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Genetic factors exist behind the high prevalence of reduced high-density lipoprotein cholesterol levels in the Roma population

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ABSTRACT

Background and aims: Previous findings showed that reduced plasma high-density lipoprotein cholesterol (HDL-C) levels are more frequent in all age groups of the Hungarian Roma compared to the general population. It suggests that genetic factors may exist behind this phenomenon. Our present study was designed to test this hypothesis, i.e., to define whether genetic factors contribute to the higher prevalence of reduced HDL-C among Roma. Single nucleotide polymorphisms (N = 21) contributing to the variation in plasma HDL-C concentrations were analysed in the Hungarian Roma (N = 646) and general (N = 1542) populations.

Methods: Genetic risk scores, unweighted (GRS) and weighted (wGRS), were computed and compared. Associations between the GRSs and the prevalence of reduced HDL-C levels were analysed.

Results: The GRS and wGRS were significantly higher in the Roma compared to the general population (GRS: 22.2 ± 3.2 *vs.* 21.5 ± 3.3; wGRS: 0.57 ± 0.1 *vs.* 0.53 ± 0.1; $p<0.001$). One half per cent of Roma subjects were in the bottom fifth of the wGRS (wGRS$\leq$ 0.3) compared with 1.8% of those in the general population ($p=0.025$), while 5% of the Roma subjects were in the top fifth of the wGRS (wGRS$\geq$ 0.75) compared with 2.6% of those in the general population ($p=0.004$). The GRS showed similar correlation with reduced plasma HDL-C levels in the two populations, whilst the wGRS showed stronger correlation with the trait among Roma after controlling for confounders.

Conclusions: These results strongly suggest that genetic factors contribute to the higher prevalence of reduced HDL-C levels among Roma, so interventions aiming to improve Roma health status need to consider their increased genetic susceptibility.

1. Introduction

Investigations of the biological, environmental, social and psychological attributes associated with ethnicity will be an essential component of multidisciplinary research into the prevention of diseases, including those that differ in prevalence among ethnic groups [1]. The Roma population, which constitutes the largest ethnic minority in Europe, is the main subject of ethnicity-based studies. An estimated 10–12 million Roma are scattered throughout the continent, showing accumulation in the Central Eastern and Southern European countries (mainly in Bulgaria, Hungary, Slovakia and Romania) [2]. The Roma are frequently concentrated in severely deprived regions [3], segregated colonies characterised by very unfavourable environmental conditions [4]. According to the latest census in 2011, approximately 3.2% of the total Roma population is in Hungary; however, their estimated representation is much higher, up to 7.5% of the total population [5,6]. This discrepancy arises from the fact that due to the widespread prejudice they face, many Roma people do not declare their ethnic origin [7]. Although research on the Roma population faces many challenges in both data collection and methodology [7,8], the available data strongly suggest that Roma populations suffer from poor health, lower life expectancy and barriers in access to healthcare [9].

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Comparative studies on the risk profile for Roma adults indicate that the Roma population bears significantly higher CVD risk load in comparison with the majority of the population independently of the country where they live [10–14]. Although in a few studies the possible role of genetic factors in association with particular metabolic traits was also investigated [15–17], the contributions of environmental and genetic factors to the increased risk of CVD have not been defined in any previous studies.

Our present study is based on the findings of our recent health examination survey that estimated and compared the prevalence of metabolic syndrome, the most robust indicator of risk of non-communicable diseases among Roma adults, and its components on representative random samples of the Hungarian general population and Roma living in segregated colonies. It was found that the prevalence of metabolic syndrome, especially a reduced plasma HDL-cholesterol (HDL-C) level, was significantly more frequent in all age groups of the Hungarian Roma population compared to Hungarian general counterparts (<1.03 mmol/l in males and <1.29 mmol/l in females) [18]. On the basis of these results, as well as of the high level of consanguinity in the Roma population [19,20] it is reasonable to suppose that ethnic disparities exist not only in the prevalence of reduced HDL-C level but also in the prevalence of related gene polymorphisms. The high endogamy was proved by the gipsy origin of male partners in 90% of couples. The occurrence of first cousin couples was 16 times higher than that of the Hungarian population at large [21].

Research on the genetic determinants, however, faces certain challenges. The effect sizes of individual risk alleles associated with the trait are small, with most genotype relative risks in the range of 1.1–2.0; consequently, the predictive value afforded by these single variants is likely to be very low at best. However, the recent development of a polygenic/genetic risk score (GRS) that sums up the total effect of several single nucleotide polymorphisms (SNPs) utilizing effect size estimates from published genome-wide association studies (GWAS) offers an important new opportunity [22].

The aim of our present study was to define whether in addition to the effect of unfavourable environmental factors (diet, smoking, alcohol consumption) being more common among Roma [23,24], genetic susceptibility also contributes to the higher prevalence of reduced HDL-C level among Roma. The answer to this question has important implications for planning and delivering - hopefully effective - preventive interventions, which in case of genetically defined susceptibility to reduced HDL-C, may considerably forestall the clinical manifestation of the trait in at-risk patient groups.

2. Materials and methods

2.1. Study design

Our study involved subjects of representative samples investigated during recent cross sectional surveys [18,25]. The subjects included 646 Hungarian Roma individuals living in segregated colonies (Roma) in North-East Hungary where Roma are concentrated and 1542 individuals from the Hungarian general population (General).

2.2. Sample populations

2.2.1. Roma living in segregated colonies

Participants were enrolled from North-East Hungary (Hajdú-Bihar and Szabolcs-Szatmár-Bereg counties), where the majority of Roma colonies can be found, using a stratified multistage sampling method. The details of the sampling methodology and data collected are described elsewhere [18]. As a part of this health examination survey, medical histories and socio-demographic characteristics were recorded and physical examinations were carried out for each participant. Blood samples were taken for laboratory and genotype investigations. The present study used 646 samples where complete clinical records of 20–64-year-old Roma adults were available.

2.2.2. Hungarian general population

A population-based disease monitoring system, the General Practitioners' Morbidity Sentinel Stations Programme (GPMSSP), provided the Hungarian reference sample [26]. Samples were drawn from the population of counties participating in the GPMSSP. The methods of sampling applied and survey data collected are described in the Hungarian Metabolic Syndrome Survey (HMSS) [25]. As part of a health examination survey, medical histories and socio-demographic characteristics were recorded and physical examinations were carried out for each participant. Blood samples were taken for laboratory tests and for DNA isolation. The present study used DNA samples from 1542 20–64-year-old adults with complete records to create the reference dataset. The sample is representative of the Hungarian adult population in terms of geographic, age and sex distributions.

2.3. DNA isolation

DNA was isolated using a MagNA Pure LC system (Roche Diagnostics, Basel, Switzerland) with a MagNA Pure LC DNA Isolation Kit–Large Volume according to the manufacturer's instructions. Extracted DNA was eluted in 200 μl MagNA Pure LC DNA Isolation Kit-Large Volume elution buffer.

2.4. Selection of SNPs

A systematic literature review on the PubMed, HuGE Navigator and Ensembl databases was conducted to identify SNPs most strongly associated with HDL-C synthesis and cholesterol transport using different combinations of the following keywords: high-density lipoprotein cholesterol, cholesterol transport and synthesis, single nucleotide polymorphism, candidate gene, and meta-analysis. Studies applying a candidate gene approach were used to identify SNPs resulting in susceptible/protective alleles, as well as the strengths of their effect on plasma HDL-C levels. Reports were selected if they considered the HDL-C level as an outcome, were original research publications and the study was conducted in humans. The references of selected articles and reviews were also examined to identify additional related studies. Special attention was given to publications on meta-analyses. SNPs were selected if they were found to be consistently associated with plasma HDL-C levels (across study populations) in samples with biostatistically acceptable size. The results of the literature search, i.e., the selected SNPs, their genes and related references, are summarized in Supplementary Table 1, Step 1.

The adequate sample size for the study groups was computed using the online calculator OSSE (http://osse.bii.a-star.edu.sg/calculation1.php), assuming a power of 80%, an alpha-level of 0.05 for a 1:2.5 case: control ratio. The allele frequencies for CEU (Utah Residents (CEPH) with Northern and Western Ancestry) and for GIH (Gujarati Indian from Houston, Texas) populations from 1000 genome project, phase 3 were applied in the sample size estimation considering the fact that Roma population of Europe arrived to the Balkans from North-India and then migrated to Europe [27].

The literature search resulted in the selection of 33 SNPs influencing plasma HDL-C levels. During the assay design, a pool of 23 SNPs was created for genotyping by the service provider (Mutation Analysis Core Facility of the Karolinska University Hospital,