vs. 27.45 ± 6.48 kg/m^2, $p = 0.0004$).

BMI, body weight, total cholesterol, and blood sugar were also evaluated in relation to the studied polymorphisms. A significant dependence between body weight and BMI relative to polymorphism of the *MC4R* gene was found in the Roma minority, but not for the majority population (Table 2). The highest BMI was found in Roma MC4R minor homozygotes (32.2 ± 7.92 kg/m^2), differing considerably from carriers of the major allele ($p = 0.0039$). The MC4R minor homozygotes also had the greatest body weight (87.4 ± 22.6 kg, $p = 0.02$). BMI and body weight values of the control group did not differ relative to analyzed polymorphisms. Blood sugar and total cholesterol also did not differ relative to the studied genes, in either group.

The average total cholesterol in the non-Roma (control group B) was 5.1 ± 1.1 mmol/l and in the Roma, 5.1 ± 1.4 mmol/l (n.s.). The average value of systolic blood pressure in the Roma group was 124.3 ± 20.9 mm Hg and in the non-Roma (control group A), 124.9 ± 14.4 mm Hg (n.s.); the average diastolic blood pressure in the Roma was 76.6 ± 12.7 mm Hg and in the control group A, 77.2 ± 10.6 mm Hg (n.s.); and as expected these characteristics were not been associated with the analyzed polymorphisms.

Randomly measured blood sugar values were considerably higher in the Roma group (Table 3) – the percentage of Roma with blood sugar values above 6 or above 7 mmol/l (31.8% vs. 17.9%, $p < 0.05$, and 26.8% vs. 6.4%, $p < 0.001$, respectively), was also considerably higher than in control Caucasians (B).

DISCUSSION

The estimated number of Roma in the Czech Republic is 200,000–300,000; in Slovakia, it is 400,000–500,000, which represent about 8.5% of the Slovak population. As other publications have shown, the average age of the Roma population is generally younger than that of the non-Roma population, and the overall health of the Roma appears worse than the non-Roma population. This is partially demonstrated by higher child mortality, which has been confirmed in several countries. The newborn mortality in Romania in 2003 was 27.1 per 1,000 live births, while in the Roma population it was 72.8 per 1,000 live births. The Czech and Slovak Repub-

Tab. 1. Prevalence of gene polymorphisms.

	Roma persons *n* = 296[a] (%)	Control A *n* = 295[b] (%)	*P*
MC4R CC	17 (5.74)	14 (4.75)	n.s.
MC4R CT	114 (38.51)	93 (31.53)	n.s.
MC4R TT	165 (55.74)	188 (63.73)	n.s.
TMEM TT	211 (70.33)	212 (71.38)	n.s.
TMEM TG	81 (27)	71 (23.91)	n.s.
TMEM GG	8 (2.67)	14 (4.71)	n.s.

n.s., not significant
[a] 6 Roma did not provide all data needed to be included in this analysis.
[b] 3 members of non-Roma control group A did not provided all data needed to be included this analysis.

Tab. 2. Relation of *MC4R rs17782313* gene to BMI and other variables in the Roma group.

	MC4R CC *N* = 17	*MC4R CT* *N* = 114	*MC4R TT* *N* = 165	*P*
BMI (kg/m^2)	32.2 ± 7.92	27.3 ± 6.50	26.9 ± 5.75	0.0039
Weight (kg)	87.4 ± 22.60	74.8 ± 18.0	74.7 ± 17.70	0.02
T. cholesterol (mmol/l)	5.1 ± 0.97	5.3 ± 1.64	5.0 ± 1.30	n.s.
Blood sugar (mmol/l)	6.0 ± 0.68	6.5 ± 1.97	6.6 ± 2.04	n.s.

BMI, Body Mass Index; mmol, millimole; l, litre; n.s., not significant

Tab. 3. Blood sugar – Roma vs. Control group B.

Blood sugar mmol/l	Roma *n* = 302 (%)	Control B *n* = 78 (%)	*P*
<5	7.9	35.9	<0.001
5–6	33.4	39.7	n.s.
6–7	31.8	17.9	<0.05
≥7	26.8	6.4	<0.001

n.s., not significant; mmol, millimole

lics have reported newborn mortality in the Roma to be double of that of the non-Roma majority population, while in Bulgaria, it is thought to be 6 times higher (Sepkowitz 2006). After 1989, the Roma were offered new opportunities (including educational programs) to better integrate into the society of the majority population; unfortunately, despite the huge effort, the expected results are far below expectations (Koupilova *et al.* 2001).

Obesity is considered a high-risk factor for many diseases, predominantly cardio-vascular diseases. Each new finding with regard to its etiology is therefore beneficial for potential prognostic estimates. For example data from Serbia indicate that Roma mortality from cardio-vascular diseases increase with the life style modernization (Bogdanović *et al.* 2007). By trying to help minorities, the majority population may actually enable them to live in ways they might not otherwise choose or find inaccessible (e.g. very easy access to food, ease of travel, high social assistance, including financial assistance, which varies between individual countries). These efforts to balance the economic inequities may force new life style opportunities on minority populations faster than they can culturally respond and adapt.

Our measurements confirm the higher BMI of Roma compared to the majority Caucasian population. Studies from Slovakia have shown that the average BMI of Roma is higher (32 kg/m^2), as is the waist/hips ratio (0.91 × 0.87), than in the non-Roma Slovak population (Vozarova *et al.* 2003).

The similar prevalence of polymorphisms, of the analyzed genes, that we found in the Roma minority and in the majority population adds important information regarding the role of the *MC4R* and *TMEM* genes in the Roma minority. The polymorphism within the *TMEM18* gene, which may be related to obesity, has been found in different ethnic groups, i.e. Caucasians, and Indians of the Pima tribe (Hainerová *et al.* 2007).

MC4R is an intensively studied receptor related to monogenic obesity. We have found the highest BMI associated with the *MC4R* gene in our Roma population. Literature offers reports of its influence on obesity, for example describing *N274S* mutation in a man with a BMI of 41.7 kg/m^2 (Mergen *et al.* 2001). Such reports support our findings.

We did not find any relationship among BMI, body weight, blood sugar, and cholesterol values relative to the TMEM18 gene in either the Roma or control group B. Total cholesterol values did not appear to be linked to any of the studied polymorphisms in either group; however, we did not evaluate a full lipidogram, but other works have shown that Roma women have rather higher triglyceride values compared to non-Roma women (Zeljko *et al.* 2013) and that Roma have higher triglycerides values, atherogenic indexs, insulin resistance, and reduced HDL cholesterol (Valachovicova *et al.* 2009). The total cholesterol and blood sugar values, evaluated in this study, were mainly for general comparison purposes. Blood samples were not taken on an empty stomach and we were not able to determine accurately the time from the last meal; therefore we cannot comment on those values in more detail (but total cholesterol value is rather not influenced by postprandial status of individual). To compare these values, we used a control group from the non-Roma majority population, created using snowball sampling. Within the limitations mentioned above, we found clearly higher blood sugar values in the Roma. In this context, we did not think it appropriate to make comparisons to the non-Roma control group B, which would have to be created using a different method, since the majority population, per our experience from other epidemiological examinations, shows (Vitásková *et al.* 2013; Zvolska *et al.* 2014) that participants arrive either on an empty stomach or after a long interval since the last meal; thus comparisons would not be valid. The Roma participants also included a considerably higher percentage of individuals with blood sugar values between 6–7 mmol/l, which demonstrates higher blood sugar values on an empty stomach and probably also a higher prevalence of prediabetic individuals. Blood sugar tolerance tests could not be performed. The blood sugar values from the Roma participants correspond to higher BMIs, which allows us to conclude that the energy intake of food is high and sugar consumption is also rather higher.

We did not find any difference in the distribution of the polymorphisms within the *MC4R* and *TMEM18* gene between our groups.

There are some reports of differences within other genes being investigated e.g. examination of the P450 cytochrome has shown that *CYP2B6* c.516G>T is found more frequently in the Roma population than in the Hungarian majority population; 33.6% vs. 21.4%, $p < 0.001$; the homozygous form is found in 9.9% of Roma and 5.6% of the majority population (Weber *et al.* 2015). Mačeková *et al.* (2012) reported that the *FTO rs9939609SNP* polymorphism, located in the first intron, was linked to obesity in Roma ($n = 312$; odds ratio, 1.55; 95% confidence interval, 1.129–2.128; $P = 0.007$). In addition, mutations in genes involved in the immune response have also been analysed; and it turned out that some mutations occurred when the Roma arrived to the Iberian Peninsula (Mancebo *et al.* 2008). In the Greek Roma, differences on the Y chromosome have been confirmed (Deligiannidis *et al.* 2006); the Portuguese Roma population showed differences in the Y chromosome not only compared to the majority population but also relative to Bulgarian Roma, although there were no differences compared to Spanish Roma (Gusmao *et al.* 2008). Similar differences in genetic variations have been confirmed in the Slovak Republic if STR locus has been analysed. The difference existed even between Roma from Eastern Slovakia compared to those from Eastern Hungary (9 loci out of 15), which shows that Roma communities are very separated, even relative to other Roma communities (Soták *et al.* 2008). Analysis of

the incidence of important individual polymorphisms for different genes is under way, but the information has been rather fragmentary so far, often yielding conflicting results. The Roma ethnic group is not the only minority group whose genetic particularities have not been fully described for the purposes of prediction of health problems and preventive measures. Many other countries face the same problem; there were reports regarding risk factors of diseases of majority populations in South Asian regions, which failed to include the relatively large minority of the Somalian men living there (Johnson *et al.* 2011). Data regarding the poorer health conditions of migrating communities has also come from Ireland, where there still exists a relatively large group of people who maintain a migratory life style. While lower quality of health and higher mortality has been confirmed for this group, there is no indication that they live below the poverty line (Kelleher *et al*. 2012). Irish migrants are probably the closest, in life style, to the Roma of the Czech Republic; but no prospective international comparison of health conditions has been performed, and in view of the life style of these groups, such a study is difficult to conceived, at least for the present. The problem of many diseases, particularly those of the circulatory system, is closely related to life style (e.g. nutritional recommendations).

A study from 1997 shows that in Bulgaria, 84% of the Roma population lived at the poverty line (Ringold 2000). An issue that arises as a consequence of social conditions is that of health conditions in Roma settlements, which have their own specific problems. Although there is only incomplete data available, it is estimated that about 2/3 of the 400,000 Slovak Roma live in Roma settlements and they live below the poverty line (Ruzicka 2012). Such an unsatisfactory socioeconomic position certainly constitutes one of the aspects that negatively affect overall health (Kolarcik *et al*. 2009). Another difference can be seen in eating habits; for example, Czech non-Roma children consume 2.6 portions of milk/dairy products per day on average, while Roma children consume only 1 portion. Roma also consume only 44% of the recommended daily allowance of vitamin C (Brazdova *et al*. 1998). To improve the conditions of the Roma minority living in Europe, twelve European countries joined the "Decade of Roma Inclusion 2005–2015" initiative (Molnár *et al.* 2012). The project represents a very important step towards real efforts to improve health conditions of the Roma population; however, the effort invested so far have yet to produce the successes originally envisioned.

CONCLUSION

The results of research has gradually added fragments of knowledge that have revealed that the Roma minority lead a uniquely different life style with unique health challenges, which are partly associated with distinctive genetic make-up of the Roma population. If this genetic predisposition may constitute a risk factor for shorter lifespans, increased neonatal mortality, and more aggressive effects of the atherosclerosis needs further studies. So far efforts to minimize these risks in a culturally sensitive way have had only limited success.

Conflict of interest

The authors report no conflicts of interest.

ACKNOWLEDGEMENT

The article is related to the research grant project number LD14114, implemented with financial support from the Ministry of Education, Youth and Sports within the COST (Cooperation on Scientific and Technical Research) project named "Obesity and overweight in the Roma minority in the South Bohemia Region" and by the project (Ministry of Health, Czech Republic) for the development of research organisation 00023001 (IKEM, Prague, Czech Republic) – Institutional support.

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