ABSTRACT

Introduction: The *SHOX* gene is located in pseudoautosomal region 1 at the end of the short arm of the X and Y chromosomes (Xp22.32/Yp11.32) and encodes a transcription factor that regulates chondrocyte proliferation and differentiation in growth plate. *SHOX* haploinsufficiency is associated with Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). In most cases, it is caused by a CNV (copy number variation) involving *SHOX* exons and/or its regulatory elements; however, in approximately 30% of cases, the cause of the pathological phenotype remains undiscovered. Compared to deletions involving *SHOX* regulatory elements, the significance of duplications remains controversial in some cases. Duplications involving regulatory elements in the *SHOX* region have been described in patients with the LWD/ISS phenotype, but also in healthy individuals. Under normal conditions, the CpG islands flanking the *SHOX* gene are hypomethylated, which corresponds to the fact that *SHOX* escapes inactivation. However, some studies assume that changes in the DNA methylation level of genes can be associated with rearrangements in their surroundings, the role of changes in the DNA methylation profile in the etiopathogenesis of some diseases is also known. The aim of this thesis was to verify the possible influence of duplications involving regulatory sequences of the *SHOX* gene on the DNA methylation status of this gene, in patients with the LWD phenotype. Another goal was to verify whether changes in *SHOX* methylation profiles in LWD/ISS patients without mutations could be responsible for the pathological phenotype.

Material and Methods: Material consisted of: a) 20 patients with LWD phenotype and duplication involving *SHOX* regulatory elements; b) 30 patients with LWD/ISS phenotype without any structural or point mutations in the *SHOX* region; c) 23 healthy individuals. We focused on the methylation status of two CpG islands in the up-stream region of the *SHOX*. Genomic DNA samples were first subjected to bisulfite conversion, then amplified using the polymerase chain reaction, and the reaction products were finally sequenced using the Sanger sequencing method. Determination of the level of methylation of individual CpG dinucleotides was performed using the ABSP tool software.

Results: Our results indicate, that CpG islands in the *SHOX* region show a lower level of methylation in patients carrying a duplications than in healthy individuals, but only one island showed a statistically significant difference (CpG 1: $p = 0{,}4224$; CpG 3: $p < 0{,}0001$). The results of methylation profiling of CpG islands in patients without a point or structural mutation indicate that the methylation indexes of patients differed from the average of the group of healthy individuals by at least +/- 2SD in 86,6 % of patients in the

case of CpG 1 island, and in 70 % of patients in the monitored section of CpG 3 island. However, the difference in methylation level was observed for individual CpG dinucleotides, not for all CpGs in the island simultaneously.

Conclusion: Based on the analysis, we found that duplications in the regulatory regions of the *SHOX* gene in patients with the LWD phenotype lead to a statistically significant decrease in the level of DNA methylation only in the CpG 3 island. However, the biological effect of this difference will probably be insignificant due to the generally very low level of methylation. The methylation indexes of patients with the LWD/ISS phenotype without any mutations deviate from the mean of healthy individuals; however, these differences are in units of percentage points. Molecular-genetic mechanisms other than DNA methylation are likely to be responsible for the pathological phenotype in these patients. Questions for further research include the effects of specific duplications depending on their location, the strength of the real biological effect of the observed methylation changes, and the significance of changes in the methylation profile in the etiology of LWD and ISS.

Key words: DNA methylation, CpG islands, haploinsufficiency, *SHOX* gene, Léri-Weill dyschondrosteosis