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Inbreeding and Learning Disability in Croatian Island Isolates

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A B S T R A C T

The aim of this study was to investigate the prevalence of learning disability (LD) in isolate populations with different inbreeding coefficients (F). Prevalence of LD and F were determined in 10 villages from five Croatian islands: Brač, Hvar, Korčula, Lastovo and Susak. For the purpose of this study, LD was defined as the inability to attend the public school system. As the elementary schools (grade 1–8) in the place of the study are both public and compulsory, the assessment of child's inability to attend the school is performed at the age of six. This is required by all children in the country based on standard set of tests of cognitive performance defined by the Ministry of Education and Culture of the Republic of Croatia. The average inbreeding coefficients in each village population (F) were estimated in a random sample of 20–30% adults in each of the 10 villages based on 4 ancestral generations and using Wright's path method. Prevalence of LD ranged from 0.43% to 2.47%, and the inbreeding coefficients ranged from 0.8% to 4.9%. The Pearson's correlation coefficient between F and LD prevalence was 0.80 ($p<0.01$). Although the relative risk per 5% inbreeding appeared very high (about 10), the absolute risk only increased from 0.18% to 1.77%. The genetic effect of inbreeding (GEI) was approximately 0.69% and the population-attributable fraction 76.6%. A review of the literature and the results of this study lead to a conclusion that a very large number of predominantly recessive genetic factors might mediate the genetic susceptibility to various forms of LD in these populations.

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Introduction

Despite the common belief that consanguineous unions are associated with increased risk of some form of learning disability (LD) in their children, a review of scientific evidence in support of this association[1–22] failed to identify rigorous evidence in its favor[23,24]. The origins of this belief certainly lie in a distant past, as this association has been widely mentioned in various historical works of literature in many cultures[25]. We were only able to identify 22 well-designed studies investigating the effect of inbreeding on cognitive performance. Only 2 of these were published after 1980, suggesting that this has been a topic avoided by the research community, possibly due to its sensitive nature. This is unfortunate since advances in the understanding of human disorders made possible in this post-genomic era provide realistic hope that the mechanisms of disease may be better understood and lead to new prevention and treatment strategies. We believed that an investigation into whether the observed increase in LD in inbred communities was due to numerous recessive genetic variants of small effect, or a small number of rare variants of large effect, or simply cultural or socio-economic bias would be a useful contribution to improving understanding of the disease mechanisms which underlie LD.

In this paper, we present one approach to the study of LD that aimed to study the relationship between inbreeding patterns and LD whilst attempting to correct for cultural and socio-economical bias. We further aimed to determine the relative and absolute risk of LD that might be attributable to inbreeding. The studied population included 10 isolate villages from the eastern Adriatic islands of Hvar, Brač, Korčula, Lastovo and Susak, in Croatia, a resource well characterized through a long-term multidisciplinary anthropologic and biomedical research[26–30].

Materials and Methods

Study design

The prevalence of learning disability was determined in 10 isolate villages on 5 different Croatian islands (Figure 1). These villages are characterized by reduced environmental variation and their inhabitants share very similar environmental factors (climate, nutrition, socio-economic status, occupation, education, housing), as it has been demonstrated in previous studies[28,31]. In theory this should create a favorable setting for study since it should help limit socio-economic and cultural bias in the interpretation of the results.

Another favorable characteristic of these populations for our study is the diversity of the attitudes towards inbreeding[26,28]. This was influenced by geographic isolation, political privileges in the past and socio-cultural reasons and resulted in a range of inbreeding coefficients present at both individual and population level[26,27].

Previously conducted studies compared the prevalence of LD in an inbred cohort with non-inbred controls. This raises issues about the social and cultural comparability of controls and the possible clustering of a Mendelian disease (a single large effect gene) in the inbred cases. In contrast, this study investigated 10 populations with similar environment and culture but with a spectrum of inbreeding coefficients and quite different founding populations.

We hypothesized that if the study found comparable prevalence of LD in all 10 populations, this would not support any inbreeding effect and the LD prevalence will be assumed to be determined mainly by factors related to environment. However, if we found a consistently positive correlation between inbreeding levels and LD prevalence across 10 villages, this would clearly point to an effect of inbreeding. A further advantage of having 10 distinct

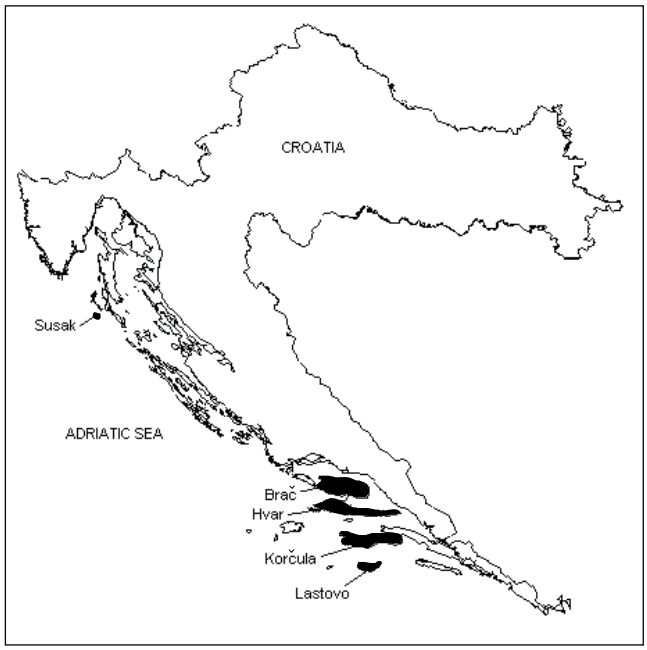

Fig. 1. Geographic location of the investigated islands of Brač, Hvar, Korčula, Lastovo and Susak.

populations under investigation is to rule out the possibility of a rare Mendelian disease clustering, as it is unlikely that the same rare variant would be present in all 10 populations having very different founding populations and ethnohistory.

We further hypothesized that if a modest increase in sharing of genes identical by descent (e.g. an increase in inbreeding coefficient at the level of entire population from 0% to 5%) led to a significant change in prevalence of LD across several isolate populations that share very similar environmental effects, then this would be most consistent with the model including a very large number of genomic loci influencing the disease, as Morton has suggested in his review of the problem[19].

Estimation of the prevalence of learning disability

For the purpose of this study, learning disability was defined as the inability to attend the public school system. As the elementary schools (grade 1–8) in the place of the study are both public and compulsory, the assessment of child's ability to attend the school is performed at the age of six. The assessment is based on standard set of tests, as required by the Ministry of Education and Culture of the Republic of Croatia[32]. These tests include: (a) perception test, test of point linkage, test of knowing facts, drawing test and numerical test; (b) intelligence test based on drawing a human image; (c) »Bender Gestalt« test; (d) Raven's progressive colored matrices[32]. Data on the individuals unable to attend school were retrieved from local general practitioners and were considered to be complete. The prevalence of LD was calculated as the proportion of these individuals in the total population of each village (as of January 2001). Ethical approval for this study was obtained from the Ethics Committee of

the Institute for Anthropological Research, Zagreb, Croatia.

Computation of inbreeding coefficients (F)

Genetic characterization of these villages included the computation of average inbreeding coefficient of each village based on reconstruction of genealogies of a sample of examinees which formed 20–30% of adult village population. The pedigree information on 2–3 ancestral generations was recorded for each examinee during the fieldwork between 1979–1981 performed by the Institute of Anthropological Research in Zagreb. It was later 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[33]:

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

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.

Statistical data analysis

Linear regression analysis of LD prevalence on F was performed using the data from all ten villages. The corresponding Pearson's coefficient of correlation (r) and the regression coefficient (b) were determined using the SYSTAT 7.0 software.

The observed prevalence of LD in each of the studied populations was considered to approximate reasonably well the absolute risk of LD in pre-school age in each population. The relative risk for each unit increase of 5% inbreeding was inferred from the slope of the linear regression curve as the ratio of the expected LD prevalence at the points of F = 5% and F = 0%.

As pointed out by Freire-Maia[34], in certain instances the absolute and relative risk measures can be artificially low. An index – called »the genetic effects of inbreeding« (GEI) – was suggested as an alternative[34], and is calculated as:

$$GEI = (Pi–Po) / (1–Po),$$

where Pi is a probability of the event (in this case LD) in an inbred person (in this case a village with an average F greater than 3%), and Po is the probability of the event in a non-inbred person (in this case a village with an average F less than 1%).

The population-attributable fraction (PAF) was calculated by logistic regression, noting each village's probability LD prevalence value if their F was set equal to zero. The sum of all such probabilities, is an estimate of the LD prevalence in the absence of inbreeding. Then:

$$PAF = 1 – Psum / Npop,$$

where Npop is the total population size[35].

Results

Table 1 presents the data on studied villages coded from A to J and their respective total populations, the average coefficients of inbreeding (computed as above), the number of cases of LD and the prevalence of LD in each village. The inbreeding coefficients in these villages ranged from 0.8% to 4.9%, and the prevalence of LD from 0.43% to 2.47%.

Figure 2 presents the linear regression between F and LD and the corresponding Pearson's correlation coefficient, which was 0.80 ($p<0.01$). Although the relative risk per unit increase of 5% inbreeding appeared to be quite high (about 10), the absolute risk (defined as prevalence at the intercept of the regression line with F=0% and F=5%) only increased from 0.18% to 1.77%. The genetic effect of inbreeding (GEI) was 0.69% which relates to the difference in ex-

TABLE 1
TOTAL POPULATION OF 10 STUDIED VILLAGES (AS OF JANUARY 2001), AVERAGE POPULATION INBREEDING COEFFICIENT (F) DETERMINED FROM GENEALOGIES, NUMBER OF LEARNING DISABILITY (LD) CASES AND THE PREVALENCE OF LEARNING DISABILITY

Village (island)	N (Population)	F (Genealogical)	LD Cases	LD Prevalence
A (Hvar)	153	0.049	2	1.31%
B (Susak)	81	0.047	2	2.47%
C (Korčula)	326	0.044	6	1.84%
D (Korčula)	464	0.032	2	0.43%
E (Korčula)	290	0.027	3	1.03%
F (Brač)	214	0.013	1	0.47%
G (Korčula)	354	0.012	3	0.85%
H (Lastovo)	899	0.011	5	0.56%
I (Korčula)	866	0.008	4	0.46%
J (Hvar)	375	0.008	2	0.53%

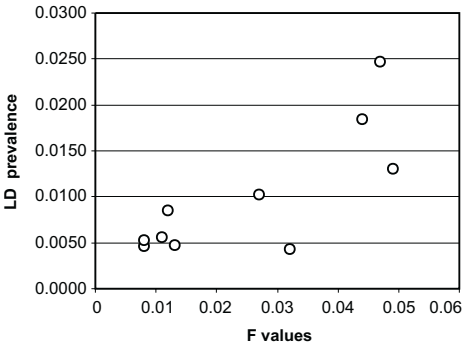

Fig. 2. Plot of the values of prevalence of learning disability (LD) against average coefficients of inbreeding (F) in ten studied villages.

TABLE 2
PEARSON'S COEFFICIENT OF CORRELATION BETWEEN INBREEDING COEFFICIENT AND PREVALENCE OF LEARNING DISABILITY, REGRESSION COEFFICIENT, ESTIMATES OF RELATIVE RISK, GENETIC EFFECT OF INBREEDING (GEI) AND POPULATION ATTRIBUTABLE FRACTION (PAF)

Measure	Value
Pearson's coefficient of correlation (r)	0.80
Regression coefficient (b)	0.33
Relative risk (F=5% vs. F=0%)	10.23
GEI (F3% vs. F1%)	0.69%
PAF	76.6%

pected prevalence of LD when the villages with F greater than 3% and less than 1% are compared. The population attributable fraction of cases due to inbreeding in all ten populations was high and it amounted to 76.6% (Table 2).

Discussion

Early work on the relationship between inbreeding and cognitive performance was reported by Penrose in 1938[1], but the most influential studies were carried out in 1970's by Schull, Neel and their coworkers in Japan[4,7,8,10,11,15], Danielov in Russia[12,13,17] and Costeff in Israel[9,16]. These three groups published about a half of all the available studies in the world literature to date. The results of these studies were in very close agreement: they all found statistically significant effects of the inbreeding on cognitive performance (measured as a quantitative trait) or the prevalence of learning disability (measured as a qualitative trait). Although the associated relative risks were also rather large, it still needs to be

understood that in absolute terms any risk attributable to inbreeding was still small. The average regression coefficient of inbreeding on IQ found in these studies, after weighting by the reciprocal variance, was –44.0 (12.3)[15]. In addition, the risk of some form of LD in offspring of non-inbred marriages was estimated to about 1.2%, and in the offspring of first-cousin marriages to about 6.2%[19]. Although the risk in the latter is reportedly increased 5 times, this still means that 94 of 100 children born in such unions did not suffer of any form of LD.

In our study, learning disability was measured as a qualitative trait. The relative risk (RR) per unit increase 5% inbreeding seemed to be about 10, although this was based on the intercept of our linear regression line with F = 0 as we only studied inbred populations. However, when this is replaced by the prevalence of LD in general Croatian population, kindly provided by the Croatian Ministry of Education and Culture, the more realistic estimate of absolute risk in non-inbred population of about 4.0 is observed. This is in close agreement of RR = 5 per 6.25% inbreeding, reported in previous studies. The basal prevalence of LD in non-inbred populations in our study was somewhat smaller than in other reports or in general Croatian population (0.43–0.56% in comparison to about 1.2%), but this can be explained by random fluctuations in relatively small populations of the villages in this study. Thus, if we accept the conclusion that an absolute increase in inbreeding of about 4–5% (from F = 0.005 to 0.05) could be responsible for the observed 4-fold increase in prevalence of LD, the central question becomes what does it tell us about the genetic mechanisms underlying this complex syndrome. An effect of inbreeding implies that the susceptibility is most probably controlled, at least partly, by recessive genetic variants. In addition, if we accept that the total number of human genes is between 30,000 and 40,000, then an absolute increase in inbreeding of 5% would correspond to having about 1,750 random genes across the genome identical by descent. If this unrecombined homozygosity in only 5% of genes could lead to a measurable effect in the prevalence of LD, then there are two main mechanisms that could explain it: (a) this brings together some rare major effect genes in a simple Mendelian fashion, or (b) the genes controlling this trait are of small effect but very numerous, scattered across the genome. The design of this study provides evidence against the first explanation. Major effect genes arise after mutations that are considered to be extremely rare, as the probability of random mutation causing a 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 indicate. In addition, under this assumption the differences between inbred and non-inbred individuals would normally be much larger than was the case in our study, where the differences were very small but consistent across many distinct populations. We conclude, therefore, that it is more likely that the genetic susceptibility to learning disability, a highly heterogeneous group of syndromes, is at least in part controlled by a large number of recessive genes.

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SRODIVANJE I POTEŠKOĆE U UČENJU MEĐU STANOVNIŠTVOM HRVATSKIH OTOKA

SAŽETAK

Cilj ovoga istraživanja je odrediti prevalenciju poteškoća u učenju (LD) u izoliranim otočnim populacijama s različitim vrijednostima koeficijenta urođenosti (F). Prevalencija poteškoća u učenju i vrijednosti F određene su u deset sela s pet različitih hrvatskih otoka: Brača, Hvara, Korčule, Lastova i Suska. Za potrebe ovoga istraživanja, LD je definirana kao nemogućnost pohađanja sustava osnovnog obrazovanja. Kako su osnovne škole (1.–8. razred) u mjestu istraživanja državne i obvezne, procjena nemogućnosti svakog djeteta da pohađa osnovnu školu vrši se u dobi od 6 godina. Testiranje je obvezno za svu djecu a temelji se na skupu testova kojeg propisuje Ministarstvo prosvjete. Prosječni koeficijent urođenosti u svakom selu izračunat je na temelju genealoških podataka 20–30% odraslog stanovništva upotrebom Wrightove »path«

metode. Prevalencija LD kretala se između 0.43% i 2.47%, a vrijednost koeficijenata urođenosti između 0.8% i 4.9%. Pearsonov koeficijent korelacije između F i prevalencije LD bio je 0.80 ($p<0.01$). Iako je relativni rizik za svakih 5% urođenosti bio velik (oko 10), apsolutni rizik pritom raste s procijenjenih 0.18% na samo 1.77%. Genetički učinak srođivanja procijenjen je na 0.69% a pripisivi populacijski udio na 76.6%. Pregled raspoložive literature i rezultati ovoga istraživanja ukazuju na zaključak da bi vrlo velik broj pretežno recesivnih genetskih čimbenika mogao biti odgovoran za genetsku sklonost različitim tipovima LD među stanovništvom.