

INDIVIDUAL PHENOTYPE TRAIT VARIABILITY AS GENETIC MARKERS OF GENDER SUSCEPTIBILITY TO SPINA BIFIDA

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ABSTRACT

We compared individual trait variability in 65 male and 81 female patients with spina bifida occulta (SBO) or spina bifida aperta (SBA) against 170 male and 200 female subjects randomly selected Serbian subjects without these conditions. Variability was evaluated by direct observation of 15 homozygous recessive traits (HRT), while gender was evaluated separately. Individual trait variations between genders in SBO patients (4/15 HRT) and in SBA patients (12/15 HRT) showed remarkable differences. Individual trait variations between the male control group and SBO (9/15 HRT), between the female control group and SBO (5/15 HRT), between the male control group and SBA (8/15 HRT), between the female control group and SBA (9/15 HRT), between male SBO and SBA patients (6/15 HRT), between female SBO and SBA patients (6/15 HRT), also indicated remarkable differences. These differences could be explained by differ-

ent expression of genes that may contribute to expression of spina bifida (SB).

Key words: Spina bifida (SB); Gender; Homozygous recessive traits (HRT); Variability

INTRODUCTION

Spina bifida (SB) is the incomplete closure of the neural tube during fetal development [1]. The prevalence of SB is related to geographic location (higher in northern Russians than in Norwegians), time of the conception, gender (higher in females than in males), mother's age, folic acid consumption and other factors [2-6]. It is a multifactorial disease involving genetic and environmental factors [6]. In support of a genetic component of SB are reported the gender differences in prevalence at birth and the higher incidence in monozygous twins and in relatives of affected patients [7]. Genes with a suggested linkage to neural tube defects and SB include those located at 17q11.2-q12, 1p13, 6q27, 14q24, 1p36.3, 5p15.3-p15.2, 1q43 (OMIM numbers 601634, 182940) and on the X chromosome (OMIM number 301410) [8-10]. Changes in the skin, particularly in spina bifida occulta (SBO), such as hyperpigmentation, hypertrichosis and dermal sinuses [11], have led Cvjeticanin *et al.* [12] to investigate the frequency of recessive homozygosity and genetic variability in patients with SB. A higher frequency of recessive homozygosity and decreased ge-

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netic variability was reported in patients with SB than in healthy matched subjects [12]. Increased recessive homozygosity in several conditions including diabetes mellitus, congenital hip dislocation, bronchogenic carcinoma and chronic lymphocytic leukemia, have also been reported [13,14]. Genetic inheritance [6], increased incidence of certain skin markers as phenotype traits [8] and gender differences in prevalence at birth [7], have led us to investigate the individual phenotype trait variability in male and in female patients with SBO and/or spina bifida aperta (SBA) and their value for the prediction of this disease.

MATERIALS AND METHODS

We have evaluated 65 male lumbosacral SB patients, and 170 male unaffected subjects, and 81 female SB patients, and 200 female unaffected subjects. Radiographic imaging was used to confirm diagnosis and lesion level and to differentiate SBO from SBA. The unaffected were healthy individuals, taken randomly from the school population. All parents or legal guardians of the participants signed informed consent forms. The patients were classified into SBO and SBA groups. Gender was analyzed separately for all subjects. Patients with SB were Serbian and 3 to 18 years of age. Unaffected subjects were Serbian and 4 to 18 years of age and of similar socioeconomic status as the patients.

To estimate the proportion of homozygous recessive traits (HRT), based on the reports of previous investigators who were evaluating the nature of inheritance, we used the HRT-test [15-17]. This included 15 HRT, whose extreme appearance was taken to represent homozygosity [17,18]. These were: 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 and opposite hair whorl orientation (OMIM number 139400); color blindness (gene location Xq28, OMIM number 303800); ear without Darwinian notch; 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); right thumb over left thumb (hand clasping) (OMIM number 139800) and top joint of the thumb $>45^\circ$ [8].

The frequency of an HRT in each group, was recorded by whole numbers and percentages. The chi

squared test (χ^2) was used to compare frequencies of HRT by gender between the groups. Statistical significance was set on $p < 0.05$.

RESULTS

Variability of Homozygous Recessive Traits by Gender in Controls. Only four (26.7%) of the HRT differed significantly, of which two (13.3%) (opposite hair whorl orientation and left-handedness) were significantly more frequent in males, while two (13.3%) HRT (continuous hairline and index finger longer than the ring finger) were significantly more frequent in females (Tables 1 and 2). The individual variations of 15 HRT between genders in the control group [$\chi^2 = 46.3$; degree of freedom (df) = 14, $p < 0.01$] were significantly different.

Variability of Homozygous Recessive Traits by Gender in Spina Bifida Occulta Patients. Homozygous recessive traits that significantly differed [four (26.7%)] were remarkably higher in females than in males (opposite hair whorl, continuous hair line and attached ear lobe, top joint of the thumb $>45^\circ$) (Tables 1 and 2). The individual variations of 15 HRT between genders in SBO subjects ($\Sigma\chi^2 = 66.9$; df = 14, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits by Gender in Spina Bifida Aperta Patients. There were 12 (80.0%) HRT that significantly differed, of which six (40.0%) HRT were remarkably frequent in males (straight hair, continuous hairline, ear without Darwinian notch, color blindness, right thumb over left thumb and top joint of the thumb $>45^\circ$), and six (40.0%) HRT were remarkably frequent in females (opposite hair whorl, soft hair, attached ear lobe, blue eyes, proximal thumb extensibility and index finger longer than the ring finger) (Tables 1 and 2). The individual variations of 15 HRT between genders in SBA subjects ($\Sigma\chi^2 = 165.9$; df = 14, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Male Controls and Spina Bifida Occulta Patients. We found nine (60.0%) HRT that differed significantly, of which four (26.7%) HRT were remarkably frequent in the male control group (opposite hair whorl orientation, attached ear lobe, ear without Darwinian notch and right thumb over left thumb), while five (33.3%) HRT were remarkably frequent in male patients (blond hair, straight hair, soft hair, blue eyes and proximal thumb extensibility) (Tables 1 and

Table 1. Cases of homozygous recessive traits in male and female subjects

| HRT (frequencies) | Males | | | Females | | |
|--|--------------------------------|--------------------------|--------------------------|--------------------------------|--------------------------|--------------------------|
| | Controls <i>n</i> = 170 (%) | SBO <i>n</i> = 42 (%) | SBA <i>n</i> = 23 (%) | Controls <i>n</i> = 200 (%) | SBO <i>n</i> = 53 (%) | SBA <i>n</i> = 28 (%) |
| Blond hair | 35 (20.6) | 16 (38.1) | 10 (43.5) | 42 (21.0) | 19 (35.9) | 12 (42.9) |
| Straight hair | 92 (54.1) | 31 (73.8) | 17 (73.9) | 98 (49.0) | 36 (67.9) | 15 (53.6) |
| Two hair whorls | 21 (12.4) | 4 (9.5) | 1 (4.3) | 21 (10.5) | 3 (5.7) | 1 (3.6) |
| Opposite hair whorl orientation | 44 (25.9) | 1 (2.4) | 3 (13.0) | 25 (12.5) | 8 (15.1) | 6 (21.4) |
| Soft hair | 60 (35.3) | 23 (54.8) | 10 (43.5) | 91 (45.5) | 28 (52.8) | 20 (71.4) |
| Continuous hairline | 53 (31.2) | 14 (33.3) | 13 (56.5) | 89 (44.5) | 29 (54.7) | 9 (32.1) |
| Attached ear lobe | 31 (18.2) | 3 (7.1) | 1 (4.3) | 51 (25.5) | 7 (13.2) | 4 (14.3) |
| Ear without the Darwinian notch | 21 (12.4) | 2 (4.8) | 4 (17.4) | 16 (8.0) | 3 (5.7) | 1 (3.6) |
| Blue eyes | 39 (22.9) | 15 (35.7) | 8 (34.8) | 48 (24.0) | 15 (28.3) | 15 (53.6) |
| Color blindness | 8 (4.7) | 1 (2.4) | 2 (8.7) | 4 (2.0) | 1 (1.9) | 0 (0.0) |
| Right thumb over left thumb | 82 (48.2) | 14 (33.3) | 10 (43.5) | 86 (43.0) | 22 (41.5) | 7 (25.0) |
| Top joint of the thumb >45° | 67 (39.4) | 13 (31.0) | 8 (34.8) | 62 (31.0) | 26 (49.1) | 6 (21.4) |
| Proximal thumb extensibility | 20 (11.8) | 10 (23.8) | 3 (13.0) | 37 (18.5) | 11 (20.8) | 10 (35.7) |
| Left handedness | 32 (18.8) | 8 (19.0) | 4 (17.4) | 19 (9.5) | 11 (20.8) | 6 (21.4) |
| Index finger longer than the ring finger | 58 (34.1) | 18 (42.9) | 5 (21.7) | 103 (51.5) | 29 (54.7) | 13 (46.4) |

3). The individual variations of 15 HRT between both groups ($\Sigma\chi^2 = 95.7$; $df = 14$, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Female Controls and Spina Bifida Occulta Patients. We found 5 (33.3%) HRT that differed significantly between these groups, of which 1 (6.7%) HRT was remarkable frequent in the control group (attached ear lobe), while 4 (26.7%) HRT were remarkable frequent in the patients (blond hair, straight hair, top joint of the thumb > 45° and left-handedness) (Tables 1 and 3). Individual variations of 15 HRT between both groups ($\Sigma\chi^2 = 56.1$; $df = 14$, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Male Controls and Spina Bifida Aperta Patients. We found eight (53.3%) HRT that differed significantly between these groups. Male controls had four (26.7%) HRT that were significantly frequent (two hair whorls, opposite hair whorl orientation, attached ear lobe and index finger longer than the ring finger), and male SBA patients had four (26.7%) HRT that were remarkably frequent (blond hair, straight hair, continuous hairline and blue eyes) (Tables 1 and

3). Individual variations of 15 HRT between the male control group and SBA patients ($\Sigma\chi^2 = 90.1$; $df = 14$, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Female Controls and Spina Bifida Aperta Patients. We found nine (60.0%) HRT that differed significantly between these groups. Female controls had three (20.0%) HRT that were remarkably frequent (two hair whorls, attached ear lobe and right thumb over left thumb), and female SBA patients had six (40.0%) HRT were remarkably frequent (blond hair, opposite hair whorl orientation, soft hair, blue eyes, proximal thumb extensibility and left-handedness) (Tables 1 and 3). Individual variations of 15 HRT between the female control group and SBA patients ($\Sigma\chi^2 = 140.1$; $df = 14$, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Male Spina Bifida Occulta and Spina Bifida Aperta Patients. We found six (40.0%) HRT that significantly differed between these groups, of which three (20.0%) HRT were remarkably frequent in SBO patients (two hair whorls, proximal thumb extensibility and index finger longer than the ring finger), and

Table 2. Differences in prevalence of homozygous recessive traits between male and female subjects

| HRT (χ^2) | Males/Females | | |
|--|-------------------|-------------------|--------------------|
| | Controls (%) | SBO (%) | SBA (%) |
| Blond hair | 0 | 0.1 | 0 |
| Straight hair | 0.5 | 0.5 | 6.7 ^a |
| Two hair whorls | 0.3 | 2 | 0.1 |
| Opposite hair whorl orientation | 10.7 ^a | 39.0 ^a | 4.4 ^b |
| Soft hair | 2.6 | 0.4 | 14.4 ^a |
| Continuous hairline | 4.9 ^b | 4.9 ^b | 14.5 ^a |
| Attached ear lobe | 2.5 | 4.0 ^b | 15.1 ^a |
| Ear without the Darwinian notch | 2 | 0.2 | 31.9 ^a |
| Blue eyes | 0.1 | 1.7 | 8.4 ^a |
| Color blindness | 2.6 | 0.1 | 4.4 ^a |
| Right thumb over left thumb | 0.6 | 1.8 | 10.8 ^a |
| Top joint of the thumb >45° | 2.1 | 8.7 ^a | 6.8 ^a |
| Proximal thumb extensibility | 3.1 | 0.4 | 27.0 ^a |
| Left handedness | 6.9 ^a | 0.2 | 0.8 |
| Index finger longer than the ring finger | 7.4 ^a | 2.9 | 20.6 ^a |
| $\Sigma\chi^2$ | 46.3 ^a | 66.9 ^a | 165.9 ^a |

^a $p < 0.01$. ^b $p < 0.05$.

three (20.0%) HRT were remarkably frequent in SBA patients (opposite hair whorl orientation, continuous hairline and color blindness and) (Table 1 and 3). Individual variations of 15 HRT between male SBO and SBA patients ($\Sigma\chi^2 = 89.4$; $df = 14$, $p < 0.01$) were significantly different.

Variability of Homozygous Recessive Traits Between Female Spina Bifida Occulta and Spina Bifida Aperta Patients. We found six (40.0%) HRT that differed significantly between these groups, of which three (20.0%) HRT were remarkable frequent in SBO patients (continuous hairline, right thumb over left thumb and top joint of the thumb >45°), and three (40.0%) HRT were remarkably frequent in SBA patients (soft hair, blue eyes and proximal thumb extensibility) (Tables 1 and 3). Individual variations of 15 HRT between female SBO and SBA patients ($\Sigma\chi^2 = 90.4$; $df = 14$, $p < 0.01$) were significantly different.

Phenotype Traits Distribution Between Different Male and Female Groups of Participants. In the male gender there is a continuous increase in the pro-

portional presence with the least proportion in the control group and the highest proportion in SBA patients for three (20.0%) HRT, while two (13.3%) HRT had a continuous decrease (Table 1). In the female gender, six HRT (40.0%) showed a continuous increase from the control group to SBA patients, while three HRT (20.0%) showed a continuous decrease in the proportional presence (Table 1). Even though color blindness in the female gender had a continuous decrease, we have excluded this trait from this study since the difference between percentages was 0.1% between the control group and SBO patients (Table 1). We found that 2/12 HRT had the same tendency of proportional presence in both genders, while 10/12 HRT were different (3/12 HRT for the male gender and 7/12 HRT for the female gender).

DISCUSSION

Our findings indicate a difference in individual trait variability between males and females for the pre-

Table 3. Differences in prevalence of homozygous recessive traits between evaluated groups of same gender subjects

| HRT (χ^2) | Males | | | Females | | |
|--|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|
| | Controls/ SBO | Controls/ SBA | SBO/SBA | Controls/ SBO | Controls/ SBA | SBO/SBA |
| Blond hair | 14.9 ^a | 25.5 ^a | 1.8 | 10.6 ^a | 22.8 ^a | 1.3 |
| Straight hair | 7.2 ^a | 7.2 ^a | 0 | 7.3 ^a | 0.4 | 3.4 |
| Two hair whorls | 0.7 | 5.3 ^b | 4.6 ^b | 2.2 | 4.6 ^b | 1.1 |
| Opposite hair whorl orientation | 21.3 ^a | 6.4 ^b | 27.7 ^a | 0.5 | 6.3 ^b | 2.3 |
| Soft hair | 10.8 ^a | 1.9 | 2.6 | 1.2 | 14.7 ^a | 5.7 ^b |
| Continuous hairline | 0.1 | 15.9 ^a | 12.9 ^a | 2.3 | 3.5 | 12.6 ^a |
| Attached ear lobe | 6.8 ^a | 10.6 ^a | 1.5 | 5.8 ^b | 4.9 ^b | 0.1 |
| Ear without the Darwinian notch | 4.7 ^b | 2 | 1.7 | 0.7 | 2.5 | 1.1 |
| Blue eyes | 7.2 ^a | 6.2 ^b | 0 | 0.8 | 36.5 ^a | 17.3 ^a |
| Color blindness | 1.1 | 3.4 | 10.6 ^a | 0 | 2 | 1 |
| Right thumb over left thumb | 4.6 ^b | 0.5 | 2.8 | 0.1 | 7.5 ^a | 8.8 ^a |
| Top joint of the thumb >45° | 1.8 | 0.5 | 0.5 | 10.6 ^a | 3 | 25.8 ^a |
| Proximal thumb extensibility | 12.2 ^a | 0.1 | 7.0 ^a | 0.3 | 16.0 ^a | 8.5 ^a |
| Left handedness | 0 | 0.1 | 0.1 | 13.4 ^a | 14.9 ^a | 0 |
| Index finger longer than the ring finger | 2.3 | 4.5 ^b | 15.6 ^a | 0.2 | 0.5 | 1.4 |
| $\Sigma\chi^2$ | 95.7 ^a | 90.1 ^a | 89.4 ^a | 56.1 ^a | 140.1 ^a | 90.4 ^a |

^a $p < 0.01$. ^b $p < 0.05$.

disposition to SB and to the expression of SB (SBO or SBA). A difference in genetic variability between healthy individuals and those with a diagnosed SB was reported by Nikolic *et al.* [19], but the possible role of gender for the SB individuals has not yet been observed regarding the individual traits variability.

The present variability in the proportion of certain observed traits between genders in the control groups have been noticed in previous studies as well. For example, in the study of Ziering and Krenitzky [20], frequent opposite hair whorl orientation was observed in the male population. Reiss and Reiss [21] have presented that left-handedness was more frequent in males, while in the study of Gillam *et al.* [22], a longer index finger than the ring finger was frequently recorded in the female population.

Our findings clearly show that there are differences in the distribution of certain phenotype traits between genders in control groups and in SBO and

SBA patients, giving us the assumption of different predisposition to SBO and SBA between genders and the possibility of a correlation with different combinations of polygenes in these subjects. Such differences, stress the possibility of different, more specific phenotype existence for SBO or SBA regarding gender. From this point of view, it could be assumed that certain genes determining observed phenotypes that differ remarkably between genders in these subjects could have influence, to a certain degree, on the adaptability potential of the organism with the present SBO or SBA, to the specific environmental factors that could interfere during embryo genesis processes [14]. Since the genes for evaluated HRT are located on different chromosomes, they are considered not just the markers of such chromosomes, but the markers of the groups of surrounding polygenes that may have influence to a different degree on the development of SB and expression of SBO or SBA.

Even though we have noticed different presentations of phenotype traits distribution between genders for SBO or SBA patients, phenotype traits differ remarkably between the control groups and SBO patients, and the control groups and SBA patients for the same gender. These findings could possibly explain the different predisposition to the SBO or SBA in the same gender group. The presence of different distributions of HRT that significantly differ indicates how high the variability of genetic homeostasis in humans can be, with a high possibility of extreme genotypes to be exposed to specific metabolic and developmental malformations [14].

Since SB is a multifactorial disease with reported locations of several genes that suggest the linkage to formation of neural tube defects [8-10], it should be stressed that further evaluation of the possible influence of environmental factors is necessary to better understand the processes of SB etiopathogenesis and to establish proper preventive measures and programs [23,24]. Age limitation for the inclusion of evaluated subjects in the study was due to the establishment of clear presentation of index finger-ring finger ratio. The previous studies have demonstrated that such a ratio is genetically controlled but the full expression is expected when the individual reaches 2 years of age [22]. Since we have included extreme appearance of evaluated HRTs, therefore the lower age for the inclusion was 3 years of life.

Even though it is delicate to establish expected phenotype variations in humans, our results clearly show that distinction in such variations persist between different groups of individuals (diseased and healthy, male and female gender). Therefore, the applied methodology that we used in this study could be in a certain degree of benefit as a potential early screening tool in diseased humans with an identifiable genetic predisposition with other diagnostic tests.

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