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Effects of Inbreeding, Endogamy, Genetic Admixture, and Outbreeding on Human Health: A "1001 Dalmatians" Study

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Aim "1001 Dalmatians" research program collects biomedical information from multiple small isolated populations ("metapopulation") on Adriatic islands, Croatia, and investigates health effects of human population isolation, inbreeding, admixture, and outbreeding.

Methods We collected random samples of 100 individuals from 9 island settlements and an additional sample of 101 immigrants to the islands, pooled from all study populations. According to their personal genetic histories, the examinees were categorized as inbred, autochthonous, admixed, and outbred. A total of 76 inbred individuals from a total sample of 1001 examinees were matched to 76 autochthonous, 76 admixed, and 76 outbred controls by gender, age (±5 years), village of residence, education, and socio-economic status. We investigated the effects of presumed individual genome-wide heterozygosity predicted from personal genetic histories on the following 10 traits: systolic and diastolic blood pressure, body mass index, high and low density lipoproteins and total cholesterol, triglycerides, uric acid, creatinine, and blood glucose.

Results Personal genetic history significantly affected systolic blood pressure (Spearman ρ=0.157, P = 0.006), while the effect on cholesterol (ρ=0.105, P = 0.069), and high density lipoprotein cholesterol (ρ=0.104, P = 0.071) was suggestive. Admixed individuals and immigrants consistently showed values associated with lower health risk. When inbred and autochthonous samples were merged and compared with the admixed and outbred samples to increase the power of the study, the effects on the three traits above and also on body mass index and diastolic blood pressure became statistically significant. The medians for all 10 medically relevant traits in inbred and autochthonous group, with lower values of presumed individual genome-wide heterozygosity, were less favorable in terms of health.

Conclusion The combined effects of founder effect, genetic drift, and inbreeding can increase the frequency of detrimental rare variants in human metapopulations, leading to overall worsening of population health, whereas admixture and outbreeding appear to have the opposite effect.

Traditional epidemiological research has mainly focused on the association between different environmental, biological, or lifestyle variables and health status (1). In most of these studies, genetic structure of the investigated populations was presumed to have little or no effect on measured health outcomes. Human genetic structure is still constantly being affected by processes such as urbanization and migration ("outbreeding"), rapid population expansion, admixture between populations, isolation of specific sub-populations, and rapid non-random depopulation ("bottleneck effect," ref. 2). However, it has not yet been systematically evaluated whether these changes in human genetic structure can affect the health-related quantitative biological traits or incidence of common human diseases.

In the Republic of Croatia, there are 15 Adriatic Sea islands with a population of more than 1000 inhabitants. Some of the villages on these islands have been genetically isolated for centuries from other villages and the outside world. The isolation was one of the reasons why their ethnic history, demography, population biology, and genetic structure have been investigated for more than 50 years. The research resulted in more than 100 publications in international journals (3-7). The potential of this isolate resource for research into disease etiology was then outlined (3) and confirmed by initial successes in finding a genetic basis of known monogenic (Mendelian) diseases in these populations (8,9). Further research, based on historically collected data, was carried out to help design future studies into genetic architecture of common complex diseases of late onset (such as cardiovascular diseases, cancer, diabetes, psychiatric disorders, and osteoporosis) (10-14).

The "1001 Dalmatians" research program was launched in 2001 with an aim to collect biomedical data from multiple small isolated populations (a human "metapopulation") that share similar environment and lifestyle (3). Such a resource would approximate characteristic demographic organization of human populations in more distant past, before urbanization and increase in population size took place (15). Our resource was situated in isolated villages of Adriatic islands, Croatia. The ethnic history, demography, lifestyle, human biology, and genetic structure of these populations have been extensively studied over the past 30 years, which makes them very suitable for the proposed research (3-5). The scope of the program is to investigate health effects of changes in population genetic structure, such as inbreeding, isolation, admixture, and outbreeding, under very similar environmental conditions.

In this study, we explored whether inbred subjects showed inbreeding depression for any of the 10 measured health-related quantitative traits: systolic and diastolic blood pressure, body mass index, high and low density lipoproteins, total cholesterol, triglycerides, uric acid, creatinine, and blood glucose (16-18). We also studied whether admixed and outbred individuals showed the beneficial effects of heterosis (19-22). In literature, we could not identify any studies in human populations that simultaneously compared health effects of changes in population genetic structure (eg, inbreeding, admixture, and outbreeding) on quantitative traits of health significance, such as blood pressure, body mass index, cholesterol, and glucose levels.

Subjects and methods

Study population

The "1001 Dalmatians" research program aims to study multiple small isolated populations of Croatian islands that share similar environment and lifestyle (3). The 9 settlements were carefully chosen in 2002 based on their current population sizes, historic records, demographic information and accessibility of genealogical records to present a wide range of different ethnic histories, fluctuations in population size, admixture,

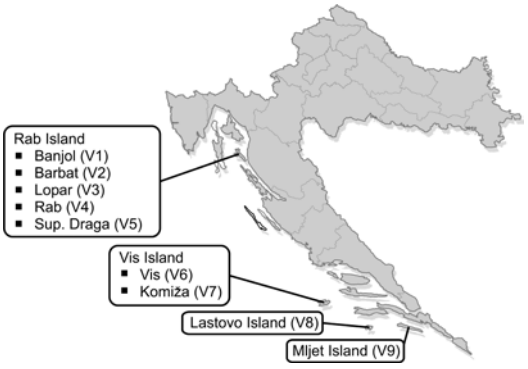

Figure 1. Geographic location of the investigated islands of Rab, Vis, Lastovo, and Mljet. Villages on the islands are study populations. Immigrants into the islands originate from mainland Croatia.

and bottleneck events (Figure 1). Current population size in the studied villages ranges from about 500 to about 2000. The fluctuations in size through history were large, even during the 20th century (Table 1).

The field study, measuring health variables and the genetic history variables of the population, was performed during 2002 and 2003 by a team from the Andrija Štampar School of Public Health, Zagreb University School of Medicine, and the Institute for Anthropological Research in Zagreb, Croatia. In each of the 9 villages, a random sample of 100 adult inhabitants was recruited (Table 2). 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). Additional 101 examinees were recruited from the immigrants from all 9 villages, to form a genetically diverse control population that shared the same environment. The ethical approval for this research was obtained from appropriate research ethics committees in Croatia and Scotland. Informed written consent was obtained from all participants in the study. The degree of recent isolation of the studied villages was assessed as the percentage of subject's grandparents who were born in the same village, ranging from 39.4 to 98.4%. The percentage of persons who were apparently inbred, based on 2-generation parental genealogy, ranged from 3 to 26% (3-5,15) (Table 2).

Measurement of health-related variables

The examinees were first interviewed by one of the trained surveyors, on the basis of a questionnaire that was developed for this research program. The questionnaire was designed to obtain personal information (eg, name, date and place of birth, gender, marital status, occupation, and different lifestyle variables), personal medical history, health complaints, drug intake, and hospitalization records. It also included World Health Organization (Rose) angina questionnaire (23), World Health Organization claudication questionnaire (24), World Health Organization noncommunicable diseases questionnaire (25), Eu-

Table 1. Basic demographic parameters recorded for each studied village (V1-V9)

	Village								
Parameter	Banjol (V1)	Barbat (V2)	Lopar (V3)	Rab (V4)	Sup.Draga (V5)	Vis (V6)	Komiža (V7)	Lastovo (V8)	Mljet (V9)
Years since the foundation of the population	1600	1450	1600	3000	950	3000	640	1200	1200
Number of major admixture episodes*	3	2	3	4	1	4	0	0	0
Time since the last admixture episode (years)	350	350	350	350	350	350	640	1200	1200
Last bottleneck event† (in years before present)†	550	550	550	550	550	25	25	25	25
Reduction in population size during the last bottleneck event (%)	60	60	60	95	60	53	44	32	43
Maximum achieved population size	1971	1300	1500	5000	1164	4300	3572	1602	2106
Year in which maximum size was achieved	2001	1950	1400	1400	2001	1910	1910	1931	1948
Demographic trend 1 (percentage of pop. in 2001 vs 1750)	340	402	657	55	333	127	585	76	101
Demographic trend 2 (percentage of pop. in 2001 vs 1875)	229	280	505	62	162	58	68	83	77
Demographic trend 3 (percentage of pop. 2001 vs 1925)	167	110	208	64	116	55	46	58	57
Population size (based on 2001 census)	1971	1205	1191	554	1164	1776	1523	835	1111

*Admixture episodes – events in history when a major influx of immigrants of different genetic background (at least 10% of the population) occurred within a single generation.
†Bottleneck event – event in history where a reduction in population size of at least 50% occurred within a single generation.

Table 2. Basic characteristics of the random samples obtained from each village (V1-V9)

Parameter	Village								
	Banjol (V1)	Barbat (V2)	Lopar (V3)	Rab (V4)	Sup. Draga (V5)	Vis (V6)	Komiža (V7)	Lastovo (V8)	Mljet (V9)
The size of random sample from population	100	100	100	100	100	100	100	100	100
Male-to-female ratio within the sample	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Median age and range within the sample	55.0 (20-80)	59.5 (21-76)	54.0 (22-87)	50.0 (19-80)	56.5 (22-82)	62.0 (24-80)	60.0 (18-83)	66.0 (27-88)	51.5 (18-78)
Proportion of inbred individuals*	7	13	10	4	5	12	27	3	11
Proportion of autochthonous individuals†	16	67	83	36	21	32	53	58	71
Proportion of admixed individuals‡	17	4	0	9	13	10	4	1	3
Proportion of outbred individuals§	60	16	7	51	61	46	16	38	15
Percentage of grandparental endogamy‖	42	90	98	39	47	88	91	72	94

*An examinee with the same (non-marital) surname, highly specific of the settlement found in at least one of their father's and one of their mother's parents, and if further genealogical information, ie the complete information on 2-3 ancestral generations for each individual included in the study indicated inbreeding.
†An examinee with all four of their grandparents born in the examinee's village of residence, but surnames did not indicate any inbreeding.
‡An examinee with both father's parents born in one small village, and both mother's parents in another, different small village.
§An examinee with either 3 or all 4 grandparents born in different larger settlements on the Croatian mainland.
‖Proportion of all grandparents of the examinees who were born in the same village as the examinees.

ropean Union respiratory health questionnaire (26), two simple questionnaires on socio-economical status and nutrition habits developed for this specific population (Appendix 1), and SF-36 questionnaire on quality of life (27). It usually took 25-40 minutes per examinee to complete the questionnaire.

Blood pressure (mmHg), height (mm), and body mass (kg) were each measured by a single observer in local health centers and dispensaries between 8 and 11 AM, following standard procedures (28). Blood pressure was measured on the right forearm in sitting position. Two measurements of both systolic and diastolic blood pressure were taken 5 minutes apart in each individual, and the mean value obtained from two readings was analyzed. Height and body mass were measured using a single anthropometer (Hospitalija, Zagreb, Croatia). Biochemical analyses of creatinine, uric acid, high and low density lipoproteins, total cholesterol, triglycerides, and blood glucose were done from fasting blood samples taken from the examinees between 7 and 9 AM (20 mL EDTA, [BD Vacutainer Systems, Franklin Lakes, NJ, USA], either for DNA extraction or liquid nitrogen storage of 2 × 0.5 mL aliquots for future transformation; 4.5 mL citrate for clotting factors and 10 mL clotted blood for serum biochemistry). Plasma and serum were rapidly frozen and stored at -20°C in 200 μL aliquots using standardized sample handling procedures. They were then transported frozen, within a maximum of 3 days, to a single biochemical laboratory based in Zagreb. The laboratory was chosen as it was internationally accredited for performing this type of analysis and included in internal quality assessment by Roche and Olympus, as well as in external monitoring programs by Croatian reference center for biochemical measurements and RIQAS international agency for quality control (29).

Defining personal genetic histories

Before the field study was undertaken, all randomly selected subjects were sent a form to complete their individual two-generation pedigree, including dates of birth, birthplaces, full names, and marital surnames of their both parents and all 4 grandparents. With respect to their personal genetic histories, the individuals included in this study were classified into one of the 4 categories based on their presumed individual genome-wide heterozygosity as follows: inbred, autochthonous, admixed, and outbred.

An examinee was considered inbred when the same (non-marital) surname, highly specific of the settlement, was found in at least one of their father's and one of their mother's parents,

and if further genealogical information, ie the complete information on 2-3 ancestral generations for each individual included in the study indicated inbreeding. Examinees were classified as autochthonous if all four of their grandparents were born in the examinee's village of residence, but surnames did not indicate any inbreeding. An examinee was strictly considered admixed when both father's parents were born in one small village, and both mother's parents in another, different small village. An examinee was strictly considered outbred when either 3 or all 4 grandparents were born in different larger settlements on the Croatian mainland.

Although personal genetic history is not an ordered categorical variable in strict terms, we could expect differences in mean individual genome-wide heterozygosity between the categories. This is because in closed isolated communities surnames are not polyphyletic and the genetic parameters computed from isonymy are highly correlated with the actual values (3,13). Category which includes the recently inbred examinees, would be expected to have the lowest mean value of individual genome-wide heterozygosity, followed by category of autochthonous examinees, in which cryptic homozygosity resulting from complex patterns of consanguinity in more distant past is likely present along with increased homozygosity due to population structure. Category of admixed examinees and especially category of outbred examinees would be expected to have increasing mean values of individual genome-wide heterozygosity.

Study design

The "1001 Dalmatians" data set contains information on 100 individuals from each of 9 different island villages and 101 individuals who moved into the 9 villages from the mainland, thus representing a genetic control population sharing the same environment. The 16 variables recorded for each individual were the following: village of residence, gender, age, personal genetic history (inbred, autochtonous, admixed, outbred), height, weight, body mass index, systolic blood pressure, diastolic blood pressure, creatinine, uric acid, cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, and glucose.

To address the hypotheses of interest, while controlling for several possible confounding effects, the study was designed as follows: first, all the inbred individuals in a larger sample of 1001 persons were identified based on self-reported information on the degree of relationship among their ancestors (the criterion was to have parents related as fourth cousins or closer). A total of 92 such individuals were identified (9.2% of the total sample). Then, for each examinee from the inbred group, an appropriate control was chosen from the autochthonous, admixed, and outbred categories. The controls were matched to inbred examinees by gender, age (±5 years), village of residence, level of education, and socio-economic status in all cases where this was strictly possible. The level of education was measured as the number of completed years in the education system (range: 4-20). Socioeconomic status was determined on the basis of information whether the examinee's family possessed a car, whether they acquired it in the past 5 years, whether they possessed a washing machine, or a color TV (range: 0-4).

Statistical analysis

For 76 inbred examinees it was strictly possible to find an appropriate autochthonous, admixed, and outbred control in the larger sample of 1001 examinees. The process of selection of the examinees into 4 categories with 76 matched individuals in each category was designed to minimize the "noise" from the likely confounding effects of age, gender, village of residence, education, and socio-economic status on health, thus maximizing the power of the study to detect true effects of personal genetic history on medically relevant quantitative traits. The association between per-

sonal genetic history and measured quantitative traits was then explored in two ways:

First, in the final sample of 304 individuals, the personal genetic history variable (defined as ordered categorical variable based on presumed increase in individual genome-wide heterozygosity between the 4 groups, with examinees in inbred group having an assigned value of 1, autochthonous of 2, admixed of 3, and outbred of 4) was correlated with each of the 10 measured quantitative traits using Spearman ρ.

Second, to further increase the power of the study to detect differences in health status attributable to personal genetic history, the individuals in inbred and autochthonous group were merged (n = 152) and compared with the 152 individuals in admixed and outbred group. The results for all 10 medically relevant quantitative traits were presented as medians and inter-quartile ranges, and the statistical significance of the differences was assessed using Mann-Whitney test (30). This test was chosen because the values of quantitative traits were expectedly showing multimodal distributions that did not satisfy the criterion of normality even after repeated attempts of logarithmic transformation. Statistical analysis was performed using SPSS 12.0.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

We examined the correlation between personal genetic history (defined as ordered categorical variable according to increasing presumed individual genome-wide heterozygosity) and 10 medically relevant quantitative traits (Table 3). Positive Spearman coefficient of correlation (ρ) implied that increasing presumed individual genome-wide heterozygosity had an increasing effect on trait value. The ρ value was negative for all traits except urate and high density lipoprotein cholesterol (Table 3). The only correlation that reached statistical significance was between personal genetic history and systolic blood pressure ($P = 0.006$). However, the effects on total cholesterol measurements ($P = 0.069$), high density lipoprotein cholesterol ($P = 0.071$), and diastolic blood pressure ($P = 0.113$) were also suggestive.

To increase the power of the study and possibly overcome some of the disadvantages, inbred and autochthonous samples were merged (n = 152) and the medians for all 10 traits were compared to the admixed and outbred samples (Table 4). When this was done, the effect of personal genetic history on systolic blood pressure remained strong and statistically significant ($P = 0.005$), whereas the effects on body mass index, diastolic blood pressure, and high density lipoprotein cholesterol also approached formal statistical significance ($P = 0.065$, $P = 0.063$, and $P = 0.071$, respectively). More importantly, this analysis revealed that the observed medians in 10 quantitative traits in inbred and autochthonous

Table 3. Correlation of personal genetic history* and 10 medically relevant quantitative traits

Quantitative trait	Spearman ρ	*P*
Body mass index	-0.072	0.211
Systolic blood pressure	-0.157	0.006
Diastolic blood pressure	-0.091	0.113
Creatinine	-0.079	0.170
Urate	0.017	0.770
Cholesterol	-0.105	0.069
Triglycerides	-0.035	0.543
HDL cholesterol	0.104	0.071
LDL cholesterol	-0.061	0.288
Glucose	-0.031	0.596

*Defined as ordered categorical variable according to increasing presumed individual genome-wide heterozygosity: 1 – inbred individual; 2 – autochthonous individual; 3 – admixed individual; 4 – outbred individual.

Table 4. Differences in observed values of 10 medically relevant quantitative traits between 152 inbred or autochtonous individuals and 152 admixed or outbred individuals (matched by age, gender, village of residence, education, and socio-economic status)

Quantitative trait (median and interquartile range)	Inbred or autochthonous (n = 152)	Admixed or outbred (n = 152)	*P**
Body mass index	28.2 (4.8)	27.5 (5.1)	0.065
Systolic blood pressure	150 (40)	140 (35)	0.005
Diastolic blood pressure	90 (15)	85 (15)	0.063
Creatinine	78 (19)	76 (19)	0.174
Urate	296 (100)	284 (128)	0.712
Cholesterol	6.30 (2.1)	6.10 (1.9)	0.256
Triglycerides	1.30 (0.8)	1.20 (0.7)	0.575
HDL cholesterol	1.00 (0.27)	1.10 (0.28)	0.071
LDL cholesterol	4.40 (2.1)	4.30 (2.1)	0.718
Glucose	5.40 (1.2)	5.30 (1.1)	0.237

*Mann-Whitney test.

group, with lower values of presumed individual genome-wide heterozygosity, were consistently less favorable in terms of health for all 10 measured traits. The value of high density lipoprotein cholesterol was decreased in the group with lower values of presumed individual genome-wide heterozygosity, whereas the values of all other nine traits were increased.

Matching of individuals by gender, age, village of residence, level of education, and socioeconomic status eliminated potential biases such as age-specific and gender-specific effects, isolate-specific effects of environment, or differences in data collection between populations, seasonality, and assessor-related differences. Furthermore, smoking could not have acted as a major confounder, as the prevalence of smokers across the four sub-groups was 26.3%, 30.3%, 27.6%, and 28.9%, with no sub-group being significantly different from others. The analysis of differences in 25 variables from the food frequency questionnaire showed no statistically significant difference for any single item at the level of $P<0.03$ or lower (a somewhat more stringent level chosen to account for multiple comparisons).

Discussion

Our study mainly suggested that inbred individuals generally have more pathologic values of quantitative traits than those of diverse genetic background. This could be explained through action of numerous rare and recessive variants of slightly deleterious effect that have not been selected against in childhood, as they mostly affect the variation in late-onset traits. Therefore, these variants could reach appreciable frequencies at numerous quantitative trait loci and show a non-specific pathologic effect on a number of quantitative traits, even in the levels of inbreeding are relatively low, as it was in our study. We believe that these observations are genuine, as the sub-sample comparison excluded a number of potential biases and confounding effects present at the level of between-population comparisons.

Furthermore, if we studied a single isolate population, it would not be possible to distinguish the effects of inbreeding (recessive inheritance) from kinship (dominant inheritance), as inbred people in a single community frequently tend to be related. However, since the metapopulation nature of our study population ensured that our inbred cases originate from 9 genetically different and highly structured isolate populations, the observed effects are attributable to inbreeding, and not kinship. The consistency of the effect on 9 measured traits (all negatively correlated with health) and the inverse effect on HDL cholesterol (positively correlated with health) strongly suggest that the observed effects are perhaps small, but likely to truly exist. The effects on some of the quantitative traits perhaps did not reach the formal significance because of the lack of study power and the problems in study design, such as possible misclassification.

Throughout history, human population has been organized in small and sparsely scattered isolate communities tied to the land they harvested. However, in the way of life of the last 5-6 generations dramatic changes have occurred, which have also affected the genetic structure. Some of the processes involved in this change, on both regional and global level, were increase in population size, outbreeding, gene flow, and admixture, First of all, an unprecedented increase in the population size, from about 1 billion (in 1850) to more than 6 billion (in 2000) happened as a result of measures to reduce childhood mortality (vaccination, antibiotic treatment of infections, improved nutrition, and sanitation). These measures also reduced the selection in childhood, which kept the human population size reasonably constant for centuries, and are predicted to create and retain many new and rare genetic variants introduced through mutations (31). Therefore, the increase in the population size is a likely cause of increased genetic diversity of contem-

porary human populations in comparison with those that lived only two centuries ago, and the effect is expected to be largely due to rare and recently introduced genetic variation.

Another population genetic phenomenon which occurred relatively recently on a massive scale is outbreeding. Organization of humans in small rural communities and lack of transport systems limited their mate choice, which makes it probable that inbreeding could have been quite prevalent throughout human history. Up to the year 1900, the vast majority of the world's population lived in villages, and even today up to 2 billion people globally are living in areas with considerable prevalence of consanguineous marriages (32,33). Even if we take a look at two-generation pedigree of inhabitants in our set of isolate populations, which are reasonably large villages, we can see that about 10% of all individuals were inbred, although inbreeding was generally discouraged. It is obvious that, in smaller historic villages, and with deeper look into genealogies, this percentage would have been even higher. Besides that, strong positive selection (through high childhood mortality) of infectious diseases on rare variants that were shared by relatives in closed communities was causing so-called "selective sweep," where standing genetic variation in population was being decreased through constant positive selection of relatives carrying rare protective alleles (34). As opposed to this, the process of urbanization suddenly shifted a considerable proportion of the human population from villages into the cities, where they had more opportunities to mate with individuals of genetically diverse background, causing a massive outbreeding at the global scale.

Finally, international travel and large intercontinental migrations of people during the second half of 20th century led to gene flow and admixture. The effects of urbanization at the regional level are now being repeated through international migrations and mating between people of different origin at the global level. Taking into account the relatively sudden occurrence and potentially enormous magnitude of all four effects, it is very surprising that the possible public health effects of changes in genetic structure of populations have hardly been addressed, let alone investigated. A careful review of the literature performed during 2002 and 2003 implied only a handful of attempts to address various elements of this hypothesis (12,13,20-22). The extent of this effect therefore remains uncertain and it would be worth investigating. Our "1001 Dalmatians" research program was established in a rather unique resource in an attempt to serve as a model for understanding these effects on regional and global scale.

The World Health Organization has recently defined major disease risk factors in the developed world that attribute most to disease burden in the population (35). Apart from smoking, other major risks include increases in body mass index, blood pressure, cholesterol levels, and blood glucose, which are all readily measurable quantitative traits. There is a plausible theoretical argument why inbreeding (decreased heterozygosity) and outbreeding (increased heterozygosity) should cause changes in mean population values of quantitative traits (16,17,31). Associations between heterozygosity and biologically important quantitative traits and their cumulative effect on fitness have been convincingly demonstrated in a number of plant and animal populations (36-44). However, we could only identify a handful of studies in human populations that provided data on these effects in post-reproductive age (45-47). The only important human quantitative trait that has been frequently associated with decreased heterozygosity is intelligence, with reasonably large number of supportive studies from various parts of the world showing strikingly large effect on the trait (22,48,49). Therefore, the main aim of this study was to investigate if changes in population structure could be associated with shifts in population distribution of quantitative traits identified as major disease risk

factors, such as body mass index, blood pressure, cholesterol, glucose, and others. We confirmed the results of several previous large-scale studies which showed the effect of inbreeding on blood pressure (12,45-47). We are currently developing methods to measure inbreeding from the genomic information, and plan to support the findings reported here with genomic measures of individual genome-wide heterozygosity calculated from genome-wide scans, using several hundred of microsatellite polymorphisms.

In this study, we also pointed to the facts that human population was rather small, subdivided, and inbred up to several generation ago, and that it has undergone dramatic changes in genetic structure at regional and global level due to expansion in size, urbanization and subsequent outbreeding, gene flow, and admixture. The effects of these changes on public health have not been addressed, although a number of plausible theoretical hypotheses and empirical observations imply that we should expect them (50). It has been suggested that considerable part of the increase in life expectancy in both developed and developing world between the years 1930 and 1960 could not be explained by known environmental improvements and economic factors (51,52). Therefore, perhaps the dramatic changes in genetic structure of human population could represent a forgotten variable that may be at least partially responsible for the observed trends.

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