

Degree of genetic homozygosity and distribution of ABO blood types among patients with spina bifida occulta and spina bifida aperta

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Abstract

Introduction: Assuming that spina bifida (SB) is a genetically controlled disease, the aim of our study was to evaluate the degree of genetic homozygosity and the distribution of ABO blood types among patients with SB occulta and SB aperta by the homozygously recessive characteristics (HRC) test.

Material and methods: Our study included an analysis of the presence, distribution and individual combination of 15 selected genetically controlled morpho-physiological traits in a sample of 100 patients with SB (SB occulta $N = 50$ and SB aperta $N = 50$) and a control group of individuals ($N = 100$).

Results: We found a statistically significant difference between the mean values for genetic homozygosity (SB 4.5 ± 0.3 ; control 3.0 ± 0.2 , $p < 0.001$) and also differences in the presence of certain individual combinations of such traits. In 12 (80.0%) of the 15 observed characteristics, recessive homozygosity was expressed to a greater degree among the group of SB patients, while for 9 (60.0%) of the traits this level of difference was statistically significant ($\Sigma\chi^2 = 266.3$, $p < 0.001$). There was no difference in average homozygosity of such genetic markers between groups of SB occulta and SB aperta patients, but the type of individual variation in the two studied groups significantly differed. In the group of patients with SB the frequency of O blood group was significantly increased while B blood group was significantly decreased.

Conclusions: Our results clearly show that there is a populational genetic difference in the degree of genetic homozygosity and variability between the group of patients with SB and individuals without clinical manifestations, indicating a possible genetic component in the aetiopathogenesis of spina bifida.

Key words: spina bifida, genetic variability, genetic homozygosity

Introduction

Spinal dysraphism (spina bifida – SB) is a congenital deformity involving failure of normal midline fusion of the neural tube. The aetiology of SB is still unknown, but there is evidence suggesting multifactorial origin [1, 2]. A genetic component has been evaluated as a possible factor governing formation of SB including specific chromosomal or single-gene disorders [1, 3, 4]. The role of environmental factors including folic acid, vitamin B₁₂, infection, drugs, etc., has also been studied [1, 5, 6].

The genes which are so far known to be involved in determining susceptibility to SB are 1p13, 6q27, and 17q11.20q12 (OMIM number 182940), followed by 1p36.3, 1q43, 5p15.3-p15.2 and 14q24 (OMIM number 601634), while some studies have suggested X-linked inheritance (OMIM number 301410) [7].

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

Since we know only a small number of loci determining a specific morpho-physiological character, it is very delicate to estimate genetic homozygosity in humans. But knowing the type of inheritance and variability, it can be seen that a series of morpho-physiological traits are under the 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) [8-16].

The studied homozygous recessive characteristics (HRC) are obviously controlled by genes located on different human chromosomes, so they could be considered as markers of those 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 the human population, or in any specific sample of human individuals [11-15].

Numerous studies show that the percentage of the ABO blood types was found to be quite different in different samples of patients [13, 17-23]. According to the findings, we assumed that there may be a certain connection between the predisposition to SB and the frequencies of ABO blood types.

Material and methods

The HRC test for determination of genetic homozygosity in humans [8, 9] has been developed in order to define the proportion of homozygously recessive clearly expressed characteristics in every individual as markers of chromosomal homozygosities. Selected groups of people, such as a healthy control group and a sample of individuals affected by a specific disease, are compared. If they have different amounts of homo-recessive traits, this would be a manifestation of a kind of genetic load which will be present in the selected samples of the studied population [11-15].

The HRC test is a highly suitable method for estimating individual homozygosity by direct observation of defined phenotypic traits, and it takes only a few minutes to analyse the presence of 15 characteristics of the person observed (Table I). The presence of the studied genetically controlled recessive characteristics was used as a parameter for homozygosity of the corresponding genes and chromosomes.

Some homozygously recessive traits in the region of the human head are, for example: unattached ear lobe (OMIM number 128900), continuous frontal hair line (OMIM number 194000), blue eyes (gene location 19p13.1-q13.11, OMIM number 227240), 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 (phenylthiocarbamide) (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 mid-digital hair (OMIM number 157200), etc. [7].

In this study, comparative analyses were made by the same person, with equal criteria for determining extremely pronounced homozygously recessive characteristics by direct observation in tested groups of individuals. Variations in the presence of such characteristics were estimated using standard statistical procedures, and by comparing the means, the variances, and shapes of the distribution between the affected and healthy individuals. We used common tests to determine the differences in the mean values, scope and type of variability (Mann-Whitney U test, and χ^2 respectively).

Both groups of tested individuals (SB patients and controls) belong to the same ethnic group (Serbian population). The group of SB patients consisted of 100 individuals (aged between 3 and 16) who were treated in the University Children's Hospital in Belgrade. The control sample consisted of 100 randomly chosen children from Belgrade, belonging to the same age group and from the same locality. Since previous confirmation of spinal dysraphism with imaging techniques and clinical evaluation had been done by physicians, the group of SB patients was divided into the subgroups of SB occulta patients (SBO, $N = 50$) and SB aperta patients (SBA, $N = 50$).

Analyses of ABO blood type frequencies in the tested groups were undertaken with the assistance of the Saint Sava Institute for blood transfusion from Belgrade in Serbia.

For interpretation of the results in our study, a value of $p < 0.05$ was considered to be statistically significant.

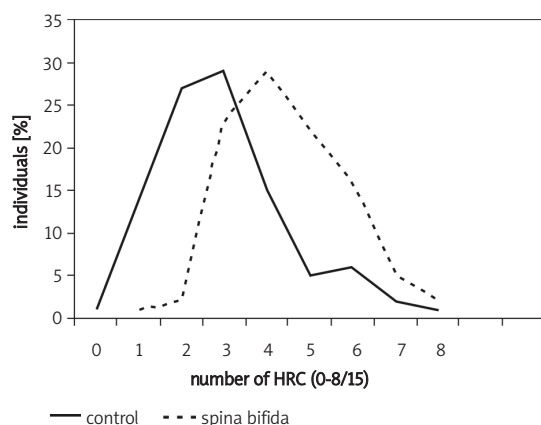


Figure 1. Frequencies of homozygous recessive characteristics in the group of patients with spinal dysraphism and the control group

Control: $N = 100$, $\bar{x}_{hro/15} = 3.0 \pm 0.2$

Affected: $N = 100$, $\bar{x}_{hro/15} = 4.5 \pm 0.3$ ($z = 6.80$, $p < 0.001$)

Results

From the data presented (Table I, Figure 1), it can be seen that HRC testing of SB patients shows an increased average homozygosity of the studied genetic markers, in comparison with healthy control individuals. It is obvious that the mean value of HRC in the complete sample of SB patients is significantly higher ($p < 0.001$) than in the control group (SB: 4.5 ± 0.3 , control: 3.0 ± 0.2). In 12 (80.0%) (blond hair, continuous hairline, straight hair, soft hair, double hair whorl, opposite hair whorl orientation, ear without Darwinian knot, blue eyes, top joint of the thumb $> 45^\circ$, proximal thumb extensibility, left-handedness and index finger longer than the ring finger) out of 15 observed characters, recessive homozygosity was expressed to a greater degree among SB patients, and in 9 (60.0%) (blond hair, continuous hairline, straight hair, soft hair, opposite hair whorl orientation, ear without Darwinian knot, blue eyes, top joint of the thumb $> 45^\circ$ and proximal thumb extensibility) of these characteristics this difference was statistically significant ($p < 0.01$ for continuous hairline, blue eyes and top joint of the thumb $> 45^\circ$, and $p < 0.001$ for the rest of the traits), while only 3 (20.0%) (attached ear lobe, colour blindness and right thumb over left thumb) are more frequent in the control group of individuals (Table I). The type

Table I. Frequencies of homozygously recessive characteristics among patients with spina bifida and individuals of control sample

Homozygously recessive characteristics	Control sample	Spina bifida affected	χ^2
	$N = 100$, n (%)	$N = 100$, n (%)	
Blond hair	9 (9)	45 (45)	144**
Continuous hairline	30 (30)	45 (45)	7.5*
Straight hair	41 (41)	67 (67)	16.5**
Soft hair	36 (36)	59 (59)	14.7**
Double hair whorl	6 (6)	8 (8)	0.7
Opposite hair whorl orientation	6 (6)	15 (15)	13.5**
Attached ear lobe	23 (23)	8 (8)	9.8**
Ear without Darwinian knot	1 (1)	5 (5)	16.0**
Blue eyes	21 (21)	33 (33)	6.9*
Colour blindness	3 (3)	2 (2)	0.3
Right thumb over left thumb	43 (43)	36 (36)	1.1
Top joint of the thumb $> 45^\circ$	24 (24)	37 (37)	7.0*
Proximal thumb extensibility	9 (9)	23 (23)	21.8**
Left-handedness	13 (13)	20 (20)	3.8
Index finger longer than the ring finger	30 (30)	39 (39)	2.7
			$\Sigma\chi^2 = 266.3^{**}$

* $p < 0.01$, ** $p < 0.001$

Control: $N = 100$, $\bar{x}_{hro/15} = 3.0 \pm 0.2$

Affected: $N = 100$, $\bar{x}_{hro/15} = 4.5 \pm 0.3$ ($z = 6.80$, $p < 0.001$)

of individual variation in the two studied groups significantly differed ($\Sigma\chi^2 = 266.3$, $df = 14$), showing that their genetic dispositions were remarkably different. Almost three quarters of SB patients had four or more homo-recessive characteristics, whereas among the control group almost three quarters of individuals had less than four homo-recessive characteristics.

Observing the distribution of HRC frequency between SB patients and the control sample of individuals, it can be seen that HRC in the group of SB patients are moving toward higher values, suggesting that genetic disposition at the polygenic level exists between two tested samples (Figure 1).

From the data presented (Table II) it can be seen that there is no difference in average homozygosity of such genetic markers between groups of SBO and SBA patients. In 8 (53.3%) (straight hair, double hair whorl, attached ear lobe, ear without Darwinian knot, right thumb over left thumb, top joint of the thumb $> 45^\circ$, left-handedness and index finger longer than the ring finger) out of 15 observed characters, recessive homozygosity was expressed to a greater degree among SBO patients, and in 2 (13.3%) (right thumb over left thumb and top joint of the thumb $> 45^\circ$) of these characteristics this difference was statistically significant ($p < 0.05$). In the group of SBA patients 6 (40.0%) (blond hair,

continuous hairline, soft hair, opposite hair whorl orientation, blue eyes and proximal thumb extensibility) out of 15 recessive characters are more frequent, and in 1 (6.7%) (blue eyes) of these characteristics this difference was statistically significant ($p < 0.001$). The type of individual variation in the two studied groups significantly differed ($\Sigma\chi^2 = 40.9$, $df = 14$).

As for the distribution of HRC frequency between groups of SBO and SBA patients, it can be seen that HRC in the group of SBA patients have a slight tendency towards higher values ($z = 0.10$; $p > 0.05$). The group of SBO patients shows two peaks of HRC frequencies while there is one peak in the group of SBA patients, suggesting that different genetic dispositions at the polygenic level exist in the two tested samples (Figure 2).

The results of ABO blood type distribution testing (Table III) show that there is a statistically significant difference in frequencies of B blood type ($\chi^2 = 4.1$, $p < 0.05$) and O blood type ($\chi^2 = 4.5$, $p < 0.05$) between the group of SB patients and the average value in the Serbian population [24].

Discussion

From the data presented in this study (Table I, Figure 1), the frequency distribution of tested homozygously recessive characteristics was

Table II. Frequencies of homozygously recessive characteristics among patients with spina bifida occulta and spina bifida aperta

Homozygously recessive characteristics	Spina bifida occulta	Spina bifida aperta	χ^2
	<i>N</i> = 50, <i>n</i> (%)	<i>N</i> = 50, <i>n</i> (%)	
Blond hair	22 (44)	23 (46)	0.1
Continuous hairline	20 (40)	25 (50)	2.3
Straight hair	35 (70)	32 (64)	0.2
Soft hair	28 (56)	31 (62)	0.6
Double hair whorl	5 (10)	3 (6)	2.3
Opposite hair whorl orientation	6 (12)	9 (18)	2.5
Attached ear lobe	5 (10)	3 (6)	2.3
Ear without Darwinian knot	3 (6)	2 (4)	0.9
Blue eyes	11 (22)	22 (44)	16.5**
Colour blindness	1 (2)	1 (2)	0
Right thumb over left thumb	21 (42)	15 (30)	4.1*
Top joint of the thumb $> 45^\circ$	22 (44)	15 (30)	5.5*
Proximal thumb extensibility	11 (22)	12 (24)	0.2
Left-handedness	11 (22)	9 (18)	0.8
Index finger longer than the ring finger	22 (44)	17 (34)	2.6
			$\Sigma\chi^2 = 40.9^{**}$

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

Spina bifida occulta: $N = 50$, $\bar{x}_{hro/15} = 4.5 \pm 0.2$

Spina bifida aperta: $N = 50$, $\bar{x}_{hro/15} = 4.4 \pm 0.2$ ($z = 0.10$, $p > 0.05$)

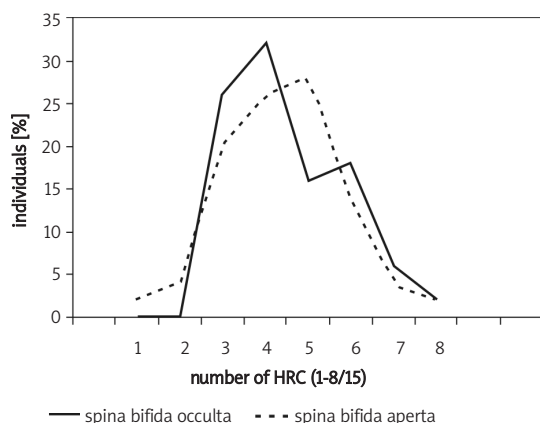


Figure 2. Frequencies of homozygous recessive characteristics in the groups of patients with spina bifida occulta and spina bifida aperta
Spina bifida occulta $N = 50$, $\bar{x}_{hro/15} = 4.5 \pm 0.2$
Spina bifida aperta $N = 50$, $\bar{x}_{hro/15} = 4.4 \pm 0.2$ ($z = 0.10$; $p > 0.05$)

Table III. Frequencies of ABO blood types among patients with spina bifida and controls

Blood type	Serbian population	Spina bifida	χ^2
	$N = 173\ 380$, n (%)	$N = 78$, n (%)	
A	71 953 (41.5)	30 (38.5)	0.2
O	58 776 (33.9)	36 (46.2)	4.5*
B	30 341 (17.5)	7 (9.05)	4.1*
AB	12 310 (7.1)	5 (6.4)	0.1

* $p < 0.05$

different between the SB group of patients and controls (SB: 4.5 ± 0.3 , control: 3.0 ± 0.2 , $z = 6.80$, $p < 0.001$), indicating the population-genetic difference that exists between them. Our results show that almost 75% of SB patients had four or more homo-recessive characteristics, whereas among the control group almost 75% of individuals had less than four homo-recessive characteristics (Figure 1). In these comparisons, characteristic groups of traits were present to a different degree among SB patients and control individuals, suggesting a correlation between different combinations of polygenes which may be involved in regulatory processes of resistance and development of spinal dysraphism.

A few possible explanations can be offered for the established differences of HRC presence in the samples of SB patients and the control group:

- a higher degree of genetic homozygosity in the group of SB patients may bring such organisms into a specific state of genetic-physiological homeostasis which enables easier expression of spinal dysraphism;
- a higher degree of genetic homozygosity may result in pleiotropic effects of specific genes

responsible for expressing predisposition to spinal dysraphism;

- increased genetic homozygosity may raise the genetic load, thus potentially causing decreased body immunity, which might predispose to development of spinal dysraphism [9-15, 25].

From the results presented (Table II, Figure 2) it can be seen that there are no differences in average homozygosity of such genetic markers between groups of SBO and SBA patients, which is already assumed since SBO and SBA are two subgroups within one general entity of SB. But the type of individual variation in the two studied groups was significantly different ($\Sigma\chi^2 = 40.9$, $df = 14$), showing that their genetic dispositions were different too. The results presented in Table II show that differences in the presence of individual homozygously recessive traits exist between patients with SBO and SBA, suggesting that there are different genetic dispositions at the polygenic level between the two tested samples.

These differences in individual variability, no matter the similar average values of genetic homozygosity, point to different expression of genes that may lead to different expression of SB (SBO and SBA). Such claims may lead to new insight into and understanding of pathogenesis of SB and the influence of environmental factors on genetic predisposition to expression of the two evaluated different subtypes of SB.

In the sample of SB patients, O blood type is significantly increased ($\chi^2 = 4.5$, $p < 0.05$) and there is a statistically significant decrease in the frequency of B blood type ($\chi^2 = 4.1$, $p < 0.05$) compared with the percentage of those blood groups in the Serbian population [24]. The significant increase in the frequency of blood type O in the group of SB patients may indicate that the presence of this blood group represents a certain predisposition to spinal dysraphism.

Beside genetic predisposition for SB inheritance and development, it is important to further evaluate the possible influence of environmental factors (folic acid, vitamin B₁₂, infection, drugs, etc.) and gender for the expression of this pathological condition [1, 5, 6].

Our results clearly show that there is a populational genetic difference in the degree of genetic homozygosity and variability between the group of patients with SB and individuals without clinical manifestations, indicating a possible genetic component in the aetiopathogenesis of spina bifida.

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