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Short communication

Polymorphisms of the 5,10-methylenetetrahydrofolate and the methionine synthase reductase genes as independent risk factors for spina bifida

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Abstract: We analyzed the role of the *C677T* polymorphism of the 5,10-methylenetetrahydrofolate and the *A66G* polymorphism of the methionine synthase reductase genes as risk factors for occurrence of spina bifida. The studied population included 106 mothers and 104 children from affected families, and a control group of 100 adults. We found statistically significant differences between the occurrence of the homozygosity in these polymorphisms in the groups of mothers and children with thoracolumbal defects (*C677T* polymorphism) and lumbosacral defects (*A66G* polymorphism). We postulate that these polymorphisms should be regarded as independent risk factors for spina bifida.

Key words: folic acid supplementation, mutation, neural tube defect.

Neural tube defects are among the most common and severe congenital defects, occuring with the average frequency of around 1 per 1000 births. The defect leads usually to death or life-long handicap in surviving children. These children need also expensive medical help for all their lives. The cause of the defects is a failure in closure of the neural tube between the 21st and the 28th day of embryogenesis, in one or more of the hypothetical closure sites (3).

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It is known that periconceptional supplementation of folic acid reduces the frequency of neural tube defects by up to 70% (2). Because of this fact, many folate-related genes have been investigated for mutations altering folate metabolism. One of the best-known mutations decreasing the activity of the folate-dependent remethylation of homocysteine to methionine, is the *C677T* transition in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene (1). The homozygosity (TT genotype) has been found to increase the risk of the occurrence of neural tube defects (4). Another mutation, which potentially alters the homocysteine remethylation pathway, is *A66G* transition of the methionine synthase reductase (*MSR*) gene (5).

The aim of our study was to evaluate the role of these mutations as risk factors for the occurrence of spina bifida (the most common form of neural tube defects) in the Polish population.

The studied population included 104 children and 106 mothers of children with nonsyndromic, isolated spina bifida, followed up in our hospital, who agreed to participate in the study (informed consent). The control population included 100 randomly chosen, adult inhabitants of Krakow. The Jagiellonian University Ethical Committee accepted the study protocol.

The participating children and the mothers were divided into two subgroups, according to the spinal neural tube closure sites (thoracolumbar defects including the region at or above the L-1 level, and lumbosacral defects at or below the L-2 level of the spine) (3). Among 104 children with spina bifida, there were 70 patients with the defect in the lumbosacral and 34 in the thoracolumbar region. Among 106 tested mothers, 72 had a child with spina bifida in the lumbosacral and 34 in the thoracolumbar region of the spine.

Genomic DNA was isolated from peripheral blood leukocytes by using standard methods. The appropriate fragments of the two investigated genes were amplified by using polymerase chain reaction (*PCR*), with a subsequent digestion with restriction enzymes *Hin*fI and *Nde*I and analysis of the restricted fragments by means of electrophoresis, as described by FROSST (1995) and WILSON (1999).

Odds ratio (OR), 95% confidence interval (CI) and Fisher's exact test were used to estimate the statistical significance of the differences between genotyping results in the tested groups.

We found statistically significant differences in the prevalence of homozygosity for the *C677T* polymorphism between the first group of mothers (thoracolumbar site) and the control (OR = 4.82; 95%CI = 1.6-14.48; p = 0.007) and between the first group of children (thoracolumbar site) and the control (OR = 3.35; 95% CI = 1.05-10.65; p = 0.049). On the other site we found statistically significant differences between the prevalence of the homozygosity for the A66G polymorphism between the second group of mothers (lumbosacral site) and the control (OR = 3.06; 95% CI = 1.04-8.97; p = 0.039) as well as children (lumbosacral site) and the control (OR = 3.16; 95% CI = 1.08-9.28; p = 0.034). The differences between the "lumbosacral" groups (*C667T* polymorphism)

Table 1. The observed prevalences of the *MTHFR* genotypes (mutation *C677T*) and *MSR* genotypes (mutation *A66G*)

Group	*677CC*	*677CT*	*677TT*	*66AA*	*66AG*	*66GG*
Mothers – thoracolumbar region	12	14	8	9	23	2
Mothers – lumbosacral region	43	23	6	31	31	10
Children – thoracolumbar region	12	16	6	17	16	1
Children – lumbosacral region	30	33	7	27	33	10
Control	56	38	6	66	29	5

and the control, and between the "thoracolumbar" groups (A66G polymorphism) and the control, did-not reach statistical significance. Table 1 shows the prevalence of the tested genotypes.

The results of our study indicate that the presence of the *677TT* and *66GG* genotypes increases the risk of occurrence of spina bifida in thoracolumbar and lumbosacral regions, respectively. In our opinion the *C677T* polymorphism of the *MTHFR* and the *A66G* polymorphism of the *MSR* genes should be considered as independent risk factors for the occurrence of spina bifida.

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