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HUMAN GENETICS

Population-Genetic Study of Balkan Endemic Nephropathy in Serbia*

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Abstract—The study of Balkan endemic nephropathy (BEN) in the affected localities of southern Serbia shows population-genetic difference between samples of BEN affected individuals and control group consisting of non-affected individuals from the same localities. Detailed population-genetic study in village Chepure, which includes 20 large families where BEN is present in 646 (from first to fourth degree) relatives of probands, shows a familial character of disease as well as significant genetic influences in expression of the illness. Our study of genetic homozygosity degree includes an analysis of the presence, distribution and individual combination of 20 to 30 selected genetically controlled morphophysiological traits in the sample of BEN patients and in the control–healthy group. Assuming that BEN is genetically controlled disease, we made a hypothesis that an increased homozygosity level, as well as the changed variability among the patients, could be population-genetic parameter for the prediction of the illness. Taking into consideration our experience, as well as the experience of numerous scientists who studied the nature of the inheritance of mono- and oligo-genically controlled qualitative traits, we applied a methodology to estimate the proportion of such homozygously recessive characters (HRC-test). This population-genetic study did not only show statistically significant difference of the mean values of genetic homozygosity (BEN: 8.7 ± 0.3; control: 7.6 ± 0.3), but of the differences in the type of distribution too, as well as the differences in the presence of certain individual combinations of such traits.

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INTRODUCTION

Balkan endemic nephropathy (BEN) is a chronical renal disease, which usually appears at the age of 40 to 60 and has a fatal ending. This illness appears in endemic foci of the settlements around Danube basin rivers in three Balkan countries: Serbia, Bulgaria and Romania.

Certain scientists support the option that BEN is environmentally induced disease caused by intoxication or infections [1–6]. On the other hand, numerous researchers give proves that the development of BEN is genetically controlled [7–11], and some cytogenetic studies discovered genetic markers connected to predisposition for BEN [12–14].

Assuming that BEN is genetically controlled disease, we made a hypothesis that a generally increased homozygosity level, as well as changed variability in the samples of such patients, could be population-genetic parameter for the prediction of this illness.

Since we know only a small number of loci determining a specific morphophysiological character, it is very delicate to estimate genetic homozygosity in humans. However, knowing the type of inheritance and variability, it can be seen that a series of morphophysiological traits are under control of one or a small number of genes. Several studies, which established distribution and frequency of a series of extremely expressed recessive traits, show quite a difference in the presence of such traits among observed groups of individuals (i.e. comparison between ill and healthy individuals, pupils from special and regular schools, carriers of different blood types) [15–22]. The studied homozygous characteristics are obviously controlled by genes located on different human chromosomes, so that they could be considered as markers of these chromosomes, as well as of numerous surrounding genes controlling different components of fitness. The amount of recessive homozygosity estimated by our HRC-test is practically an estimation of genetic loads present in human population, or in any specific sample of human individuals [9, 20–22].

In this study we submit our analyses of HRC-test on the patients with Balkan endemic nephropathy and from BEN-affected regions, to distinguish if populations from such regions should be considered to be different from the neighboring regions where BEN is absent.

* The text was submitted by the authors in English.

Table 1. Balkan endemic nephrophaty in relatives of diseased probant individuals in Chepure village near Paracin

Probants	Inspected relatives	Percent of diseased in		
		I–II	III–IV	V–VI degree relatives
20	646	56.8	39.7	20.1

MATERIALS AND METHODS

Using specific population-genetic markers (studied homozygously recessive traits), several authors of the Belgrade population-genetic school [15–18] applied a methodology to estimate the proportion of such homozygously recessive characters (HRC-test of determination of genetic homozygosity in humans) in samples of diseased and healthy/control individuals. In a sample of tested individuals the proportion of the presence of determined characteristics was compared with another group of individuals, affected by specific diseases. Such tests have been proceeded for about 20 to 30 characteristics whose extreme appearance was manifested as homozygous state of either individual or of a group of genes. The presence of the studied genetically controlled recessive characters was used as a parameter for homozygosity of corresponding genes and chromosomes.

Some homozygously-recessive traits in the region of human head are, for example : blue eyes (gene location 19p13.1-q13.11, OMIM number 227240), unattached ear lobe (OMIM number 128900), continuous frontal hair line (OMIM number 194000), straight, soft and blond hair (OMIM numbers 139450 and 210750), double hair whorl, opposite hair whorl orientation (OMIM number 139400), as well as an inability to roll, fold and curve the tongue (OMIM number 189300), a guttural "r", insensitivity to PTC (gene location 7q35-q36, OMIM number 607751) and daltonism (gene location Xq28, OMIM number 303800). Such traits are also clearly expressed in human arms and legs, such as distal or proximal hyperextensibility of the thumb, index finger longer than the ring finger (OMIM number 136100), left-handedness (gene location 2p12-q22, OMIM number 139900), hand clasping pattern (OMIM number 139800), absence of middigital hair (OMIM number 157200), etc [23]. Numerous tests we performed also proving that those traits are inherited as recessive.

The genes which are so far known to be involved in determining susceptibility to BEN are 3q25 (OMIM number 124100) [12–14, 23], followed by 1p36, 3p25, 6q23 and 7q22.

In our present study comparative analyses were made by the same person, with equal criteria in determination of extremely pronounced homozygously recessive characters in tested groups of observed individuals. Variations in the presence of such characters were estimated using standard statistical procedures, and by comparing the means, variances, and the shapes of the distribution between samples of affected and of healthy individuals.

In this study we tested BEN affected individuals from several localities of Serbia:

from village Chepure near Paracin where BEN is present in about 200 of ca. 1500 inhabitants. In this village we analyzed twenty large families where BEN is present, with 646 (from first to fourth degree) relatives of probants;

from Nis, Leskovac and Aleksinac. The BEN-affected group consists of 60 individuals while the control sample consists of 60 (healthy) randomly chosen individuals of the same age and from the same locality;

from Loznica. The BEN-affected group consists of 64 individuals while the control sample consists of 50 (healthy) randomly chosen individuals of the same age and from the same locality.

RESULTS

Detailed population-genetic study in village Chepure near Paracin, which includes 20 large families where BEN is present in 646 (from first to fourth degree) relatives of probants, shows familial character of disease as well as significant influence of genetic factors in the expression of the illness (Tables 1 and 2).

From the data presented (Table 3, figure) it can be seen that HRC-testing of BEN-affected individuals shows an increased average homozygosity of such genetic markers, in comparison with healthy control individuals from the same area. Looking at the distribution of HRC frequency in the sample of BEN patients from Nis and Aleksinac and control group of individuals (figure), it is obvious that the mean value of 30 HRCs in the sample of 60 affected individuals is significantly higher than in control group consisting of 60 healthy individuals (BEN: 8.7 ± 0.3; control: 7.6 ± 0.3).

Table 2. The incidence of diseased progenies depending on the presence of BEN in one or in both parents in Chepure village near Paracin

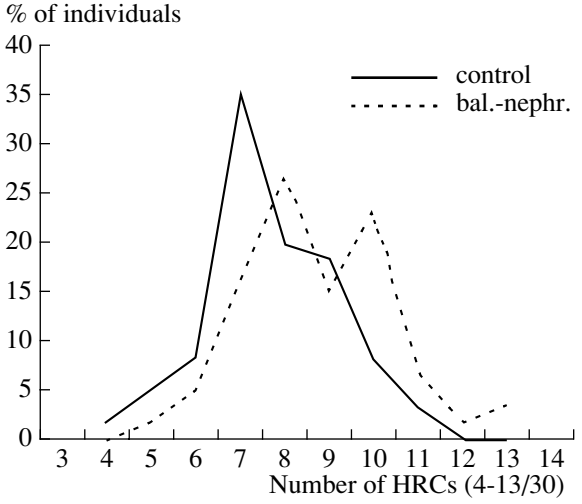
	Both parents	One parent	Father	Mother
Analyzed families	15	34	18	16
Observed progenies	56	125	69	56
% diseased	56.5	27.0	36.2	18.0

Table 3. The frequencies of homozygously recessive characteristics among patients with Balkan endemic nephropathy and individuals of control sample from Nis, Leskovac and Aleksinac

Homozygously recessive trait	Control sample, % $N = 50$	BEN affected, % $N = 50$	χ^2
1. even scalp	44	40	0.36
2. straight hair	78	98	5.13*
3. soft hair	32	86	91.13***
4. attached ear lobe	18	12	2.00
5. small forehead	14	4	7.14**
6. chin without pits	72	92	5.56*
7. blue eyes	28	38	3.57*
8. blond hair	6	10	2.67
9. guttural "r"	4	4	0
10. color blindness	2	2	0
11. inability to tongue rolling	36	40	0.44
12. right thumb over left thumb	38	40	0.11
13. thumb distal hyperextensibility	12	8	1.33
14. inserting thumb in joint	16	20	1.00
15. left-handness	4	14	25.00***
16. insensitivity to PTC	26	26	0
17. absence of middigital hair	52	66	3.77*
			$\Sigma\chi^2 = 149.21$***

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

Observing the distribution of HRC frequency between BEN patients and the control sample of individuals, it can be seen that HRCs in the group of BEN patients are moving toward higher values, suggesting that genetic disposition at the polygenic level exist between two tested samples.



HRC-test in healthy individuals and patients with Balkan Endemic Nephropathy from Nis and Aleksinac. Control: $N = 60$, $\bar{x}_{hrc/30} = 7.6 \pm 0.3$; BEN affected: $N = 60$, $\bar{x}_{hrc/30} = 8.7 \pm 0.3$ ($t = 2.44$, $p < 0.05$).

From the data presented in Table 3 it can be seen that the frequency distribution of tested homozygously-recessive characteristics was different in compared samples of diseased and healthy individuals, manifesting population-genetic difference that exists between them ($\chi^2 = 149.2$, $P < 0.001$). In each of these comparisons, characteristic groups of traits were differently present among healthy and diseased individuals, suggesting a correlation with different combinations of polygenes which may be involved in regulatory processes of resistance to different diseases. In the group of BEN patients from Nis, Leskovac and Aleksinac in 10 out of 17 observed characters recessive homozygosity was expressed to a greater degree compared with control, and in 6 of them this difference was statistically significant.

In the group of BEN-affected individuals from Loznica in 8 out of 17 observed characters recessive homozygosity was expressed to a greater degree compared to the control group of individuals, and in 4 of them this difference was statistically significant.

DISCUSSION

The results of our study show significant genetic influence in expression of BEN, which is in agreement with some other studies [9, 10], as well as with some earlier findings of ours [7, 8], showing that development of BEN is genetically controlled and that popula-

tion-genetic approach in studying such a complex and epidemiologically expressed disease is indispensable for an understanding of the nature and the origin of BEN.

From the data presented in this study, the frequency distribution of tested homozygously-recessive characteristics was different in the compared groups of individuals, manifesting population-genetic difference that exists between them. In these comparisons, characteristic groups of traits were present to a different degree among BEN patients and control group of individuals, suggesting a correlation between different combinations of polygenes which may be involved in regulatory processes of resistance to BEN.

The studied homozygous characters are obviously controlled by genes located on different human chromosomes, so that they could be considered as the markers of numerous surrounding genes which control different components of fitness.

The amount of recessive homozygosity estimated by our HRC-test is practically an estimation of genetic loads present in a human population, or in any specific sample of human individuals [9, 18, 19, 21, 22]. The fact that in those comparisons genetic homozygosity was increased within the samples of BEN patients (as markers of other numerous loci which could be also in homozygously-recessive state), gives us an impression that genetic loads are increased in such samples of human populations, which may cause decreased body resistance. Also, the genes controlling the qualitative characteristics studied here could be also pleiotropically correlated with other groups of polygenes which may have a direct impact on the development of BEN, or on the resistance to such environmental factors that provoke the appearance of characteristic signs of BEN.

The prevalence of autosomal recessive individual loci in a multigenic (i.e. oligogenic) determination of BEN development, and (or) in the resistance to BEN-affected factors, seem to be evident, proven later by location of some of these genes [12–14].

Possible explanation for established differences of HRC presence between samples of BEN-affected and healthy individuals may be that BEN patients represent part of population with somewhat different population-genetic structure because they may be groups of immigrants from particular region [2].

The increased degree of genetic homozygosity in the sample of BEN affected individuals may have in general the effects to genes responsible for the expression of BEN dependence susceptibility. Those genes would not only determine the expression of this susceptibility, but also a group of other characteristics, including possibly other HRC properties as well [15, 18, 21, 22]. The relatively large individual variation in the studied HRCs (from 4 to 13, figure), covering almost all parts of the human body, is also informative of how large a variation in genetic homeostasis may exist in human individuals, with a higher chance of extreme genotypes to be exposed to the risk of suffering from specific metabolic and developmental malformations [21, 22]. On this basis, the future application of HRC-testing can be valuable for prediction of those extremely deviant genotypes, which could be more sensitive to different diseases.

The application of noninvasive HRC-tests in apparently healthy individuals should be used as a preventive method, to discover individuals who have extremely many, or a small number of homozygous traits, which may result in some disbalances in the resistance to different diseases. Such individuals should be more carefully followed, to prevent the appearance of possible diseases in their bodies. The cooperation among medical specialists, as well as with scientists (such as geneticists and molecular biologists) is not only suggested but it is by all means necessary.

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