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Metabolic Syndrome in a Metapopulation of Croatian Island Isolates

Ivana Kolčić[1], Ariana Vorko-Jović[1], Branka Salzer[2], Mladen Smoljanović[3], Josipa Kern[1], Silvije Vuletić[1]

[1]Department of Medical Statistics, Epidemiology and Medical Informatics, Andrija Štampar School of Public Health, Zagreb University School of Medicine, Zagreb, Croatia
[2]"Labor Center" Laboratory, Zagreb, Croatia
[3]Institute of Public Health, Split, Croatia

> **Correspondence to:**

Ivana Kolčić
Department of Medical Statistics, Epidemiology and Medical Informatics
Andrija Štampar School of Public Health
Zagreb University School of Medicine
Rockefellerova 4
10000 Zagreb
ikolcic@snz.hr

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Aim To investigate the prevalence and factors associated with the metabolic syndrome in 9 isolated populations on Adriatic islands, Croatia, and in the group of immigrants to these islands.

Methods Random samples of 100 inhabitants from each village and 101 immigrants were collected during 2002 and 2003. Bivariate and multivariate methods were used in data analysis. Age, gender, village, diet, smoking habits, physical activity, education, occupational class, and personal genetic history (a pedigree-based estimate of the individual genome-wide heterozygosity level) were used as independent variables in logistic regression.

Results A total of 343 (34%) examinees met criteria of the metabolic syndrome diagnosis, with significant differences in the prevalence among villages ($P = 0.002$). Metabolic syndrome was most frequently detected on Mljet island (53%), where all examinees exhibited fasting plasma glucose over 6.1 mmol/L. Examinees with metabolic syndrome were significantly older than those without it (median age 60.0 vs 53.0; $P<0.001$). Women were more frequently diagnosed than men (39% vs 28%; $P<0.001$). The highest prevalence of the metabolic syndrome was found in the autochthonous group, whereas the lowest proportion was recorded in the admixed group (39% vs 21%, respectively, $P = 0.017$). However, only age (odds ratio [OR], 1.06; 95% confidence intervals [CI], 1.03-1.08) and having a university degree (OR, 0.18; 95% CI 0.04-0.92) were significantly associated with metabolic syndrome in the regression model.

Conclusion Metabolic syndrome was not associated with pedigree-based individual genome-wide heterozygosity estimate, after controlling for a number of confounding factors. More precise marker based genomic measures are needed to provide a clear answer whether metabolic syndrome development is influenced by the population genetic structure.

The metabolic syndrome refers to the clustering of cardiovascular risk factors that greatly increase an individual's risk for developing diabetes, cardiovascular disease, and renal disease (1,2). It is defined as a concurrence of impaired glucose and insulin metabolism, overweight and abdominal fat excess, dyslipidemia, and hypertension, associated with subsequent development of type 2 diabetes mellitus and cardiovascular disease (3). Other frequently used terms for the metabolic syndrome are syndrome X and insulin resistance syndrome. Although insulin resistance is not a defining component of the metabolic syndrome in the definition proposed by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol Adult Treatment Panel III (4), it is considered to be its core feature (5,6).

Metabolic syndrome is a substantial public health problem across the world (1,7). Its diagnosing criteria, such as high blood pressure and obesity, are globally among the ten leading risk factors (7). Croatian population does not present an exception from this finding, with elevated blood pressure, smoking, physical inactivity, high alcohol intake, inadequate nutrition, and obesity being identified as the most prevalent cardiovascular risk factors in the general population (8).

Beside widely investigated environmental and behavioral risk factors, a number of studies have identified a genetic contribution to the metabolic syndrome development. Metabolic abnormalities related to the metabolic syndrome aggregate in families, suggesting a common genetic component (9). Evidence for the genetic basis of type 2 diabetes and the metabolic syndrome has been derived from various family, twin, and population studies. Identification of genes associated with disease pathogenesis is currently under way, using techniques such as genome scanning by positional cloning and the candidate gene approach (10).

Multitude of various risk factors renders epidemiological investigation of metabolic syndrome difficult. Reduced genetic and environmental heterogeneity of isolated human populations could theoretically be useful in the investigation of metabolic syndrome. Isolated populations residing in villages of Croatian islands were already proven to be good models for the investigation of common complex diseases of late onset (11-13). The aim of this study was to investigate the prevalence of metabolic syndrome and factors associated with it, namely personal genetic history in 9 isolated populations of Croatian Adriatic islands, as well as immigrants to the islands. These island populations exhibit a wide range of inbreeding and endogamy, reduced genetic variation at both individual and (sub)population levels, and a relative uniformity of environment (11).

Subjects and Methods

Subjects

This study involved subjects from the "1001 Dalmatians" research program, which was performed during 2002 and 2003. Research program "1001 Dalmatians" gathered biomedical information from multiple small isolated populations (metapopulations) on Adriatic islands in Croatia, for genetic epidemiological research (14,15). The aim of the program was to investigate health effects of the changes in population genetic structure, such as inbreeding, isolation, admixture, and outbreeding, under very similar environmental conditions (15).

Nine villages for the study were carefully selected to represent a wide range of differing demographic histories, fluctuations in population size, admixture, and bottleneck events (14). The rationale for selecting particular villages was described in detail by Rudan et al (15). A random sample of 100 adult inhabitants older than 18 was collected in each of the 9 villages; Banjol, Barbat, Lopar, Rab, and Supetarska Draga (Rab island), Vis and Komiža (Vis island), Lastovo,

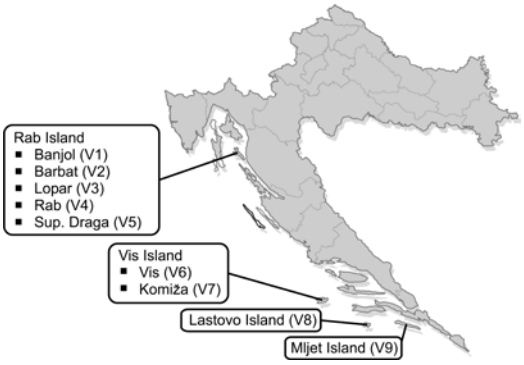

Figure 1. Geographic location of the investigated islands of Rab, Vis, Lastovo and Mljet. Investigated villages on Rab and Vis islands are bulleted.

and Mljet (Figure 1). Sampling was based on computerized randomization of the most complete and accessible population registries in each village, which included medical records (Mljet and Lastovo islands), voting lists (Vis island), and household numbers (Rab island) (14,15). The samples were considered reasonably representative for each of the island populations.

These 900 adults from multiple small isolated populations represent a human metapopulation, defined as several small and isolated population patches that may interact. Additional 101 examinees were recruited from the immigrant population to these 9 villages, to form a genetically diverse control group that shares the same environment with the indigenous population (15). The immigrants mainly originated from mainland Croatia. Further details on the "1001 Dalmatians" research program are given elsewhere (14,15).

Measurements

Variables included in the analysis were age, gender, diet, smoking habits, physical activity, personal genetic history, village, education, and occupational class. Personal genetic history, a pedigree-based estimate of the individual genome-wide heterozygosity level, was based on the two-generation ancestral pedigrees. Examinees were divided in 5 categories, according to the number of individual's grandparents who were born in the same settlement. Categories were defined as inbred (n=92), autochthonous (n=437), admixed (n=70), outbred (n=90), and others (n=312). Examinees considered inbred had the same (non-marital) surname, highly specific of the settlement, in at least one of their father's and one of their mother's parents. Autochthonous category included examinees whose four grandparents were born in the subject's village of residence, but there was no indication of inbreeding according to the surnames. Admixed category included examinees with grandparents on their father's side and grandparents on their mother's side born in two different villages. Outbred category included examinees whose 3 or 4 grandparents were born in different settlements on the Croatian mainland. Category "others" included examinees that could not be classified into any of the previous categories. They usually had between 1 and 3 grandparents born in the same village, whereas other grandparents were either born in the neighboring villages, or on the mainland, or any combination of the two (15). Category "inbred" included the individuals who were recently inbred, and would be expected to have the lowest mean value of individual genome-wide heterozygosity, followed by category "autochthonous," in which cryptic homozygosity, resulting from complex patterns of consanguinity in more distant past, is probably present. Category "admixed" and especially "outbred" would be expected to have higher mean values of individual genome-wide heterozygosity. Finally, the mean value of individual genome-wide heterozygosity in category "others" cannot be estimated in relation to other four categories, but it was expected to be higher than in the inbred and autochthonous category, and lower or similar to the admixed and outbred category (15).

All biochemical analyses were performed on fasting blood samples taken from the examinees between 7 and 9 AM. Plasma and serum were rapidly frozen and stored, and transported in fro-

zen state within a maximum of 3 days to the single internationally accredited biochemical laboratory in Zagreb (15). Diet index was defined as a binary variable, indicating dietary pattern on the basis of five unhealthy dietary habits. Examinees who reported at least three of the following criteria were considered as having unhealthy dietary habits: eating fruit and vegetables less than two times a week, eating fish less than three times a week, and consuming excessively salted or sweetened foods more than four times a week. Pack-years were counted as the number of cigarettes smoked daily multiplied by years of smoking and divided by 20. Physical activity was calculated as the average value of self-reported daily work and leisure physical activities (classified as ordinal variable with 4 classes). Education (classified in four classes; without completed primary school, completed primary school, completed secondary school, and university degree) and occupation class (classified as binary variable; white- or blue-collar occupations; excluding retired people and students) were used as socio-economic variables.

Detailed protocols and questionnaires used in "1001 Dalmatians" research program were described elsewhere (15). The ethical approval for the research was obtained from appropriate research ethics committees in Croatia and Scotland. Informed written consent was obtained from all participants in the study.

Diagnosis criteria

We used slightly modified metabolic syndrome diagnosis criteria proposed by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol Adult Treatment Panel III (ATP III) (4,16). Waist circumference was not measured, and therefore obesity criterion was determined according to the World Health Organization definition (16); examinees whose body mass index exceeded 30 kg/m^2 were considered eligible for the metabolic syndrome diagnosis.

Final metabolic syndrome diagnosis was made if at least three of the following five criteria were present: body mass index >30.0 kg/m^2; triglycerides ≥1.7 mmol/L; high-density lipoprotein (HDL)<1.03 mmol/L for men, and <1.29 mmol/L for women; fasting plasma glucose ≥6.1 mmol/L; and blood pressure ≥130/85 mm Hg. Examinees with diabetes type 1 were not considered eligible for metabolic syndrome diagnosis, and examinees that used oral hypoglycemic medications were considered as having positive fasting plasma glucose criterion.

Statistical analysis

An initial data analysis was performed using bivariate methods. χ^2 test was used in the analysis of categorical data, whereas Mann-Whitney test was used in the analysis of numerical data that exhibited non-normal distribution. A binary logistic regression model was used, with the metabolic syndrome as the dependent variable, whereas age, gender, diet index, smoking, physical activity level, village, education, occupation class, and personal genetic history (categorical variable) were used as independent variables. Personal genetic history group "others" was excluded from the logistic regression analysis, due to classification uncertainties. Examinees from the island of Mljet were also excluded, because they, for unknown reason, had uniformly positive glucose diagnostic criterion.

Analysis was performed by the SPSS 12.0.0 (SPSS Inc., Chicago, IL, USA), with significance level set at $P<0.05$.

Results

The final sample consisted of 996 examinees (data for 5 examinees were incomplete). A total of 343 (34%) of them fulfilled the metabolic syndrome diagnosis criteria. The number of examinees with the metabolic syndrome significantly varied across 10 investigated populations ($\chi^2_9=26.5$, $P=0.002$) (Table 1). Met-

Table 1. Prevalence of metabolic syndrome in 1001 subjects from Croatian Adriatic villages in 2002-2003

	Percent (95% CI) of persons from each village with positive diagnostic criterion †						
Island/village	age (median, range)	BMI	triglycerides	HDL cholesterol	glucose	blood pressure	diagnosis of metabolic syndrome
Rab:							
Banjol	55.0 (20-80)	40 (39-41)	26 (25-27)	65 (64-66)	11 (10-12)	72 (71-73)	37 (36-38)
Barbat	59.5 (21-76)	30 (29-31)	23 (22-24)	74 (73-75)	9 (8-10)	55 (54-56)	31 (30-32)
Lopar	54.0 (22-87)	33 (32-34)	18 (17-19)	73 (72-74)	19 (18-20)	76 (75-77)	42 (41-43)
Rab	50.0 (19-80)	23 (22-24)	23 (22-24)	69 (68-70)	8 (7-9)	60 (59-61)	25 (24-26)
Sup. Draga	56.5 (22-82)	24 (23-25)	28 (27-29)	73 (72-74)	10 (9-11)	59 (58-60)	30 (29-31)
Vis:							
Vis	62.0 (24-80)	29 (28-30)	20 (19-21)	68 (67-69)	14 (13-15)	74 (73-75)	34 (33-35)
Komiža	60.0 (18-83)	24 (23-25)	26 (25-27)	70 (69-71)	10 (9-11)	76 (75-77)	33 (32-34)
Lastovo	66.0 (27-88)	45 (44-46)	28 (27-29)	66 (65-67)	11 (10-12)	38 (37-39)	30 (29-31)
Mljet	51.5 (18-78)	16 (15-17)	36 (35-37)	73 (72-74)	98 (100)	42 (41-43)	52 (51-53)
Immigrants‡	45.0 (18-79)	21 (20-22)	20 (19-21)	83 (82-84)	28 (27-29)	44 (43-45)	29 (28-30)
χ^2_9	N/A	35.07	14.16	12.43	408.04	81.23	26.50
P	N/A	<0.001	0.122	0.190	<0.001	<0.001	0.002
total	56.0 (18-88)	29 (29-29)	25 (25-25)	72 (72-72)	22 (22-22)	60 (60-60)	34 (34-34)

*Abbreviations: N/A – not available; 95% CI – confidence intervals; BMI – body mass index; HDL – high density lipoproteins.
†Metabolic syndrome diagnostic criteria: BMI>30 kg/m^2; triglycerides ≥1.7 mmol/L; HDL cholesterol <1.03 mmol/L for men, <1.29 mmol/L for women; glucose ≥6.1 mmol/L; blood pressure >130/85 mm Hg; metabolic syndrome was diagnosed if at least three diagnostic criteria were recorded in an examinee.
‡Genetically diverse control group that mainly originates from mainland Croatia and shares the same environment with the indigenous population.

abolic syndrome was the most prevalent on Mljet island (n=52; 53%), and the least prevalent in the village of Rab (n=25; 25%) and among the immigrants (n=29; 29%) (Table 1). Metabolic syndrome diagnostic criteria varied across villages, especially for body mass index (χ^2_9=35.07, P≤0.001), fasting plasma glucose (χ^2_9=408.04, P<0.001), and elevated blood pressure (χ^2_9=81.23, P<0.001) (Table 1). Whereas only 8 (8%) examinees in Rab and 9 (9%) examinees in Barbat met the increased glucose diagnosis criterion, all examinees in Mljet fulfilled that criterion (Table 1).

More women (n=215, 39%) were diagnosed with the metabolic syndrome than men (n=128, 28%; χ^2_1=13.39, P<0.001). Examinees with diagnosed metabolic syndrome were significantly older, with median age 60.0 (interquartille range 17.0), compared with examinees without metabolic syndrome, with median age 53.0 (interquartille range 25.0; Mann-Whitney Z=-6.56, P<0.001). Autochthonous examinees were most likely to be diagnosed with metabolic syndrome, with significant differences among the personal genetic history categories (χ^2_4=12.06, P=0.017) (Table 2).

Table 2. Personal genetic history and diagnosis of metabolic syndrome in 1001 subjects from Croatian Adriatic villages in 2002-2003

	No (%) of examinees*		
Personal genetic history†	metabolic syndrome	no metabolic syndrome	total
inbred	34 (38)	56 (62)	90 (9)
autochthonous	170 (39)	265 (61)	435 (44)
admixed	15 (21)	55 (79)	70 (7)
outbred	27 (30)	62 (70)	89 (9)
others	97 (31)	215 (69)	312 (31)
total‡	343 (34)	653 (66)	996 (100)

*Metabolic syndrome was diagnosed if at least three of the following diagnostic criteria were recorded in an examinee: BMI>30 kg/m^2; triglycerides ≥1.7 mmol/L; HDL cholesterol <1.03 mmol/L for men and <1.29 mmol/L for women; glucose ≥6.1 mmol/L; blood pressure >130/85 mm Hg.
†Autochthonous category included examinees whose four grandparents were born in the subject's village of residence, but there was no indication of inbreeding from the surnames. Admixed category included examinees with grandparents on their father's side and grandparents on their mother's side born in two different villages. Category outbred included examinees whose either 3 or 4 grandparents were born in different settlements on the Croatian mainland. Examinees from the category others had between 1 and 3 grandparents born in the same village and other grandparents born in the neighboring villages or on the mainland, or any of the combinations.
‡χ^2_4=12.06, P=0.017.

A binary logistic regression model accounted for 23.5% variability (Nagelkerke R square), with a good data fit (Hosmer and Lemeshow test P=0.165). There were only two significant results (Table 3): age (OR, 1.06; 95% CI, 1.03-1.08) and education; with examinees with a university degree much less likely to have metabolic syndrome compared with those who did not complete primary school (OR, 0.18; 95% CI, 0.04-0.92).

Table 3. Multivariate logistic regression analysis with metabolic syndrome as dependent variable in 1001 subjects from Croatian Adriatic villages in 2002-2003

Independent variable	*P*	Odds ratio (95% CI)
Age	<0.001	1.06 (1.03-1.08)
Gender:		
male (referent)		1.00
female	0.108	1.50 (0.92-2.47)
Diet index	0.167	0.57 (0.26-1.27)
Smoking (pack years*)	0.540	0.99 (0.98-1.01)
Physical activity	0.084	1.36 (0.96-1.93)
Personal genetic history: †		
inbred (referent)		1.00
autochthonous	0.284	1.45 (0.74-2.85)
admixed	0.378	0.62 (0.21-1.80)
outbred	0.144	2.44 (0.74-8.04)
Village:‡		
immigrants (referent)		1.00
Banjol	0.074	3.21 (0.89-11.54)
Barbat	0.894	0.92 (0.25-3.41)
Lopar	0.121	2.68 (0.77-9.30)
Rab	0.273	2.04 (0.57-7.29)
Sup. Draga	0.897	1.09 (0.31-3.88)
Vis	0.665	1.34 (0.35-5.09)
Komiža	0.490	1.57 (0.44-5.62)
Lastovo	0.843	0.89 (0.26-2.99)
Education:		
without primary school (referent)		1.00
completed primary school	0.902	1.04 (0.57-1.90)
completed secondary school	0.709	0.81 (0.27-2.42)
university degree	0.039	0.18 (0.04-0.92)
Occupation class:		
blue-collar (referent)		1.00
white-collar occupations	0.424	0.76 (0.38-1.50)

*Pack-years were counted as the number of cigarettes smoked daily multiplied by years of smoking and divided by 20.
†Personal genetic history group others was excluded from the logistic regression analysis, due to classification uncertainties.
‡Examinees from Mljet were excluded from the logistic regression analysis due to inability to clearly state the reason for their uniformly increased fasting plasma glucose.

Discussion

A third of the population investigated in this study had the metabolic syndrome. The prevalence of the metabolic syndrome in the adult population differs depending on the diagnosis criteria and ethnicity, and usually varies between 22 and 39% (17). Reported prevalence of the metabolic syndrome in other Mediterranean populations was 24% in adult Greek population (18), 25% in Italian adults (19), and 17% in the Spanish province of Segovia (20).

Unfortunately, the lack of published data on the metabolic syndrome prevalence in general Croatian population makes the comparison between the investigated island populations and general population difficult. However, a recent study of the cardiovascular risk factors in the general population reported that 58% of men and 45% of women in the Adriatic area had blood pressure above 130/85 mm Hg (8). The same study reported obesity in 19% of men and 14% of women in the same region. Our study found higher prevalence of obesity and slightly higher prevalence of elevated blood pressure in the population of Croatian island isolates. A total of 29% examinees were obese, and 60% of examinees met the elevated blood pressure criterion. The reasons for this may reside in the difference in the age structure between island and mainland population, or in the genetic effects of the isolation (genetic drift and fixation) (11,12,15).

Although inappropriate nutrition, insufficient physical activity, and tobacco use are known behavioral risk factors associated with metabolic syndrome (21), these factors did not exhibit significant association with the metabolic syndrome in this study. This could be explained partly by the homogeneity of environmental factors in the metapopulation (22). However, we detected a significant association with education, suggesting that some behavioral patterns might have a substantial effect in the metabolic syndrome development. The immigrant population was considered to be a genetically diverse control group that shares the same environment with the indigenous population. In such a setting, we expected that genetic effects might be somewhat easier to detect than in other, "open" populations. However, the analysis that controlled for most obvious confounding factors did not find an association of the genetic background with the occurrence of the metabolic syndrome in the investigated metapopulation.

Although many studies attempted to map the genes associated with the metabolic syndrome, an unequivocal explanation of its pathogenesis has not yet been offered. Various studies reported a multitude of loci identified in different chromosomes, varying across populations and re-

flecting substantial complexity of the metabolic syndrome etiology (23-29).

One of the limitations of the study is a rather small sample size that might have exhibited a substantial random variation. Unfortunately, the metabolic syndrome was not in the study focus during the design phase, and therefore waist circumference was not measured. The definition of the metabolic syndrome was not based on the waist circumference but body mass index threshold, which makes direct comparisons with other studies difficult. Furthermore, there was no clear explanation for uniformly increased fasting plasma glucose on the Mljet island, possibly indicating a systematic error, which we tried to eliminate by excluding this sub-sample from the logistic regression model.

Detrimental effects of inbreeding have been identified in a number of studies investigating different traits in both animals and humans. Beside early onset traits, recent studies suggested that inbreeding might also have negative effects on the late-onset traits (30). These negative effects occur as a consequence of the loss of possibly adaptive genetic variability and the fixation of deleterious mutations (31). Inbreeding depression is a widely recognized phenomenon, which reduces fitness among inbred organisms via homozygosity at loci affecting fitness or by reducing heterozygote advantage (32). In contrast to inbreeding, increased heterozygosity is believed to be protective and beneficial, because it acts in the opposite direction to inbreeding. The results of this study showed a significant association between personal genetic history and the metabolic syndrome diagnosis in bivariate analysis. Interestingly, the proportion of metabolic syndrome was the highest in the autochthonous population, closely followed by the inbred individuals. The lowest proportion was recorded in admixed individuals who are expected to be more heterozygous, supporting the hypothesis that increased heterozygosity and admixture might be beneficial for various human traits (33). Both outbred category and category others exhibited comparable metabolic syndrome prevalence, which was between the lowest (admixed) and the highest (autochthonous and inbred) recorded values. However, personal genetic history did not exhibit a significant association with the metabolic syndrome in the multivariate analysis. This might be a consequence of the use of pedigree defined heterozygosity measure, a rather imprecise measure which should be replaced by a more precise and more reliable marker-based heterozygosity estimate.

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