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Original scientific paper

Inbreeding and Susceptibility to Osteoporosis in Croatian Island Isolates

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A B S T R A C T

The aim of this study was to investigate a recessive genetic component in susceptibility to osteoporosis (OP) by comparing its prevalence in isolated villages of three Croatian islands: Brač, Hvar and Korčula with different levels of inbreeding. A random sample of 20–30% adults from 14 villages was obtained, including a total of 1,389 examinees. The average inbreeding coefficient (F) of examinees from each village population was estimated using Wright's path method (based on genealogical information). The morphometry of the metacarpal bones was performed on hand-wrist radiographs of both hands in all examinees. OP was defined as values of cortical index smaller than 2 standard deviations based on distribution of values in examinees of the same sex under 45 years of age. Mean values of cortical index (CI) and prevalence of OP (both standardized by age and weighted for the sample size) in each village were correlated to the mean inbreeding coefficient (F). The coefficient of correlation (r) between F values and CI was –0.28 in males (p=0.08) and –0.42 in females (p=0.005), and between F and OP prevalence 0.32 in males (p<0.001) and 0.43 in females (p<0.001). These results indicate a trend of increased susceptibility to osteoporosis with increasing level of inbreeding in isolated communities of Croatian islands.

***Key words**: inbreeding, cortical index, osteoporosis, isolate populations, Croatia*

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Introduction

Osteoporosis is a common public health problem of post-reproductive age, characterized by reduced bone mass, changes in micro-architecture of the bone tissue and increased risk of fractures subsequent to those changes[1,2]. Similarly to many other common complex diseases of late onset, most cases in population probably result from the action of many different genes and their interaction with the environment[2,3]. However, osteoporosis seems to have much stronger genetic basis than most of the other late-onset diseases[4–7]. Twin and family studies indicate that a large majority of variance in quantitative traits such as bone mineral density can be explained by hereditary factors, and there is also a high correlation between siblings in skeletal geometry and bone turnover[1,4]. Some cases in population arise as a consequence of single-gene disorders, e.g. »osteoporosis-pseudoglioma syndrome«. Genome-wide linkage studies identified multiple candidate loci on chromosomes 1, 2, 5 and 11, but those associations seem to be modified by dietary calcium and vitamin D intake, which makes them difficult to repeat[4]. Significant non-hereditary risk factors include low calcium intake, vitamin D deficiency, physical inactivity, cigarette smoking, excessive consumption of protein, caffeine and alcohol, low body mass index and use of bone-resorbing medications[8–13].

There is great current interest in understanding genetic architecture of common complex diseases such as osteoporosis, as this is expected to lead to the development of genetic markers of increased disease risk and new therapeutical targets[3]. In this paper, we present an approach to study of osteoporosis that could provide a support for its predominantly genetic determination, with susceptibility mediated through a number of recessive genetic variants, most of them having a small individual effect on disease risk. The reasoning is simple: if a modest increase in number of genes identical by descent (e.g. an increase of inbreeding coefficient from 0% to 3%, predicted to affect about 800 genes) leads to significant changes in prevalence of osteoporosis, this is only consistent with large number of genomic loci influencing the disease. This conclusion is more valid if the study is conducted in an isolate population, in which the variation in environmental pressures is minimal and consanguinity is prevalent. Therefore, the studied population included 14 isolate villages from the eastern Adriatic islands of Hvar, Brač and Korčula, Croatia, an isolate resource well characterized through long-term multidisciplinary researches[14–17].

In this unique metapopulation of distinct human isolates, there is a long history of anthropological research into the determinants of skeleton-related biological traits. Initially, comparisons of within-population and between-population variation in traits such as metacarpal bone dimensions were used along with a larger number of other traits to assess population structure. The studies performed by the staff of the Institute for Anthropological Research in Zagreb, Croatia, consistently showed excellent compliance with models of population structure such as »isolation by distance« in several island populations, i.e. better »fit« to the model than observed for most of the other studied biological, bio-cultural and socio-cultural traits[18–27]. This group also characterized in detail the effects of gender and aging on bone loss[24–30] and reported on specific populations in which the expected effects of age and gender could not be shown[31]. The effects of occupation on bone loss and osteoarthritis were investigated[32,33]. An attempt to analyse genetic basis of the metacarpal bone dimensions was initially made through the analyses of their latent structure[34]. Recently, this

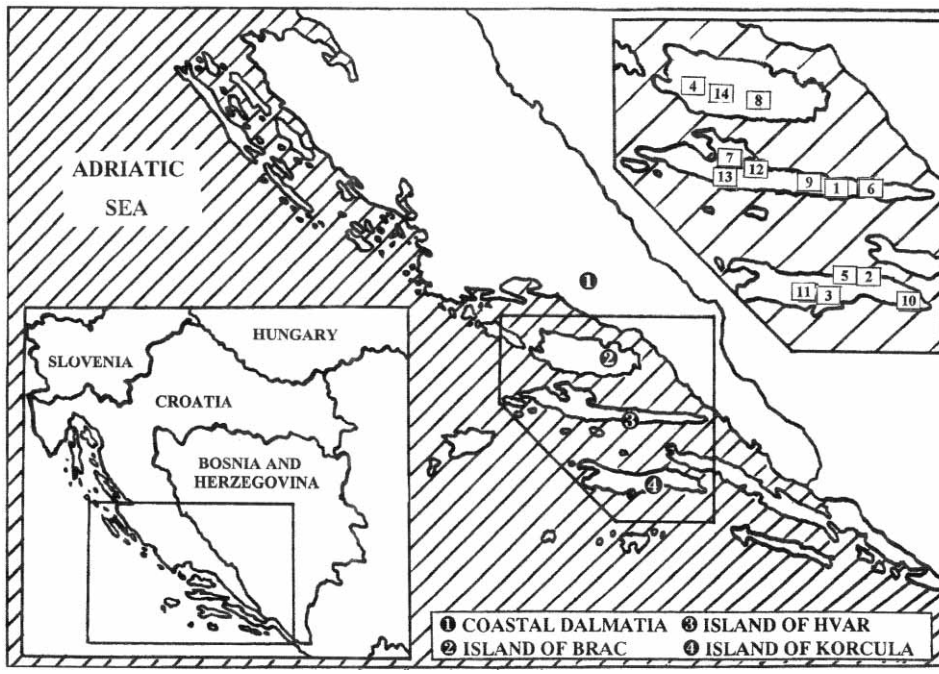

Fig. 1. Map of Dalmatian island genetic isolates showing study islands and villages (1–14).

group has also made progress in studying genetic determinants of bone-related measurements using pedigree data analyses[5,35].

Materials and Methods

A. Population choice

Croatia has 15 Adriatic Sea islands with population greater than 1,000. The villages on the islands have unique population histories and relative isolation from their neighboring villages and from the mainland through centuries. The 14 village populations of the three neighboring islands of the eastern Adriatic, Middle Dalmatia, Croatia (Brač, Hvar and Korčula, Figure 1) have been investigated by the Institute for Anthropological Research in Zagreb since early 1970's. More than 200 studies on these populations have been published to date in collaboration with many international scientists. The long-term researches included characterization of ethnohistory, migration patterns, genealogical reconstruction, analyses of many quantitative and qualitative biological traits, records of medical problems and study of environmental, socio-cultural and genetic characteristics. The most informative overviews of the results can be found in the papers of Rudan et al.[14–16,27], Bennett et al.[17] and Waddle et al.[36].

In Table 1, we list main sources that provide detailed information on population structure, inbreeding effects, monogenic (Mendelian) diseases and rare genetic variants found in particular islands and reported in the literature[37–69].

The 14 villages chosen for this study on the islands of Brač, Hvar and Korčula were founded during one of the three pe-

TABLE 1
BRIEF REVIEW OF POPULATION GENETIC RESEARCH UNDERTAKEN IN CROATIAN ISLAND ISOLATES IN THE PAST 33 YEARS

Type of research	Island	Ref.
A. Studies of population genetic variation		
STR polymorphisms	Krk, Brač, Hvar, Korčula	37–40
VNTR polymorphisms	Hvar	41
Y-chromosome haplogroups	Krk, Brač, Hvar, Korčula	42
mtDNA haplogroups	Krk, Brač, Hvar, Korčula	43,44
HLA markers or immunoglobulin allotypes	Krk, Hvar, Silba, Olib, Pag	45–48
Serogenetic polymorphisms	Brač, Hvar, Korčula, Silba, Olib,	49–52
B. Reports on autochthonous Mendelian diseases		
Dwarfism	Krk	53–55
Albinism	Krk	55
Progressive spastic quadriplegia	Krk	55
Familial cognitive dysfunction	Susak	56
Familial congenital hip dislocation	Lastovo	57
Familial ovarian cancer	Lastovo	58,59
Keratoderma palmoplantaris transgrediens	Mljet	60
C. Reports of high population frequencies of extremely rare genetic variants		
Deleted/triplicated alpha-globin gene	Silba	61
PGM1*W3 phosphoglucomutase-1 variant	Olib	62
mtDNA haplogroup F	Hvar	63
Y-chromosome haplogroup P*	Hvar	42
D. Studies of inbreeding effects		
Incidence of cancer	Brač, Hvar, Korčula, Vis, Lastovo	59
Prevalence of hypertension	Brač, Hvar, Korčula	64,65
Prevalence of 10 complex chronic diseases	Brač, Hvar, Korčula	66
Prevalence of learning disability	Brač, Hvar, Korčula, Susak	67
Prevalence of nephrolithiasis	Brač, Hvar, Korčula	68
Prevalence of malocclusion	Hvar	69

riods: BC era (by admixture of Illyrians, Greeks and succeeding Romans), 7th century AD (by Croats who immigrated from Asia) and 16–18th century AD (by Croats who fled from Balkans peninsula fearing Ottoman expansion). The subsequent tendency towards inbreeding in each village was influenced by a combination of geographic reasons (isolation), political reasons (»*The Paštrović Privileges*«) and socio-cultural reasons which were all extensively discussed elsewhere[14–17,27,70–72].

B. Field work and sample collection

During the field research between 1978 and 1987 undertaken by the Institute for Anthropological Research in Zagreb, the information was collected and various measurements performed on 1,389 adult individuals (682 males and 707 females) selected randomly from voting lists to form approximately 20–30% of the total population of these 14 villages. The collected data characterizing environmental variation included proportion of inhabitants with some college education (EDU), occupation in agriculture and fishery (OCC), regular consumption of traditional Mediterranean diet (NUT), smoking habits (SMO) and measurement of body mass index (BMI) (Table 2). The details on nutritional status assessment based on BMI are reported by Smolej Narančić[73]. Table 2 supports the hypothesis of decreased variation in most of the studied characteristics related to environment. This is important, as socio-economic status, occupation, diet, obesity and climate are usually highlighted as potential environmental risk factors for many common late onset diseases.

C. Estimation of mean inbreeding coefficients in each village

Genetic characterization of the 14 villages (Figure 1) included the computation of average inbreeding coefficient of each village based on three different methods: (i) reconstruction of genealogies for each examinee, (ii) analysis of parental isonymy from surname distribution and (iii) analysis of genotype distributions of MN, Ss and Kk serogenetic polymorphisms from blood samples obtained from all the examinees. Individual inbreeding coeffi-

TABLE 2
PREVALENCE OF FACTORS RELATED TO SOCIO-ECONOMIC STATUS, LIFESTYLE AND ENVIRONMENT IN 14 VILLAGES UNDER STUDY

Village[1]	EDU (%)	OCC (%)	NUT (%)	SMO (%)	BMI ($\bar{x}$)
Gdinj (H)	2.3	80.8	93.8	4.6	25.1
Pupnat (K)	2.1	89.7	91.8	9.3	26.7
Čara (K)	1.5	89.0	94.9	22.6	26.4
Dračevica (B)	2.4	85.7	90.5	23.8	28.6
Račišće (K)	1.9	87.5	83.6	23.1	27.3
Bogomolje (H)	2.5	84.0	93.8	4.9	23.0
Vrisnik (H)	2.1	82.3	91.6	13.5	25.5
G. Humac (B)	2.5	81.3	87.5	38.8	27.3
Zastražišće (H)	4.0	86.3	93.6	5.9	25.1
Lumbarda (K)	4.7	85.2	89.8	21.3	26.2
Smokvica (K)	3.1	81.4	81.4	27.8	26.0
Svirče (H)	2.0	80.1	83.2	12.8	25.7
Dol (H)	4.2	75.8	86.3	8.4	26.1
Nerežišća (B)	1.8	60.7	79.5	34.8	28.0

EDU = percentage of the population with higher education degree; OCC = percentage of the population working in agriculture/fishery; NUT = percentage of the population consuming »Mediterranean diet«; SMO = percentage of the population smoking; BMI = mean value of body mass index. The capital letters next to village names represent the islands (B=Brač, H=Hvar, K=Korčula).

cients were computed independently for each of 1,389 individuals. The pedigree information on 2–3 ancestral generations that was recorded for each examinee during the initial field work (1978–1987) was expanded during 1997–2000 through insight into the parish registries stored in local churches to allow the completion of the information on 4 ancestral generations in each examinee. The individual inbreeding coefficients (F) were then computed according to Wright's path method[74]:

$$F = \sum_{(1\rightarrow c)} (1/2)^{(n_i+m_i+1)}$$

where m and n refer to the number of paths from a common ancestor, and c refers to the number of common ancestors. The genealogical inbreeding coefficient for each village was then computed as the average of all individual F values. To further support these estimates, F was calculated from isonymy as[75,76]:

$$F = (P - \Sigma p_k q_k) / 4(1 - \Sigma p_k q_k) = (\Sigma p_k q_k) / 4 - (P - \Sigma p_k q_k) (\Sigma p_k q_k) / 16(1 - \Sigma p_k q_k)$$

where p_k is the frequency of the surname k in males, q_k in females, P is the proportion of marriages between spouses carrying the same surname among all marriages, and the summation is over all surnames. Apparently, in this approach the units of investigation are marital pairs of examinees' parents and not the examinees themselves. Finally, the inbreeding in each village was also assessed from the departure in the frequency of heterozygotes from the expectations based on Hardy-Weinberg equilibrium[77], where F is calculated as:

F = 1 – (observed proportion of heterozygotes / expected proportion of heterozygotes)

and where the expected proportion of heterozygotes calculated from allelic frequencies p and q of 2 alleles present in the population (MN, Ss and Kk) equals $2pq$. F values calculated from each of the 3 polymorphisms were added and divided by 3 to obtain the average F value for each village. The process of blood sample collection, storage, transport and analysis at the University of Newcastle upon Tyne was described in detail by Roberts et al.[49]. Table 3 reviews the average coefficients of inbreeding obtained by the applied three methods. Tables 2 and 3 indicate that the selected village populations represent an excellent model for studying effects of inbreeding, as a wide range of inbreeding coefficients is present while the concern over considerable confoundings related to environmental variance is reduced.

D. Cortical index and osteoporosis prevalence estimation

The osteometric dimensions of metacarpal bones are an efficient and practical method for investigation and monitoring bone mass. At the time when data used in the present analysis have been gathered, the radiogrammetry of the metacarpal bones has been widely used as the best available screening method of bone status in population studies. Procedure of metacarpal bones osteometry, as thoroughly described by Barnett and Nordin[78], was followed in field studies performed on all three investigated islands. Hand-wrist radiographs were taken using a single portable X-ray. Total diaphysis width (D) and medullary canal width (d) of the second left metacarpal bone was determined by a single, experienced observer[78]. Measurements were performed by one investigator using a millimeter ruler and a magnifying glass (×10) with a scale permitting 0.05-mm accuracy. Measurements were rounded to 0.1 mm. For each individual and for each bone, the cortical index (CI) was computed as:

$$CI = (D - d) \times 100 / D.$$

Tables 4 and 5 show the sample sizes and descriptive statistics of age and corti-

TABLE 3
GENETIC STRUCTURE OF 14 VILLAGES UNDER STUDY. THE VILLAGES ARE RANKED ACCORDING TO THE ESTIMATED AVERAGE INBREEDING COEFFICIENT CALCULATED FROM THE GENEALOGICAL DATA (*Fgen*)

Village	F(gen)	F(iso)	F(sgp)
Gdinj (H)	0.049	0.107	0.041
Pupnat (K)	0.044	0.034	0.108
Čara (K)	0.032	0.040	0.016
Dračevica (B)	0.031	0.031	0.049
Račišće (K)	0.027	0.034	0.004
Bogomolje (H)	0.025	0.030	0.018
Vrisnik (H)	0.015	0.023	0.013
G. Humac (B)	0.013	0.016	–0.016
Zastražišće (H)	0.013	0.013	–0.021
Lumbarda (K)	0.012	0.025	–0.010
Smokvica (K)	0.008	0.019	–0.107
Svirče (H)	0.008	0.008	–0.018
Dol (H)	0.005	0.004	–0.041
Nerežišća (B)	0.002	0.004	–0.076

The corresponding estimated based on isonymy *(Fiso)* and codominant serogenetic polymorphisms MN, Ss and Kk *(Fsgp)* are presented to support the results obtained through Wright's »path«-method *(Fgen)*. The capital letters next to village names represent the islands (B=Brač, H=Hvar, K=Korčula).

cal index by village in male and female examinees. As expected, the larger effect of age on cortical index and on osteoporosis prevalence was observed in females than in males (Figures 2 and 3). It is apparent that the effects of age are minimal in younger subjects (plateau) and around menopausal ages a decline in cortical index could be observed. This trend is more pronounced in females, which is in accordance with the findings in other populations. Complex segregation analysis of cortical index of the metacarpal bones has been performed by Ginsburg et al.[35] using pedigree data from the same populations. CSA model implemented included sex-specific parameterizations of inflection points of cortical index data and results – being 45 years for males and females in two most parsimonious models – which allows us to use 45 years as the reliable referent point for current analysis.

Prevalence of osteoporosis was established according to the statistical criteria. It was based on the distribution of cortical index values in people under the age of 45 in each gender separately. A »cut-off« value of cortical index was defined as the mean minus two standard deviations of the distribution in each sex. As this criterion has been widely used, and the measurements were performed by a single device and technician and analyzed by a single experienced assessor, we believe that the likelihood of substantial procedural errors is minimal.

TABLE 4
DESCRIPTIVE STATISTICS OF AGE AND CORTICAL INDEX BY VILLAGE IN MALE EXAMINEES.

Village	N	Age (yrs.)				Cortical index (%)			
		$\bar{x}$	SD	Min.	Max.	$\bar{x}$	SD	Min.	Max.
Gdinj (H)	51	54.53	11.72	24	81	55.97	7.89	37.78	72.22
Pupnat (K)	46	42.76	12.91	23	71	55.17	6.84	44.85	68.35
Čara (K)	63	42.59	11.87	20	71	53.11	8.42	32.37	72.07
Dračevica (B)	20	46.80	16.37	23	74	66.28	7.15	47.62	80.00
Račišće (K)	40	43.30	11.22	20	62	55.14	7.30	36.23	73.11
Bogomolje (H)	46	57.85	15.65	24	81	48.90	6.17	32.14	64.44
Vrisnik (H)	44	37.68	10.52	23	54	57.22	6.77	42.11	69.89
G. Humac (B)	27	51.04	16.81	22	85	62.87	6.92	49.49	76.14
Zastražišće (H)	69	50.09	14.78	20	77	53.41	7.45	32.43	76.09
Lumbarda (K)	53	43.21	11.36	22	77	59.70	7.60	41.09	77.83
Smokvica (K)	52	40.87	10.53	24	59	53.46	7.36	37.75	69.41
Svirče (H)	70	39.94	11.10	21	55	59.36	9.78	39.81	82.76
Dol (H)	50	41.44	10.37	20	56	56.73	6.15	40.00	70.51
Nerežišća (B)	51	45.59	13.86	22	81	62.31	9.07	44.12	82.02
Total	682	45.25	13.73	20	85	56.50	8.59	32.14	82.76

TABLE 5
DESCRIPTIVE STATISTICS OF AGE AND CORTICAL INDEX BY VILLAGE IN FEMALE EXAMINEES.

Village	N	Age (yrs.)				Cortical index (%)			
		$\bar{x}$	SD	Min.	Max.	$\bar{x}$	SD	Min.	Max.
Gdinj (H)	71	53.48	12.28	27	82	55.64	9.99	37.21	84.15
Pupnat (K)	50	44.76	11.74	21	61	56.84	8.67	34.53	75.00
Čara (K)	75	43.63	12.38	20	63	56.29	8.74	37.63	83.09
Dračevica (B)	23	52.48	15.82	21	87	65.11	12.46	42.22	92.41
Račišće (K)	63	43.29	11.83	21	71	58.22	8.58	40.18	77.14
Bogomolje (H)	32	58.50	15.62	19	78	50.44	9.89	29.35	75.00
Vrisnik (H)	28	41.43	8.69	23	54	61.20	9.28	43.37	76.40
G. Humac (B)	44	49.09	13.81	25	82	64.93	11.24	39.76	91.21
Zastražišće (H)	54	52.37	12.93	24	83	54.46	9.41	37.76	83.33
Lumbarda (K)	55	43.09	12.27	22	74	59.58	9.94	37.70	80.00
Smokvica (K)	42	44.48	12.41	23	61	58.28	11.17	32.08	82.45
Svirče (H)	65	39.25	10.88	20	55	63.90	9.81	40.26	87.18
Dol (H)	43	41.86	10.08	22	56	63.30	9.29	46.07	84.00
Nerežišća (B)	62	44.76	12.15	19	77	65.02	10.82	37.21	91.55
Total	707	46.15	13.21	19	87	59.31	10.60	29.35	92.41

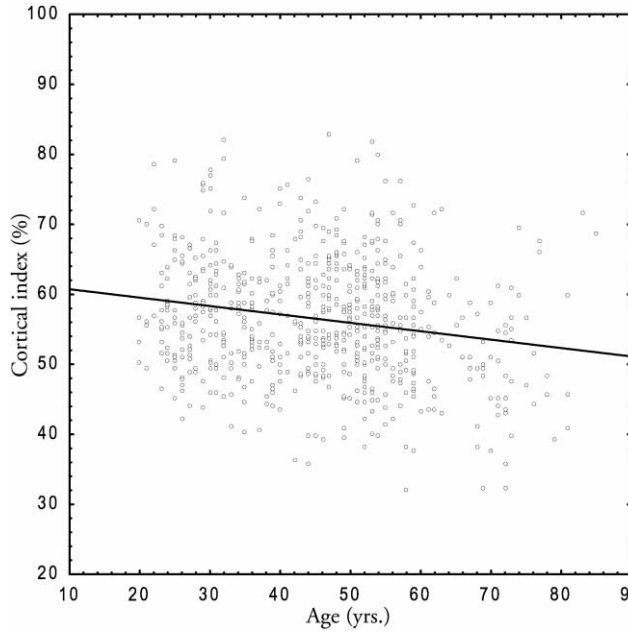

*Fig. 2. Effect of age on cortical index in males (r = –0.19, p<0.001, y = 61.932 – 0.120*x).*

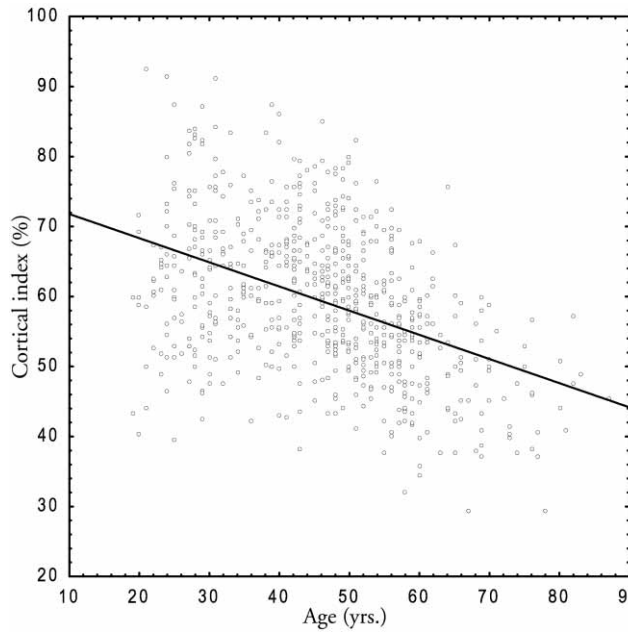

*Fig. 3. Effect of age on cortical index in females (r = –0.43, p<0.001, y = 72.251 – 0.345*x).*

E. Statistical analyses

To investigate the relationship between inbreeding and the cortical index and prevalence of osteoporosis, the ecologic epidemiological design was used[79]. The cortical index values and the prevalence of osteoporosis were compared among 14 villages with various levels of mean inbreeding. The cortical index values were adjusted for age effects in each sex by means of multiple regression (age, age^2) and mean values of standardized residuals were calculated for each village. As the estimated OP prevalence could be considerably influenced by variations in sex and age distribution of the sample sizes of different village they were adjusted according to the age and sex distribution in total sample and the results of the weighted data were presented separately for each sex. All statistical analyses were performed using »Statistica 6« software.

Results

In present study, the possible influence of inbreeding on bone mass was tested using ecologic-epidemiological design. Cortical index of metacarpal bones has been used as an indicator of total bone mass and good screening tool for susceptibility for osteoporosis appropriate for the population studies.

The coefficient of correlation (Figures 4 and 5) between F-values of each investigated village and mean values of the standardized age-adjusted residuals of metacarpal cortical index was –0.28 in males (p=0.08) and –0.42 in females (p=0.005). Correlations between F-values and estimated prevalence of osteoporosis weighted for sample sizes (Figures 6 and 7) were 0.32 in males (p<0.05) and 0.43 in females (p<0.001). Although the correlations in both traits were more significant in females than in males, there is a general concordance between values obtained in both sexes from the same village, which supports the hypothesis that the findings are indeed due to village-specific effects.

Obtained results clearly show a tendency of increased susceptibility to osteoporosis with increasing level of inbreeding

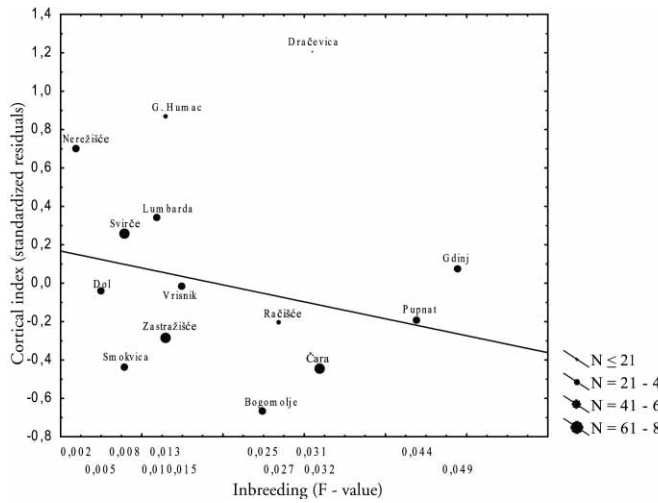

*Fig. 4. Effect of average inbreeding coefficient (F) on cortical index (standardized age-adjusted residuals weighted according to sample size) in male examinees in 14 villages (r = –0.28, p = 0.077, y = 0.168 – 8.823*x).*

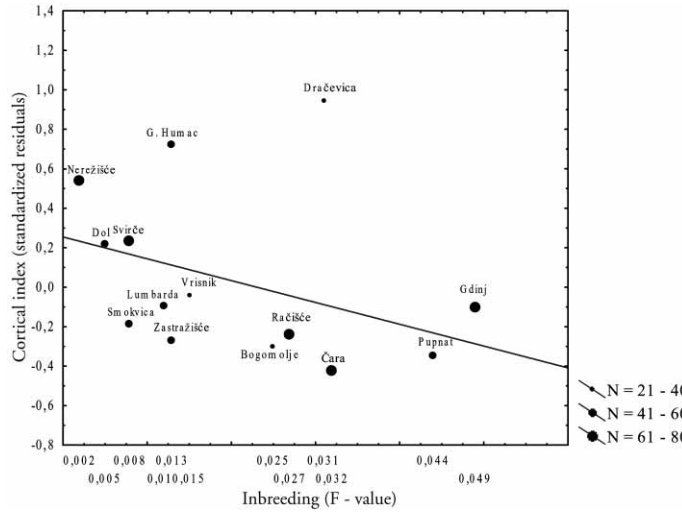

*Fig. 5. Effect of average inbreeding coefficient (F) on cortical index (standardized age-adjusted residuals weighted according to sample size) in female examinees in 14 villages (r = –0.42, p = 0.005, y = 0.255 – 11.057*x).*

in isolated communities of three Croatian islands. However, few villages are displaying as outliers in this general trend: Dračevica (Brač) with exceptionally high cortical index values and Čara (Hvar) with exceptionally high prevalence of osteopo-

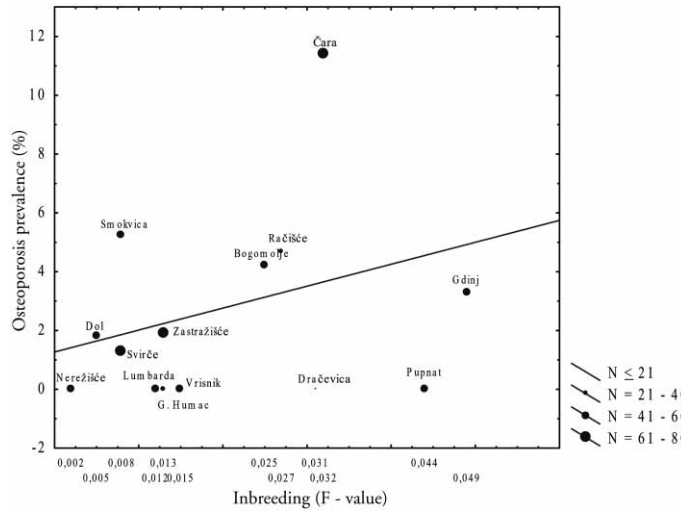

*Fig. 6. Effect of average inbreeding coefficient (F) on prevalence of osteoporosis (standardized by age, weighted according to sample size) in male examinees in 14 villages (r = 0.32, p<0.001, y = 1.269 + 74.598*x).*

rosis. We suspect that those villages are the sites of some possibly very intriguing genetic effects (drift) and therefore could be the promising sites for incoming studies of susceptibility/protection genes osteoporosis.

Discussion

There are several possible reasons why the effect of inbreeding on late-onset complex chronic diseases has not been widely evaluated to date. Firstly, in countries where inbreeding is prevalent in the population, life expectancy is generally considerably shorter than in western communities, and late-onset diseases do not represent the main public health problem. Therefore, the effects of inbreeding were mostly investigated in small isolated communities (geographic, cultural, linguistic, ethnic or religious isolates) in developed countries, which could be more easily reached. In most of the developed countries, however, the isolated human populations characterized by inbreeding often do not have the same level of access to health care as general (especially urban) population where the public health sector is well developed and the majority of epidemiological studies are being undertaken. Therefore, the health status of human isolates is not easily evaluated from medical records or communicated with their local physicians. In addition, there are not many isolate popu- lations world-wide with well-preserved parish registries from which reliable estimates of inbreeding coefficients can be determined based on the familial relationships over at least several ancestral generations. Furthermore, there are usually multiple concerns over confounding factors, as it is often quite difficult to find a non-inbred control population which would match the studied inbred isolate in environmental exposures and differ significantly only in genetic structure. Contemporary isolates usually share specific climate and environment, as well as a multitude of common socio-cultural factors such as diet, lifestyle, religion practices and socio-eco-

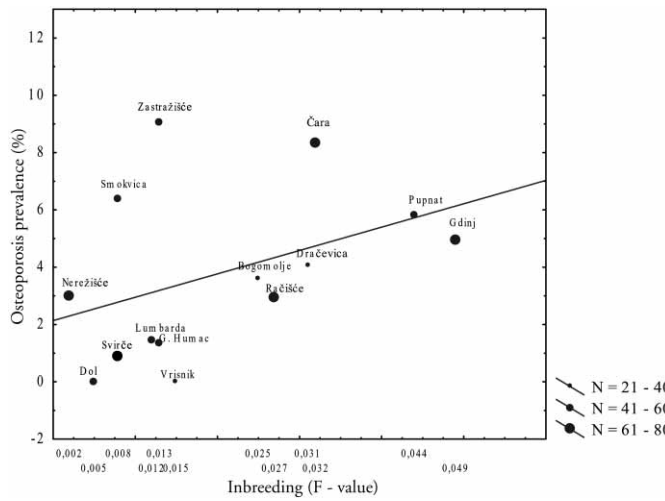

*Fig. 7. Effect of average inbreeding coefficient (F) on prevalence of osteoporosis (standardized by age, weighted according to sample size) in female examinees in 14 villages (r=0.43, p<0.001, y = 2.136 + 81.568*x).*

nomic status for which it is very difficult to control. In small and isolated communities the phenomena such as founder effect, genetic drift and inbreeding can significantly affect allele and genotype frequencies, which invalidates comparisons to control populations where genotype frequencies follow Hardy- Weinberg equilibrium[68]. Khoury concluded that, despite rare attempts, there is still hardly any study that would satisfactorily deal with all the usual concerns including small sample sizes, unreliable inbreeding estimates, doubtful disease dia- gnoses and inappropriate control populations[80]. We recently attempted to conduct a number of studies that would satisfactorily deal with those issues[64–69].

In this study, several precautions were taken to control for potential confounding effects. Primarily, a unique population was chosen, well characterized geneticall- ly and epidemiologically through almost 3 decades of continuing research by both local and international anthropologists. The subdivision of the islanders of the eastern Adriatic into small villages and a diversity of their attitudes towards inbreeding influenced by geographic isolation, political privileges in the past, as well as socio-cultural reasons resulted in a range of inbreeding coefficients present at both individual and population level. At the same time, the environmental variation within and between these populations is reduced (shared climate, religion, lifestyle, nutrition, predominant occupation and the level of education), as shown in Table 2. Therefore, we believe that these populations represent exceptionally good setting for undertaking the study of the effects of the inbreeding on complex chronic disease such as osteoporosis[59,64–69].

Further advantage of Croatian island isolates is that each of selected 14 villages in this study has its own church with well preserved records of births, marriages and deaths dating back to 1750, which helped reconstruct the genealogies and determine inbreeding coefficients. In addition, each village also has its own health clinic with the full-time local general practitioner and a nurse. The bone X-rays were performed in these clinics by the same device and same assessor from the Institute of Anthropological Research in Zagreb, as a very objective measurement of phenotype. As the results were later analyzed by a single and experienced observer, we believe that the like- lihood of substantial measurement errors is minimal (both observer-related and village-specific). An attempt was also made to avoid significant confounding effects of genetic drift and founder effect in specific villages. This has been achieved by including several villages of similar inbreeding levels from different islands into the groups with »high«, »moderate« and »low« inbreeding, as it is unlikely that the two population genetic phenomena would affect gene frequencies in the same direction in all villages[68].

The results showed a significant decreasing effect of inbreeding on cortical index (initially standardized by age) across 14 villages (Figures 4 and 5). Similarly, the prevalence of osteoporosis found in these villages appeared to increase from about 1.3% in villages with low inbreeding prevalence to nearly 4.0% in villages with average inbreeding coefficient close to F=0.05, i.e. a 3-fold increase. This result reaffirms the conclusion of our recent study where non-specific risk of inbreeding has been reported for a number of complex chronic diseases[66]. Analysis by village (Figures 6 and 7) shows that the standardized prevalence of osteoporosis follows the increase in average inbreeding coefficients, although this correlation is not really linear as there are villages with extremely increased prevalence in both sexes (e.g. Čara, with OP prevalence of about 10%). In addition, not all villages with higher rates of inbreeding reveal

high prevalence of osteoporosis (e.g. Pupnat in males). Those observations might be due to specific genetic structure of those villages which are characterized by increased or decreased frequencies of rare alleles with large effects mediating susceptibility to osteoporosis, possibly due to combined effect of genetic drift and founder effect.

If we accept the conclusion that the increase in inbreeding of about 2–3% (from F=0.005 to 0.03) could be, to some extent, responsible for the observed increase in prevalence of osteoporosis, the central question becomes what does it tell us about the genetic basis of the disease. Apparently, the susceptibility seems to be controlled, at least partly, by the recessive genetic variants that are more likely to be rare than common, as the inbreeding effects on rare variants are more apparent. In addition, if we accept that the total number of genes in the human genome, according to the most recent estimates, is about 25,000[81], then the increase in inbreeding of 3% would correspond to having about 800 random genes across the genome identical by descent. If this unrecombined autozygosity in only 3% of genes could lead to a notable effect in the prevalence of osteoporosis, there are two main mechanisms that could explain it. Firstly, inbreeding may mainly act through the rare variants of major effect, that may be enriched in frequency in each village by combined effects of founder effect and subsequent genetic drift. Second, the genes controlling this trait are of small effect and very numerous, scattered across the entire genome. The design of this study provides some arguments against the first explanation. Major effect genes arise after mutations that are considered to be very rare, as the probability of random mutation causing small effect is much greater. Therefore, even if such mutations were present in some of the studied villages, it is extremely unlikely that similar effects of inbreeding would be observed across several villages, as our results indicated. In addition, under such assumption the differences between inbred and outbred individuals would normally be much larger than it was the case in our study. It is more plausible that the genetic control of cortical index, bone mass loss and susceptibility to osteoporosis in humans is at least partly controlled by a larger number of genes of small individual effects[68].

This study attempted to discuss the genetic basis of susceptibility to osteoporosis through inbreeding study in relatively isolated communities. The results provided further evidence on polygenic basis of susceptibility for osteoporosis with detectable effects of recessive genes.

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REFERENCES

1. RALSTON, S. H., Quart. J. Med., 90 (1997) 247. — 2. STEWART, T. L., S. H. RALSTON, J. Endocrinol., 166 (2000) 235. — 3. ALBAGHA, O. M., S. H. RALSTON, Endocrinol. Metab. Clin. North Am., 32 (2003) 65. — 4. RALSTON, S. H., J. Clin. Endocrinol. Metab., 87 (2002) 2460. — 5. ŠKARIĆ-JURIĆ, T., P. RUDAN, Coll. Antropol., 21 (1997) 447. — 6. KARASIK, D., E. GINSBURG, G. LIVSHITS, O. PAVLOVSKY, E. KOBYLIANSKY, Genet. Epidemiol., 19 (2000) 410. — 7. KOBYLIANSKY, E., D. KARASIK, V. BELKIN, G. LIVSHITS, Ann. Hum. Biol., 27 (2000) 433. — 8. DEAL, C. L., Am. J. Med., 102 (1997) 35S. — 9. PRENTICE, A., Public Health Nutr., 7 (2004) 227. — 10. NGUYEN, T. V., J. R. CENTER, J. A.

EISMAN, Med. J. Aust., 180 Suppl 5 (2004) S18. — 11. MATKOVIĆ, V., K. KOSTIAL, I. ŠIMONOVIĆ, R. BUZINA, A. BRODAREC, B. E. C. NORDIN, Am. J. Clin. Nutr., 32 (1979) 540. — 12. MATKOVIĆ, V.: Influence of age, sex and nutrition on bone loss. (Ph.D. Thesis, University of Zagreb, Zagreb, 1976) (In Croat.). — 13. STINI, W. A., Coll. Antropol., 27 (2003) 23. — 14. RUDAN, P., D. ŠIMIĆ, N. SMOLEJ NARANČIĆ, L. A. BENNETT, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, M. F. LETHBRIDGE, J. MILIČIĆ, D. F. ROBERTS, A. SUJOLDŽIĆ, L. SZIROVICZA, Am. J. Phys. Anthropol., 74 (1987) 417. — 15. RUDAN, P., A. SUJOLDŽIĆ, D. ŠIMIĆ, L. A. BENNETT, D. F. ROBERTS, In: ROBERTS, D. F., N. FUJIKI, K. TORIZUKA (Eds): Isolation, Migration and Health. (Cambridge University Press, Cambridge, 1992). — 16. RU- DAN, I., H. CAMPBELL, P. RUDAN, Coll. Antropol., 23 (1999) 531. — 17. BENNETT, L. A., J. L. ANGEL, D. F. ROBERTS, P. RUDAN, Coll. Antropol., 7 (1983) 195. — 18. RUDAN, P., D. ŠIMIĆ, L.A. BENNETT, Am. J. Phys. Anthropol., 77 (1988) 97. — 19. ŠIMIĆ, D., P. RUDAN, Hum. Biol., 62 (1990) 113. — 20. RUDAN, I., P. RUDAN, L. SZIROVICZA, D. ŠIMIĆ, L. A. BENNETT, Homo, 47 (1996) 257. — 21. RUDAN, I., P. RUDAN, A. CHAVENTRE, B. JANIĆIJEVIĆ, J. MILIČIĆ, N. SMOLEJ NARANČIĆ, A. SUJOLDŽIĆ, Homo, 49 (1998) 201. — 22. RUDAN, I., P. RUDAN, In: BODZSAR, B. E., C. SUSSANNE (Eds.): Studies in Human Biology. (Eotvos University Press, Budapest, 1996, pp. 351–367). — 23. ŠIMIĆ, D., P. RUDAN, L. A. BENNETT, In: ROBERTS, D. F., A. CHAVENTRE (Eds.): Pluridisciplinary Approach of Human Isolates. (INED, Paris, 1990, pp. 149–162). — 24. RUDAN, P., J. L. ANGEL, L. A. BENNETT, B. FINKA, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, M. F. LETHBRIDGE, J. MILIČIĆ, M. MIŠIGOJ, N. SMOLEJ NARANČIĆ, A. SUJOLDŽIĆ, L. SZIROVICZA, D. ŠIMIĆ, P. ŠIMUNOVIĆ: Anthropological Investiga- tions of the Eastern Adriatic, Book 1: Biological and Cultural Microdifferentiation among the village populations of the island of Korčula and the Pelješac peninsula. (HAD, Zagreb, 1987) (In Croat.). — 25. RUDAN, P., B. FINKA, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, V. KUŠEC, J. MILIČIĆ, M. MIŠIGOJ- DURAKOVIĆ, D. F. ROBERTS, Lj. SCHMUTZER, N. SMOLEJ NARANČIĆ, A. SUJOLDŽIĆ, L. SZIROVICZA, D. ŠIMIĆ, P. ŠIMUNOVIĆ, S. M. SPOLJAR- VRZINA: Anthropological Investigations of the Eastern Adriatic, Book 2: Biological and Cultural Microdifferentiation among the village populations of the island of Hvar. (HAD, Zagreb, 1990) (In Croat.). — 26. RUDAN, P., L. A. BENNETT, B. FINKA, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, V. KUŠEC, M. LETHBRIDGE-CEJKU, J. MILIČIĆ, Lj. SCHMUTZER, N. SMOLEJ NARANČIĆ, A. SUJOLDŽIĆ, D. ŠIMIĆ, P. ŠIMUNOVIĆ, S. M. ŠPOLJAR-VRŽINA: Anthropological Investigations of the Eastern Adriatic, Book 3: Biological and Cultural Microdifferentiation among the village populations of the island of Brač. (HAD, Zagreb, 1990) (In Croat). — 27. RUDAN, P., B. JANIĆIJEVIĆ, V. JOVANOVIĆ, J. MILIČIĆ, N. SMOLEJ-NARANČIĆ, A. SUJOLDŽIĆ, L. SZIROVICZA, T. ŠKARIĆ-JURIĆ, L. BARAĆ LAUC, T. LAUC, I. MARTINOVIĆ KLARIĆ, M. PERIČIĆ, D. RUDAN, I. RUDAN, Coll. Antropol. 28 Suppl. 2 (2004) 321. — 28. KUŠEC, V., D. ŠIMIĆ, A. CHAVENTRE, J. D. TOBIN, C. C. PLATO, P. RUDAN, Coll. Antropol., 12 (1988) 309. — 29. LOVASIĆ, I., T. ŠKARIĆ-JURIĆ, B. BUDISELIĆ, L. SZIROVICZA, Coll. Antropol., 22 (1998) 307. — 30. MARTINOVIĆ KLARIĆ, I., F. LOVASIĆ, B. BUDISELIĆ, T. ŠKARIĆ-JURIĆ, L. SZIROVICZA, A. CHAVENTRÉ, Coll. Antropol., 23 (1999) 91. — 31. BEHLULI, I., M. LETHBRIDGE-CEJKU, C. C. PLATO, P. RUDAN, I. RUDAN, W. A. STINI, J. D. TOBIN, Med. Jad., 21 (1991) 55. — 32. LETHBRIDGE-ČEJKU, M.: Osteoarthritis of the hands in a rural population (Anthropological research on the island of Brac, Croatia). (Ph.D. Thesis, University of Zagreb, Zagreb, 1995) (In Croat.). — 33. LETHBRIDGE-ČEJKU, M., C. C. PLATO, P. RUDAN, Am. J. Hum. Biol., 9 (1997) 136. — 34. ŠIMIĆ, D., A. CHAVENTRE, C. C. PLATO, J. D. TOBIN, P. RUDAN, Ann. Physiol. Anthrop., 11 (1992) 3. — 35. GINSBURG, E., T. ŠKARIĆ-JURIĆ, E. KOBILYANSKY, D. KARASIK, I. MALKIN, P. RUDAN, Am. J. Hum. Biol., 13 (2001) 398. — 36. WADDLE, D. M., R. R. SOKAL, P. RUDAN, Hum. Biol., 70 (1998) 845. — 37. MARTINOVIĆ KLARIĆ, I., Am. J. Hum. Biol., 12 (2000) 509. — 38. MARTINOVIĆ KLARIĆ, I., L. BARAĆ, D. BUKOVIĆ, I. FURAČ, G. GEBER, B. JANIĆIJEVIĆ, M. KUBAT, M. PERIČIĆ, B. VIDOVIĆ, P. RUDAN, Homo, 51 (2000) 141. — 39. MARTINOVIĆ, I., L. BARAĆ, I. FURAČ, B. JANIĆIJEVIĆ, M. KUBAT, M. PERIČIĆ, B. VIDOVIĆ, P. RUDAN, Hum. Biol., 71 (1999) 341. — 40. MARTINOVIĆ KLARIĆ, I., L. BARAĆ, D. BUKOVIĆ, I. FURAČ, G. GEBER, B. JANIĆIJEVIĆ, M. KUBAT, M. PERIČIĆ, B. VIDOVIĆ PUPIĆ, P. RUDAN, Ann. Hum. Biol., 28 (2001) 281. — 41. MARTINOVIĆ, I., S. MASTANA, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, S. S. PAPIHA, D. F. ROBERTS, P. RUDAN, Ann. Hum. Biol., 25 (1998) 489. — 42. BARAĆ, L., M. PERIČIĆ, I. MARTINOVIĆ KLARIĆ, S. ROOTSI, B. JANIĆIJEVIĆ, T. KIVISILD, J. PARIK, I. RUDAN, R. VILLEMS, P. RUDAN, Eur. J. Hum. Genet., 11 (2003) 535. — 43. TOLK, H.-V., M. PERIČIĆ, L. BARAĆ, I. MARTINOVIĆ KLARIĆ, B. JANIĆIJEVIĆ, I. RUDAN, J. PARIK, R. VILLEMS, P. RUDAN, Coll. Antropol., 24 (2000) 267. — 44. TORRONI, A., H.-J. BANDELT, V. MACAULAY, M. RICHARD, M. CRUCIANI, C. RENGO, V. MARTINEZ-CABRERA, R. VILLEMS, T. KIVISILD, E. METSPALU, J. PARIK, H.-V. TOLK, K. TAMBETS, P. FORSTER, B. KARGER, P. FRANCALACCI, P. RUDAN, B. JANICIJEVIC, O. RICKARDS, M.-L. SAVONTAUS, K. HUOPONEN, V. LAITINEN, S. KOIVUMAKI, B. SYKES, E. HICKEY, A. NOVELLETTO, P. MORAL, D. SELLITTO, Am. J. Hum. Genet., 69 (2001) 844. — 45. MARTINOVIĆ, I., M. BAKRAN, A. CHAVENTRE, B. JANIĆIJEVIĆ, V. JOVANOVIĆ, N. SMOLEJ NARANČIĆ, A. KAŠTELAN, Z. GRUBIĆ, Z. ŽUNEC, D. F. ROBERTS, P. RUDAN, Hum. Biol., 69 (1997) 819. — 46. GRUBIĆ, Z., R. ŽUNEC, E. ČEČUK-JELIČIĆ, V. KERHIN- BRKLJAČIĆ, D. KAŠTELAN, L. BARAĆ, B. JANIĆIJEVIĆ, I. MARTINOVIĆ, M. PERIČIĆ, L. A. BENNETT, P. RUDAN, A. KAŠTELAN, Coll. An-

tropol., 22 (1998) 157. — 47. CAMBON-THOMSEN, A., E. SOMMER, A. SEVIN, A. CHAVENTRE, N. BOROT, E. OHAYON, P. RUDAN, Coll. Antropol., 13 (1989) 311. — 48. BOROT, N., J. M. DUGOUJON, B. JANIĆIJEVIĆ, P. RUDAN, A. CHAVENTRE, Coll. Antropol., 15 (1991) 247. — 49. ROBERTS, D. F., Z. M. NOOR, S. S. PAPIHA, P. RUDAN, Ann. Hum. Biol., 19 (1992) 539. — 50. JANIĆIJEVIĆ, B., M. BAKRAN, S. S. PAPIHA, A. CHAVENTRE, D. F. ROBERTS, Hum. Biol., 66 (1994) 991. — 51. JANIĆIJEVIĆ, B., Coll. Antropol., 12 (1988) 369. — 52. ARNAUD, J., N. BOROT, A. CHAVENTRE, B. JANIĆIJEVIĆ, A. E. SAMMARTINO, A. SUJOLDŽIĆ, P. RUDAN, R. JAMBOU, Coll. Antropol., 13 (1989) 281. — 53. KOPAJTIĆ, B., M. DUJMOVIĆ, Z. KOLACIO, V. KOGOJ-BAKIĆ, Coll. Antropol., 19 (1995) 365. — 54. KRŽIŠNIK, C., Z. KOLACIO, T. BATTELINO, M. BROWN, J. S. PARKS, Z. LARON, J. Endocr. Genet., 1 (1999) 9. — 55. ZERGOLLERN, L., Birth Defects Orig. Artic. Ser., 7 (1971) 28. — 56. BOHAČEK, N., Liječ. Vjesn., 86 (1964) 1412. — 57. MARIČEVIĆ, A., Liječ. Vjesn., 117 (1995) 126. — 58. RUDAN, I., Hum. Biol., 73 (2001) 871. — 59. RUDAN, I., Hum. Biol., 71 (1999) 173. — 60. BAKIJA-KONSUO, A., A. BASTA-JUZBAŠIĆ, I. RUDAN, M. SITUM, M. NARDELLI-KOVAČIĆ, S. LEVANAT, J. FISCHER, D. HOHL, D. LONČARIĆ, S. SEIWERT, H. CAMPBELL, Dermatology, 205 (2002) 32. — 61. TURČINOV, D., R. KRISHNAMOORTHY, B. JANIĆIJEVIĆ, I. MARKOVIĆ, C. LAPOUMEROULIE, A. CHAVENTRE, P. RUDAN, Coll. Antropol., 24 (2000) 295. — 62. BOROT, N., J. ARNAUD, P. RUDAN, A. CHAVENTRE, J. SEVIN, Hum. Hered., 41 (1991) 309. — 63. TOLK, H.-V., L. BARAĆ, M. PERIČIĆ, I. MARTINOVIĆ KLARIĆ, B. JANIĆIJEVIĆ, H. CAMPBELL, I. RUDAN, T. KIVISILD, R. VILLEMS, P. RUDAN, Eur. J. Hum. Genet., 9 (2001) 717. — 64. SMOLEJ NARANČIĆ, N., I. RUDAN, J. Physiol. Anthropol. Appl. Human Sci., 20 (2001) 85. — 65. RUDAN, I., N. SMOLEJ NARANČIĆ, H. CAMPBELL, A. CAROTHERS, A. WRIGHT, B. JANIĆIJEVIĆ, P. RUDAN, Genetics, 163 (2003) 1011. — 66. RUDAN, I., D. RUDAN, H. CAMPBELL, A. CAROTHERS, A. WRIGHT, N. SMOLEJ-NARANČIĆ, B. JANIĆIJEVIĆ, L. JIN, R. CHAKRABORTY, R. DEKA, P. RUDAN, J. Med. Genet., 40 (2003) 925. — 67. RUDAN, I., D. RUDAN, H. CAMPBELL, Z. BILOGLAV, L. SIBBETT, B. JANIĆIJEVIĆ, N. SMOLEJ NARANČIĆ, P. RUDAN, Coll. Antropol., 26 (2002) 421. — 68. RUDAN, I., M. PADOVAN, D. RUDAN, H. CAMPBELL, Z. BILOGLAV, B. JANIĆIJEVIĆ, N. SMOLEJ NARANČIĆ, P. RUDAN, Coll. Antropol., 26 (2002) 11. — 69. LAUC, T., P. RUDAN, I. RUDAN, H. CAMPBELL, J. Orthod., 30 (2003) 301. — 70. FORENBAHER, S., Coll. Antropol., 26 (2002) 361. — 71. MALNAR, A., Coll. Antropol., 26 (2002) 411. — 72. ŠKREBLIN, L., L. ŠIMIČIĆ, A. SUJOLDŽIĆ, Coll. Antropol., 26 (2002) 333. — 73. SMOLEJ NARANČIĆ, N., Coll. Antropol., 23 (1999) 59. — 74. WRIGHT, S., Am. Naturalist, 56 (1922) 330. — 75. TAY, J. S., W. C. YIP, Ann. Hum. Genet., 48 (1984) 185. — 76. ROGULJIĆ, D., I. RUDAN, P. RUDAN, Am. J. Hum. Biol., 9 (1997) 595. — 77. FALCONER, D. S., T. F. C. MACKAY: Introduction to quantitative genetics, 4th Ed. (Longman, Harlow, UK, 1996). — 78. BARNETT, L., B. E. C. NORDIN, Clin. Radiol., 11 (1960) 166. — 79. ROTHMAN, K. J., S. GREENLAND: Modern epidemiology, 2nd edition. (Lippincott, Williams & Wilkins Publishers, 1998). — 80. KHOURY, M. J., B. H. COHEN, T. H. BEATY: Fundamentals of genetic epidemiology, 1st edition. (Oxford University Press, Oxford, 1993). — 81. INTERNATIONAL HUMAN GENOME SEQUENCING CONSORTIUM, Nature, 431 (2004) 931.

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SROĐIVANJE I SKLONOST OSTEOPOROZI U IZOLIRANIM OTOČNIM POPULACIJAMA HRVATSKE

S A Ž E T A K

Cilj ovog istraživanja bio je analizirati recesivnu genetsku komponentu vrijednosti kortikalnog indeksa druge metakarpalne kosti te procijenjenu prevalenciju osteoporoze (OP) u 14 sela hrvatskih otoka Brača, Hvara i Korčule s različitom razinom srođivanja. Slučajni uzorak uključio je 20–30% odraslog stanovništva tih sela i obuhvatio ukupno 1389 ispitanika. Prosječan koeficijent urođenosti (F) ispitanika svakog sela procijenjen

je Wrightovom »path« metodom (temelji se na informacijama pohranjenima u rodoslovlju). Morfometrija metakarpalnih kostiju učinjena je na rendgenskim snimkama šake i ručnog zgloba na obje ruke u svih ispitanika. Osteoporozu smo definirali kao vrijednosti kortikalnog indeksa manju od 2 standardne devijacije temeljenu na raspodjeli vrijednosti u ispitanika istog spola i dobi ispod 45 godina. Prosječne vrijednosti kortikalnog indeksa i prevalencije osteoporoze u svakom selu (standardizirane s obzirom na dob ispitanika te ponderirane s obzirom na veličinu uzorka) korelirane su s prosječnim koeficijentom urođenosti sela. Koeficijent korelacije (r) između F vrijednosti i CI bio je –0,28 u muškaraca ($p=0,08$) i –0,42 u žena ($p=0,005$), a između F vrijednosti i standardiziranih prevalencija OP iznosio je 0,32 u muškaraca ($p<0,001$) i 0,43 u žena ($p<0,001$). Ovi rezultati ukazuju na značajnu »depresiju srođivanja« kortikalnog indeksa kao pokazatelja smanjenja koštane mase, što upućuje na povećanu sklonost osteoporozi u starijoj životnoj dobi (OP) u srođenim zajednicama hrvatskih otoka.