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Genetic Epidemiological Studies of Eastern Adriatic Island Isolates, Croatia: Objectives and Strategies

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ABSTRACT

In this paper, the authors discuss why isolate island populations represent a particularly helpful model for genetic epidemiological studies. A thorough previous anthropological research carried out in Eastern Adriatic island isolates, Croatia, in terms of ethnohistory, geography and current demography is reviewed. The major results of the studies of population genetic structure of those populations, including model-bound and model-free approaches, the analyses of serogenetic polymorphisms and most recent studies using HLA class II, VNTR and STR DNA polymorphisms, are briefly presented. The organization of health care on the islands is analyzed and some relevant details of specific medical problems and some autochtonous diseases in these island populations is noted. The authors present in outline four illustrative examples of research opportunities which are afforded by the unique circumstances found in these isolate communities. These relate to hereditary dwarfism on Krk island, Mal de Meleda on Mljet island, extreme inbreeding on Susak island and population genetics of cancer on the islands of Brač, Hvar, Korčula, Vis and Lastovo. Finally, the authors develop objectives and strategies for a long-term genetic epidemiological research of these populations and suggest that such a programme of investigation would further our understanding of the causes of (rare) diseases which are uniquely important to these communities but also of common diseases which are important contributors to the burden of disease both in these islands and throughout the world.

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Introduction

In this article, the authors will discuss why isolate island populations represent a particularly promising resource for genetic epidemiological studies. In order to set the context for these discussions key findings from previous anthropological research carried out in Eastern Adriatic island isolates, Croatia, in terms of ethnohistory, geography, current demography and population genetic structure will be briefly reviewed. Previous reports concerning specific medical problems and some autochtonous diseases in these island populations will be summarized and evaluated. Finally, the authors will develop objectives and strategies for a long-term programme of genetic epidemiological research in these populations.

Isolate island populations as a setting for genetic epidemiological studies

The ultimate aim of genetic epidemiology is to identify and to characterize population-level factors that contribute to disease. That aim is often pursued through the studies that simultaneously invoke principles in several diverse disciplines (e.g., population genetics, epidemiology and biostatistics, molecular biology, demography, evolutionary genetics, pharmacoepidemiology and ecology)[1].

The two principle approaches to gene discovery – identifying genetic fators contributing to human disease are linkage and association studies. However, the disappointing results of these approaches with respect to common diseases (as opposed to rare Mendelain disorders where there are many examples of success) has led to a review of the most promising approaches for the future. There are three main approaches which are currently favoured. Studies of affected relative pairs (or trios), most commonly affected sib pairs (non parametric linkage), linkage analysis of large (multiplex) families heavily enriched for genetic factors, and most recently, studying patients from genetic isolate populations. The first approach (sib-pair analysis) has limited power to detect the small genetic effects which are likely to operate in most common complex disorders. However, analytic procedures are well described, are relatively simple to employ and are robust to genetic heterogeneity. The studies of large families with multiply affected members have shown success in identifying the small minority of cases of some common disorders in which a major genetic effect is operating. However, statistical analysis in computationally demanding. Recent developments in methods have recently been reported by Zhao and coworkers [2–7], Schork[8], Ellsworth and Manolio[9] and Korczak and Goldstein[10].

Very recently, an entirely new approach has surprised with its power and efficiency: the collection of patients in genetic isolate human populations and the search for shared haplotypes followed by a fine-resolution mapping. This is possible because of a recent common ancestry of haplotypes in such populations, which facilitates gene mapping, with some methods (such as homozygosity mapping for recessive conditions) only requiring the study of a very few affected individuals who share a remote common ancestor. A good example is the study of Bull and coworkers[11], who mapped the FIC 1 gene, responsible for benign recurrent intrahepatic cholestasis (BRIC), in the population of Costa Rica. Such result has provoked an increased interest in isolate island populations, as a suitable setting for efficient and powerful research in genetic epidemiology.

Eastern Adriatic island isolates, Croatia: Ethnohistory

1. Illyrian period, Greek and Roman colonies – The earliest available data show that the eastern Adriatic region

was inhabited as early as the Neolithic by non-Indo-European populations, and then (around 2.000 BC) by Proto-Illyrians, and later Illyrians. Greek colonies were formed in the fourth century B.C. The succeeding Romans colonized the whole region during the 3rd century B.C., and their domination lasted until the 6th century A.D.

2. The influx of Croats – The first great influx of Croats (Slavs) into the area occurred between the 6th and 8th century. The existence of very old Croatian (Slavic) toponyms imply that the Croatians inhabited most of the Eastern Adriatic islands very early, assimilating the remains of Illyrian, Greek and Roman settlers, and thus creating a population *substratum*.

3. Venetian rule and Dubrovnik Republic – In 1409, the whole region (except the islands of Lastovo and Mljet, ruled by the Dubrovnik Republic) fell under Venetian rule, under which it remained until the fall of Venice to Napoleon Bonaparte in 1797. During that period, Venetian monopoly resulted in a limited autonomy of the islands.

4. Expansions of Ottoman Empire – While the Venetians ruled this part of the Eastern Adriatic, the Ottoman Empire's expansions took place in the continental part of the Balkans, coming as far as the coastal area of Adriatic (even to the vincinity of the island of Pag – see Figure 1). The Dubrovnik Republic was able, due to the smart policy at the time, to avoid any confrontation. However, the clashes between the Ottoman Empire and Venetian Republic produced extensive migrations from the mainland areas, especially from today's Bosnia and Herzegovina, to the eastern part of the islands of Brač, Hvar, Korčula, and Pag, creating a population *superstratum*. The newcomers brought their gene pool and a variety of cultural pecularities, among them the »*štokavian*« dialect of the Croatian language to the area where the native population spoke the »*čakavian*« dialect. The most extensive migrations to those islands occured during the Cyprian (1571–1573), Candian (1645–1669) and Morean wars (1684–1699). The newcomers to the region were given land and awarded special privileges – »*The Paštrović Privileges*« – according to which they were excused from serving on the Venetian galleys in times of wars and were exempt from many taxes and public works. Those benefits resulted in animosity, creating many socio-cultural barriers between the immigrant and indigenous populations. By marriage with natives, the newcomers would lose their privileges, which represented a barrier to gene flow between the native and immigrant population of those islands.

5. Napoleon Bonaparte, Habsburg Empire and World Wars – After 1797, most of the islands were incorporated into Napoleon Bonaparte's »Illyric provinces« and the barrier due *to »The Paštrović Privileges«* was abolished. Between 1815 and 1918, the islands came under rule of the Austrian Habsburg Empire, and in 1918 the islands and the peninsula became part of the Kingdom of Serbs, Croats and Slovenes, which was renamed »Yugoslavia« in 1928. It has to be pointed out that, during this period (19th century and first half of the 20th century), the islands were depopulized in most cases, due to very difficult economic conditions, which resulted in non-random emigration overseas, in North and South America, Australia and New Zealand. In a very rare cases, there was an immigration to the island from the nearby coastal region – the example is the the island og Pag, and the reasons are again economical (rich salt-works), so the population *adstratum* was created.

6. The Republic of Croatia – Today, the Eastern Adriatic Islands are a part of the Republic of Croatia, after its international recognition in 1992. The present pop-

ulation is derived ir. part from indigenous Croatians speaking a »čakavian« dialect, and in part from Balkan peninsula immigrants who brought in the »štokavian« dialect of Croatian language during 16th and 17th century. Both groups are Roman Catholics, and on most of the islands there are Parish Registries of births, marriages and deaths of the island population, dating back to the beginning of the 18th century (6–8 ancestral generations of current population). Linguistic and other cultural differences still exist among the villages, as a result of not only geographical isolation and/or sociocultural and biological separation, but also the hostility that still exists between those two groups of inhabitants[12–16].

Geography, current demography and organisation of health care

1. Geography and current demography – Croatian (eastern) coast of the Adriatic Sea has more than 1.000 islands, most of them very small. Among them, according to last population census (1991), 47 was inhabited, but many of them with only a few families. There are only 15 islands with area greater than 50 square kilometers, and population of at least 1.000 inhabitants. Those islands (of interest for the future epidemiological research) can be divided into two groups: northern and southern. The *northern group* comprises eight islands of the Kvarner bay, Velebit channel, and the vincinity of the Zadar city, namely: *Krk*, *Cres*, *Lošinj*, *Rab* and *Pag*, *Ugljan*, *Pašman* and *Dugi Otok* (Figure 1). The *southern group* includes additional seven islands of Middle and Southern Dalmatia, namely: *Brač*, *Šolta*, *Hvar*, *Korčula*, *Vis*, *Lastovo* and *Mljet* (Figure 2).

The total area of 47 inhabited islands is 3.138 sq. kilometers (or 5.6% of total area of the Republic of Croatia). Total population of inhabited islands is 125.281 (or about 2.6% of total Croatian population)[17].

Table 1 reveals the number of inhabitants, natality rate, mortality rate and annual increase on 15 islands with area greater than 50 sq. kilometers and population greater than 1.000.

The figures presented in Table 1 imply that depopulation represents a considerable problem in almost all of the island communities. Among total population of all inhabited islands, the share of persons older than 60 years amounted to 23% (in 1991), and today it is probably even greater. The proportion of females aged 20–29 in these islands is only 12.5%, a very low figure indeed. The main reasons of depopulation are problems with transportation to mainland, higher prices of goods on the islands, lack of economic resources and jobs, problems in education system due to small number of children, lack of health care facilities (secondary and tertiary care), etc[17].

2. Organisation of health care – The health care on the islands is organized according to their position with respect to mainland, and according to vincinity of major coastal health care facilities. There are four major groups:

a) The islands connected to the mainland with the bridge: Krk, Pag;

b) The islands very close to the mainland, and in vincinity of major coastal health care facilities: Brač, Šolta, Korčula, Ugljan, Pašman;

c) The islands relatively close to the mainland, but far from major coastal health care facilities: Lošinj, Cres, Rab;

d) Distant islands: Dugi Otok, Hvar, Vis, Lastovo, Mljet.

According to the aforementioned relations, distant islands generally have their own medical center, while the islands closer to the mainland or major coastal health facilities have only a small practice of primary health care, with a local

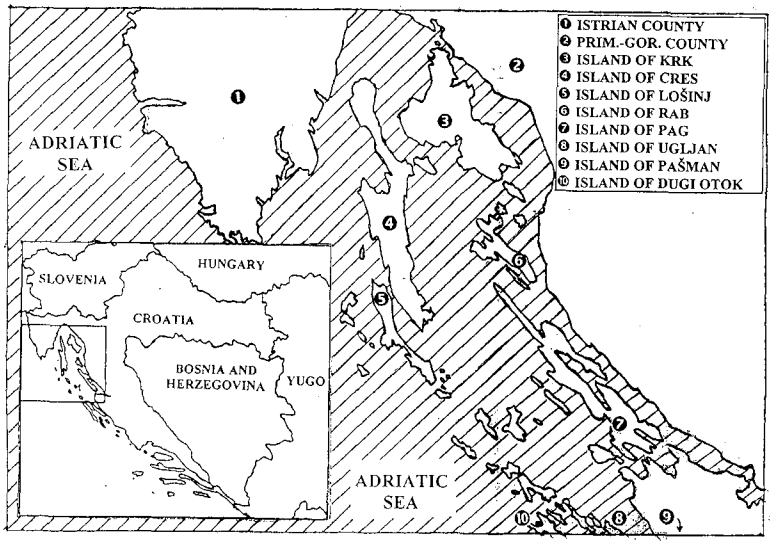

Fig. 1. Geographic location of the northern group of eastern Adriatic islands, Croatia.

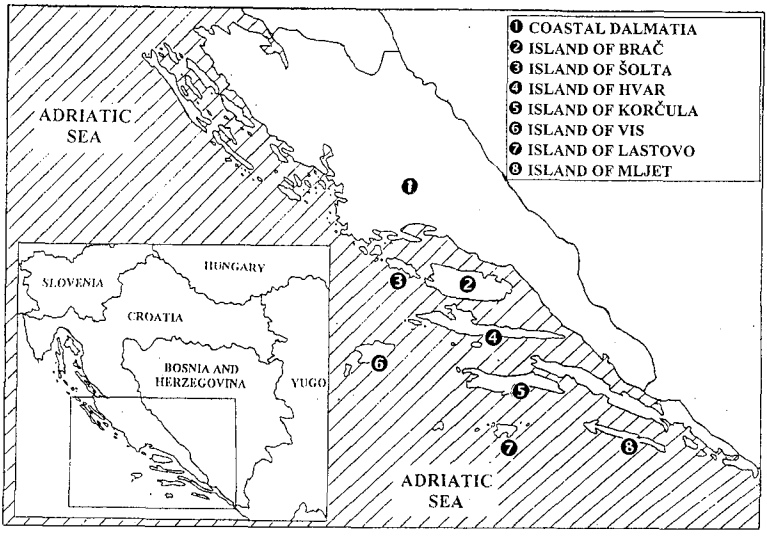

Fig. 2. Geographic location of the southern group of eastern Adriatic islands, Croatia.

GP covering all the health problems. However, it also depends on the number of total population. Some very small islands have only the schedule of regular visits of the GP's from the neighboring islands.

The islands with organized medical centers, which practice some services of

TABLE 1

NUMBER OF INHABITANTS, NATALITY RATE, MORTALITY RATE AND ANNUAL INCREASE ON 15 ISLANDS WITH AREA GREATER THAN 50 SQ. KILOMETERS AND POPULATION GREATER THAN 1.000 *(MODIFIED FROM: HEBRANG ET AL. (EDS): IMPROVEMENT OF HEALTH CARE ON CROATIAN ISLANDS. CROATIAN MINISTRY OF HEALTH, ZAGREB, 1997)*

Eastern Adriatic Island	Population (1991)	Natality rate* (per 1000)	Mortality rate* (per 1000)	Annual growth* (per 1000)
Krk	16,402	9.3	14.8	– 5.5
Cres	2,971	8.8	15.5	– 6.7
Lošinj	8,825	10.5	11.7	– 1.1
Rab	9,205	9.0	9.7	– 0.7
Pag	7,969	10.3	14.9	– 4.6
Ugljan	7,726	5.4	12.9	– 7.5
Pašman	3,349	8.4	12.8	– 4.4
Dugi Otok	2,932	7.2	15.0	– 7.8
Brač	13,824	12.1	13.2	– 1.1
Šolta	1,448	7.6	23.5	– 15.9
Hvar	11,459	10.1	11.3	– 1.2
Korčula	21,764	10.7	12.4	–1.7
Vis	4,361	9.0	16.5	–8.9
Lastovo	1,221	13.9	9.0	4.9
Mljet	1,237	12.1	18.6	– 6.5

*In the year of 1994.

TABLE 2

RANK OF MORTALITY CAUSES AND PROPORTION OF THE TOTAL DEATHS ON FOUR ISLANDS BELONGING TO SPLIT-DALMATIAN COUNTY (ŠOLTA, BRAČ, HVAR AND VIS) IN 1994, AND FOR THE REPUBLIC OF CROATIA IN 1993, NON-STANDARDIZED VALUES *(MODIFIED FROM: HEBRANG ET AL. (EDS): IMPROVEMENT OF HEALTH CARE ON CROATIAN ISLANDS. CROATIAN MINISTRY OF HEALTH, ZAGREB, 1997)*

Cause of death (disease group)	Proportion of total deaths and rank (islands Šolta, Brač, Hvar and Vis, 1994)	Proportion of total deaths and rank (Republic of Croatia, 1993)
Cardiovascular diseases	47% (1.)	48% (1.)
Malignant neoplasms	23% (2.)	20% (2.)
Respiratory diseases	11% (3.)	4% (6.)
Non-defined conditions	7% (4.)	5.5% (4.)
Injuries and poisoning	3% (5.)	12% (3.)
Urogenital tract diseases	2% (6.–7.)	1% (8.)
Digestive tract diseases	2% (6.–7.)	4.5% (5.)
Perinatal complications	1% (8.)	0.5% (9.)
Endocrinological diseases	0.5% (9.)	2% (7.)
Communicable diseases	<0.5% (10.)	< 1% (10.)

secondary health care, include Cres, Lošinj, Rab, Brač, Hvar, Korčula and Vis. Small practices of primary health care with local GP's are organized on Ugljan, Pašman, Dugi Otok, Šolta, Lastovo and Mljet. Islands of Krk and Pag, connected to mainland with the bridge, belong to health systems of the cities of Rijeka and Zadar[17].

Although the epidemiological data are sparse, in Table 2 we present the rank of the mortality causes in the year of 1994

TABLE 3
COMPARISON OF INBREEDING COEFFICIENT ESTIMATED FROM THE ISONYMY DATA IN THIS STUDY WITH THE REPORTED DATA IN SELECTED WORLD POPULATIONS

Author	Population	Inbreeding coefficient
Lasker, 1977 (18)	Peru	0.0040
Kayshap and Tiwari, 1980 (19)	Kashmir	0.0400
Wilson, 1981 (20)	Bhatia	0.0427
Hurd, 1983 (21)	Nebraska Amish	0.1129
Lasker, 1985 (22)	Reading, England	0.0003
Sujoldžić, 1989 (23)	Island of Silba, Croatia	0.0110
Sujoldžić, 1989 (23)	Island of Olib, Croatia	0.0162
Biondi et al., 1990 (24)	Greek villages in Italy (Calabria)	0.0144
Biondi et al., 1993 (25)	Albanian villages in Italy	0.0063
Sujoldžić, 1993 (26)	Island of Korčula, Croatia	0.0244
De Braekeleer, 1996 (27)	SLSJ region, Quebec, Canada	0.0069
Roguljić et al., 1997 (28)	Island of Hvar, Croatia	0.0233
Rudan et al., 1999 (29)	Island of Pag, Croatia	0.0331

(calculated as the proportion of the total deaths, non-standardized) for the islands of Brač, Šolta, Hvar and Vis (belonging to Split-Dalmatian County; the data are from the County Institute of Public Health). The proportions are shown along with the data for the population of the entire Republic of Croatia, for the year of 1993 (non-standardized). Although the data are not fully comparable (different years of study and without standardization by sex and age), a general insight into the causes of mortality can be obtained[17].

Brief review of previous anthropological studies of population genetic structure

Since the early 1970's, the staff of the Institute of Anthropological Research in Zagreb, Croatia, have been studying the isolate populations of several Eastern Adriatic islands, namely Brač, Hvar, Korčula, Pag, Krk, Silba, Olib and the Peninsula of Pelješac (Figures 1, 2). Their extensive research, performed over the fruitful collaboration with many visiting scientists and distinguished researchers from many world countries, resulted in more than 150 publications on those populations in leading anthropological journals up to date. It is far beyond the scope of this article to review major results and discoveries. Here, we will briefly list the nature of the collected material, and present the overview of results that deal with genetic structure of the island populations, namely the levels of endogamy, proportion of isonymous marriages, models of genetic structure and estimates of inbreeding coefficients.

The data collected for each examinee in each studied population (more than 10.000 subjects from 7 islands and 1 peninsula, or approximately one third of village population) included the place of birth of the examinees and their parents, basic vocabulary, anthropometric head and body dimensions, physiological (cardiorespiratory) properties, quantitative and qualitative dermatoglyphic traits (digitopalmar complex), metacarpal bone X-rays and blood specimens. All the methods of data collection and measurements of the island's population are presented in detail in the papers of Rudan et al.[12–16].

Blood samples were used to calculate *genetic distances* between villages from

differences in the frequencies of 21 polymorphic systems: erythrocyte antigens (including 10 genetic systems: ABO, Rh, MN, Ss, Duffy, Kidd, P, Kell, Colton and Lutheran), serum proteins (haptoglobin, third component of complement, properdin factor B) and erythrocyte enzyme systems (adenosine deaminase, esterase D, acid phosphatase 1, adenylate kinase 1, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, malate dehydrogenase, lactate dehydrogenase A and B, and phosphoglucomutase 1). The exception was the Island of Pag, where the genetic distances were calculated from differences in the frequencies of two polymorphic systems (Gm and Km) of immunoglobulin allotypes: G1m[1–3,17], G2m[23], G3m[5,6,10,11,13–16,21,24,28] and Km[1]. Antiallotypes were of human origin, with the exception of anti-G2m[23], extracted from monoclonal mouse, and one of two used anti-G3m[21], that was a rabbit heteroantibody. All the data are available upon request at the Institute for Anthropological Research, Zagreb, Croatia.

It has been shown that all the investigated populations are characterized by frequent consanguinity, inbreeding, and founder effect. The levels of endogamy (product of the share of mothers and fathers born in the same settlement as the proband) ranged from 60–90% (i.e., 78–95% parents from the same village). Due to some sociocultural reasons, discussed by Rudan et al.[12–16] isonymous matings are favored. The proportion of such matings among all marriages is, generally, 5–40%, depending on the island and the village. Regarding the model of genetic structure, it has been shown in majority of populations that Malecot's »isolation by distance« model can well explain the inter-populational differences in traits such as sociocultural and linguistic distances, migrational kinship (especially in females), and anthropometric and physiological properties. However, the traits presumed to be more »eco-resistant«, such as quantitative and qualitative dermatoglyphic traits, metacarpal bone dimensions and monogenic blood polymorphisms, are usually not well explained by Malecot's model.

In several island populations (namely Silba, Olib, Korčula, Hvar and Pag), the coefficient of inbreeding was estimated from isonymy data. Table 3 shows that the obtained results, in comparison to several other world populations known for consanguinity, with the inbreeding coefficient calculated using the same method, indicate that Eastern Adriatic island populations represent maybe one of the few last remaining isolates in the modern Europe, characterized by very high rates of inbreeding[18–29].

Thorough recent reviews of the general population (genetic) structure of Eastern Adriatic island populations (incorporating model-bound and model-free approaches in the study of sources of variation) have been published by Rudan et al.[30–32] and Waddle et al.[33]. Studies of genetic structure through analyses of serogenetic polymorphisms have been reported by Roberts et al.[34] and Janićijević et al.[35]; and more recently utilising *HLA class II*, *VNTR* and *STR* DNA polymorphisms by Martinović et al.[36–38].

Brief review of autochtonous diseases and other specific medical problems

A review of the world literature has pointed out that there are some autochtonuos diseases and specific medical problems resulting from the reproductive isolation and specific genetic structure of the island populations, characterized by high degree of consanguinity and inbreeding. In this section, those medical conditions will be briefly discussed. It is likely that there might also be other conditions, the data on which remained unpublished up to date.

1. Mal de Meleda syndrome (Island of Mljet) – Mal de Meleda received its name from Croatian island of Mljet (previously called Meleda), where it was originally observed. It was first described in 1826 and traced back to 1763[39]. At first, it was considered to be a form of leprosy, but subsequently it was recognized as a distinct entity, inherited on an autosomal recessive mode[40].

According to Schnyder and coworkers, the disease originated on the island of Mljet: between 1397 and 1808, the island was used for quarantining people suffering from plague and leprosy[41]. Such a setting, characterized by reproductive isolation and high inbreeding, favoured the appearance of the gene responsible for a condition.

In the past two decades, several cases of this disease have also been reported in Italy[42], Tunisia[43], western region of Saudi-Arabia[44] and United Arab Emirates[45], all regions and territories that have been within the trading zone of Dubrovnik Republic (which included the Mljet island) for centuries. Furthermore, some cases of recessive parmoplantar keratodermas have been reported in northern Sweden, Japan[46], and the Chinese family in Taiwan[47]. It is still unclear whether a palmoplantar keratoderma reported in 9 patients from four Greek families with the Naxos disease (Island of Naxos, Greece) was indeed a form of Mal-de-Meleda[48]. The features of genuine disease, reported on Mljet, are presented in Table 4.

TABLE 4
FEATURES OF THE MAL DE MELEDA SYNDROME

Autosomal recessive inheritance
Onset at birth or in early infancy
Characteristic glove-like and sock-like hyperkeratosis with sharp margination
Occasional hyperkeratotic plaques on elbows, knees or corner of the mouth
Marked hyperhidrosis, maceration and malodor
Slow progression without remissions

The study by Šalamon et al. (1994)[49], who analyzed *MN*, *Ss* and *Kk* erythrocyte antigen polymorphisms in nine patients with Mal de Meleda on the island of Mljet, tried to demonstrate the increased homozygosity in comparison to control population, but the results were inconsistent due to a very small sample.

In a recent study, Fisher et al.[50] analyzed two large consanguineous families from Algeria, including 10 affected individuals, and managed to localize a disease gene to chromosome 8qter. The maximum two-point lod score in their study was found for D8S1751, and it amounted to 8.21 at theta = 0. The analysis of homozygosity regions and recombination events placed the gene in a region of at least 3 cM, telomeric to D8S1727. A common haplotype found in both families suggested a founder effect. We suggest that shared haplotype analysis should be feasible in this genetic isolate population and homozygosity mapping could be employed to further understand the genetic aetiology of this autosomal recessive condition.

2. Hereditary dwarfism (Island of Krk) – The first report on hereditary dwarfism on the Island of Krk was written by Jauregg (1909)[51], who called the condition *'coastal maritine cretenism'*. However, later on it has been observed that dwarfs origin only from two adjacent villages, Bašćanska Draga and Jurandvor, while no cases of nanism were observed anywhere else on the island. First two dwarfs, born in 1864 and 1869 as two sons of the same parents, were the inhabitants of Bašćanska Draga, and a decade later two cases, born in 1877 and 1880, have also been observed in Jurandvor.

Fig. 3. Dwarf I. Č., born 1930, 137 cm in height, with his close relative. (Reprinted from the article: Kopajtić B., Dujmović M., Kolacio Z., Kogoj-Bakić V.: Enclaves of hereditary dwarfism on the Island of Krk, Croatia. Coll. Antropol., 2 (1995) 365).

Since then, 23 dwarfs have descended from the two villages.

This pattern attracted Swiss geneticist Hanhart[52], who called the condition *'heredodegenerative genitodystrophic nanism'*. The systematic study of the condition was performed by Vojska in 1938[53]. The last published material is available thanks to the efforts of a group from the Medical Faculty of Rijeka, who reinitiated the studies of the cases in early seventies[54–56], and published their results recently[57].

The two villages where the condition occured, Baščanska Draga and Jurandvor, are characterized by small, highly inbred population. The total population of the villages in 1973 amounted to 459 and 164 inhabitants, respectively, and the share of autochtonous inhabitants was 95.4% and 89.0%. Twice removed parental consanguinity was 11.9% and 22.0%, a rates very high indeed.

However, although the Rijeka group stated in their articles[54–57] that they performed a detailed medical analysis of the dwarfs, including the blood sampling in late 70's, physical and endocrinological examination, the results and the description of the condition itself have never been published(!). All the material written by this group considers the general health status of the population in two villages – Jurandvor and Baščanska Draga, and the results of the auxological examination in children. We believe that there are many possibilities in continuation of the research on this condition.

3. Extreme inbreeding and health outcomes (Island of Susak) – The small island of Susak is an exceptional example of extreme inbreeding. Founded by only two families of settlers, the island's population rose to 300 in the year of 1771, then to 1.111 in 1880, 1.427 in 1900, and 1.876 in 1945. After the World War II, a depopulation of the island took place, and the current population is estimated to be some three hundred inhabitants. However, almost entire emigration from the island moved to Hoboken, New Jersey, U.S.A., where they still live in the 'closed' community, marrying each other. Therefore, the population of Susak along with its emigration in Hoboken, although limited in size, represents an outstanding example of genetically very homogenous group, separated some half a century ago and continuing to live under very different environmental settings, which could be a suitable model for studies of genetic vs. environmental impacts on quantita-

tive traits such as blood pressure, blood cholesterol, body mass index or bronchial hyper-responsiveness or, alternatively, on the development of common multifactorial diseases[58].

Data on the Island of Susak were presented in a publication of the former »Yugoslav Academy of Sciences and Arts« in 1957, after the completion of thorough anthropological and biomedical studes of the population[58]. Only the brief review of the most important findings will be presented here.

Whilst studying consanguinity, the researchers were able to reconstruct 374 genealogies, which covered the entire island population, most of the emigration, and usually four (in some cases up to six) generations of ancestors. The family trees indicated very many cases of consanguinity, indicating that the island population is extremely inbred. There were only seven different surnames among almost 1.400 inhabitants (!), with a couple of 'dominant' surnames present in a vast majority of population. A basic analysis of blood polymorphysms was performed through analysis of frequency distribution of blood groups. In a sample of 200 inhabitants, frequencies were as follows: O – 46%; A – 49%; B – 4%; AB – 1%; In a subsample of 50 inhabitants, the frequency of positive Rhesus factor was 86%, and negative 14%. These data are significantly different to those found in surrounding populations, with a low proportion of blood group B, and high proportion of blood group A, in comparison to neighboring Croatian, Slovenian and Italian populations.A systematic medical check-up was performed on the entire island population forming a substantial database. Selected data (which may represent opportunities for future studies in genetic epidemiology) will be briefly highlighted here. The distribution of blood pressure values in 1.101 adult inhabitants is given in table 5, and it could be compared to current data, as well as to the emigrant population in Hoboken, New Jersey, U.S.A., in order to delineate environmental vs. genetic factors in the development of hypertension.

TABLE 5
DISTRIBUTION OF BLOOD PRESSURE VALUES IN 1.101 ADULT INHABITANTS OF THE ISLAND OF SUSAK, PERFORMED DURING OCTOBER-NOVEMBER, 1953 *(MODIFIED FROM: HAHN AND IVANČIĆ[59])*

Value (mmHg)	Systolic	Diastolic
40–49	–	0.14%
50–59	–	3.16%
60–69	–	19.09%
70–79	–	32.55%
80–89	–	27.75%
90–99	0.27%	11.54%
100–109	8.79%	3.98%
110–119	19.09%	1.24%
120–129	23.49%	0.41%
130–139	17.17%	0.14%
140–149	8.79%	–
150–159	7.28%	–
160–169	6.73%	–
170–179	3.98%	–
180–189	2.06%	–
190–199	0.82%	–
200–209	0.96%	–
210–219	0.41%	–
220–229	0.14%	–

Among 346 children examined, multiple congenital anomalies were present in 25 cases, clustering in 12 families, and a detailed description of each particular case along with the results of examination in siblings was presented. Special attention was also given to psychiatric disorders, presumably common in the island. Among the entire population (cca. 1400 at the time), there were 129 persons fulfilling criteria for some psychiatric diagnosis: 57 cases of oligophrenia (in all three forms), 33 cases of senile dementia, 21 cases of psychoses, 16 cases of schizophrenia and 2 other cases. In many of those cases, there had been striking evidence of familial clustering of the diseases[58].

4. Population genetics of cancer (Islands of Brač, Hvar, Korčula, Vis and Lastovo) – Rudan[60] investigated the incidence of cancer in isolate island populations of the Eastern Adriatic. The number of cancer cases on five islands (Brač, Hvar, Korčula, Vis and Lastovo) over the 20-year period (1971 to 1990) has been extracted from the data of the Croatian Cancer Registry. The population of coastal Dalmatia, characterized by similar environmental factors but quite different population genetic structure, represented a control population of over 800.000. The leading hypothesis was that, if there were genes or gene complexes (especially with recessive inheritance) responsible for genetic susceptibility to certain types of cancer, the incidence of those cancer types should be greater in reproductively isolated island populations than in a control population, due to increased manifestation of such genes/gene complexes caused by two factors: founder effect and inbreeding.

After the adjustment of the data by sex and age, it has been shown that island populations had greater total cancer incidence than the control population for both sexes, and that the total incidence was increasing with the distance from mainland (Table 6).

The cancer sites primarily responsible for the excess incidence were urinary bladder cancer in males, and breast, ovarian, brain and large bowel cancer in females, predominantly in the younger age-groups (Tables 7, 8). The incidence of breast cancer in the control population (26.5) was lower than in all island populations, namely in Brač (30.4), Hvar (35.7, $p < 0.05$), Korčula (41.7, $p < 0.001$), Vis (56.3, $p < 0.001$) and Lastovo (33.9). The incidence of ovarian cancer was significantly increased in Brač (11.6 vs. 6.3, $p < 0.05$) and Lastovo (42.4 vs. 6.3, $p < 0.001$). The analysis of age-specific incidence in female island populations showed that the control incidence for the age group younger than 25 years (amounting to 13.3) was exceeded in Brač (25.1, $p < 0.05$), Korčula (17.8), Vis (28.5, $p < 0.05$) and Lastovo (30.1). Similarly, for the age group between 25 and 44 years the control incidence of 91.7 was significantly exceeded in Hvar (173.1, $p < 0.001$), Korčula (144.1, $p < 0.001$), Vis (156.1, $p < 0.05$) and Lastovo (215.4, $p < 0.05$). In older female age-groups, the differences were not as prononuced as they were in younger age groups.

The excess incidence on the islands showed almost a linear correlation to the geographic distance from mainland, especially in females (Figure 4). The correlation coefficient between incidence excess and geographic distance from mainland in females was extremely high ($r = 0.991$). Future studies to explore these findings further are in progress.

TABLE 6

TOTAL CANCER INCIDENCE (BY SEX) IN CONTROL POPULATION AND FIVE INVESTIGATED ISLANDS (Modified from: Rudan, I.: Inbreeding and cancer incidence in Human Isolates. Hum. Biol., 71 (1999) 173.)

	Control Population	Island of Brač	Island of Hvar	Island of Korčula	Island of Vis	Island of Lastovo
MALES	n = 407,177	n = 6,128	n = 5,322	n = 8,773	n = 1,934	n = 477
Total incidence	189.6	192.5	197.9	198.7	210.3	*99.5
Increase	0.0	+ 2.9	+ 8.3	+ 9.1	+ 20.7	– 90.1
FEMALES	n = 422,043	n = 6,477	n = 5,730	n = 9,406	n = 2,157	n = 462
Total incidence	134.4	151.0	**164.1	***173.5	*177.0	196.0
Increase	0.0	+ 16.6	+ 29.7	+ 39.1	+ 42.6	+ 61.6

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 7
STATISTICALLY SIGNIFICANT SITE-SPECIFIC DIFFERENCES IN CANCER INCIDENCE (PER 100.000 STANDARD WORLD POPULATION) BETWEEN CONTROL POPULATION AND FIVE INVESTIGATED ISLANDS (Modified from: Rudan, I.: Inbreeding and cancer incidence in Human Isolates. Hum. Biol., 71 (1999) 173.)

Cancer Site (ICD-9)	Control Population	Island of Brač	Island of Hvar	Island of Korčula	Island of Vis	Island of Lastovo
MALES						
Stomach (151)	13.3	17.1	13.8	14.1	*25.5	–
Lungs (162)	46.5	*60.7	57.8	37.9	61.4	17.7
Bladder (188)	8.7	4.7	7.6	***20.8	15.5	13.9
FEMALES						

TABLE 8
STATISTICALLY SIGNIFICANT AGE-SPECIFIC DIFFERENCES IN FEMALE CANCER INCIDENCE (PER 100.000 STANDARD WORLD POPULATION) BETWEEN CONTROL POPULATION AND FIVE INVESTIGATED ISLANDS

Age group (Females)	Control Population	Island of Brač	Island of Hvar	Island of Korčula	Island of Vis	Island of Lastovo
<25	13.3	*25.1	8.6	17.8	*28.5	30.1
25–44	91.7	84.4	***173.1	***144.1	*156.1	*215.4
45–64	309.4	322.3	316.1	362.9	364.6	356.2
65+	646.9	795.8	782.5	**835.4	763.2	832.8

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Future genetic epidemiological studies: objectives and strategies

Identifying genes for disorders with complex inheritance patterns is one of the greatest challenges in biomedical research. These disorders include most of the diseases which make up the majority of the burden of disease in most industrialised countries. However, with currently available genetic technology, it is widely considered that studies seeking to identify genes of small or moderate sized effect responsible for these disorders may only be possible in special »genetic isolate« populations where conditions are particularly favorable for such genetic epidemiological studies. Three important aspects of these populations are the homogeneous nature of the communities with resulting reduced diversity of exposure to environmental factors; a suggested reduction on genetic heterogeneity (for example, due to alleles lost due to genetic drift in a small population or due to founder effects) which may make the study of genetic risk factors less complex; and the ability to investigate genetic factors by means of looking for shared segments coming from common ancestors.

There are very few of these populations which are known globally, characterized by specific ethnohistory, known migrations within past centuries, continuing reproductive isolation and well-documented effects of events which could influence their genetic structure, such as epidemics of diseases, socio-cultural barriers, political turmoils, etc. The Eastern Adriatic islands of the coast of mainland Croatia represent an exceptionally well characterized »genetic isolate« population. Anthropological research carried out in these islands over the past 20 years has yielded important descriptive information about the population struc-

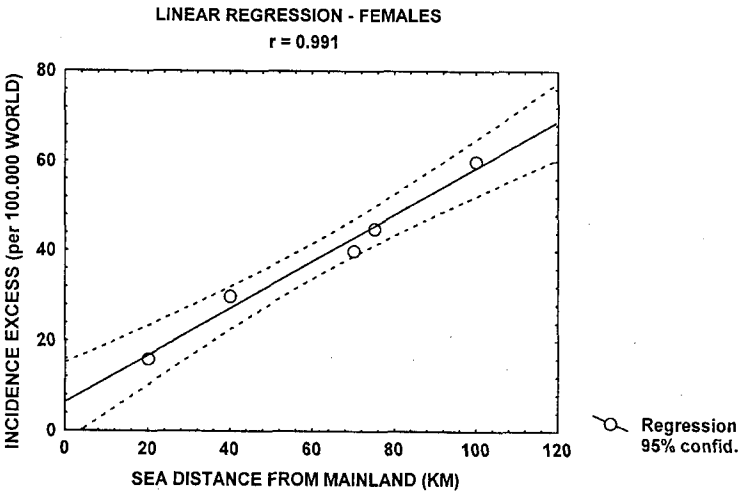

Fig. 4. Correlation between distance from the mainland and increase in the total cancer incidence in females. (Modified from: Rudan, I.: Inbreeding and cancer incidence in Human Isolates. Hum. Biol., 71 (1999) 173.)

ture, also showing that island inhabitants give informed consent and actively participate in such studies.

Our short-term aims will include following:

a) to document prevalence of disease in populations for which there was contact with primary care or hospital medical services; to record any medical data in support of the diagnosis (e.g. blood pressure level recorded, blood glucose level); to note any unusual forms of common diseases (for example, early onset, severe phenotype, or unusual or unreported common associations with other conditions);

b) to identify any rare diseases which may be peculiar to these islands and record details of the cases and their family histories; to identify and investigate further cases of rare diseases specific to these communities which have already been reported to exist (Mal-de-Meleda on Mljet island; hereditary dwarfism on Krk island; ovarian cancer families on Lastovo island); to construct detailed pedigrees of such cases and (if possible) collect blood samples from cases for DNA extraction;

c) to identify any possible pedigrees with multiple cases of common diseases (by asking local general practitioners (GP'S) and through checking medical records)

d) to document a »census« and genealogical record of island populations (age, sex, address), for possible future survey work; to discuss with local GP's the idea of setting up a prospective Registry of diseases/illneses using their records.

e) to measure quantitative traits in these communities and investigate their assocation with genetic factors by means of linkage and / or association or by more recently described ancestral haplotype reconstruction approaches.

Our long-term aim is to develop a sound research base for the (genetic epidemiological) study of common complex disease in these islands. Such studies will require the multi-disciplinary collaboration of many medical and related special-

ties. The establishment of formal links between the University departments of epidemiology in Edinburgh and Zagreb will represent an important foundation for future collaborations (including, for example, the Institute for Anthropological Research in Zagreb and Human Genetics Unit in the University of Edinburgh).

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GENETIČKO-EPIDEMIOLOŠKA ISTRAŽIVANJA IZOLIRANIH OTOČNIH POPULACIJA ISTOČNOG JADRANA: CILJEVI I STRATEGIJE

SAŽETAK

U ovom radu autori analiziraju zašto su izolirane otočne populacije posebno pogodni modeli za genetičko-epidemiološka istraživanja. Ukratko su prikazani rezultati prethodnih temeljitih antropoloških istraživanja ovih populacija, s obzirom na etnopovijest, zemljopis i sadašnju demografsku strukturu. Također su predočene osnovne spoznaje o genetičkoj strukturi stanovništva tih otoka, uključujući pristup sa i bez uporabe modela, analize serogenetičkih polimorfizama i nedavne studije *HLA klase II*, *VNTR* i *STR* DNA polimorfizama. Analizirana je sadašnja organizacija zdravstvene zaštite na otocima, kao i dosadašnja izvješća o specifičnim medicinskim problemima i nekim autohtonim bolestima u spomenutim otočnim populacijama (npr. nasljedni patuljasti rast na Krku, Mljetska bolest, posljedice srođivanja na Susku i populacijska genetika zloćudnih tumora na otocima Braču, Hvaru, Korčuli, Visu i Lastovu). Naposljetku, autori razvijaju ciljeve i strategije dugoročnog genetičko-epidemiološkog istraživanja ovih populacija.