

Letter to the Editor

Mutations of NOS1 and MLN regulatory sequences are a potential cause of infantile hypertrophic pyloric stenosis

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Infantile hypertrophic pyloric stenosis (IHPS) is one of the most common congenital defects requiring surgical intervention in the neonatal period. Infantile hypertrophic pyloric stenosis is characterized by progressive hypertrophy of the pyloric muscle. The prevalence of IHPS is 5 in 1000 live births [1]. Infantile hypertrophic pyloric stenosis shows familial aggregation and heritability, but knowledge about specific genetic risk variants is limited.

The study was conducted after obtaining the approval of the Bioethics Committee of the Medical University of Lodz and the signing of a consent form by the parents. The study was carried out on a group of 45 children (70% boys) with IHPS who underwent pyloromyotomy aged 4 to 8 weeks. Vomiting and lack of weight gain were observed between 2 and 6 weeks of age. Electrolyte imbalance and, in rare cases ($n = 3$), metabolic alkalosis were noted. All patients were discharged home after 3–5 days of hospitalization in good general condition without complications.

Biopsy specimens consisting of smooth-muscle layers were obtained at pyloromyotomy. The material was tested by immunohistochemistry and molecular analysis.

Genetic linkage sites mapped infantile hypertrophic pyloric stenosis to the short arm of chromosome 12 due to the genetic disequilibrium of the nitric oxide synthase (*NOS1*) genetic variants [2]. A second likely candidate gene is motilin (*MLN*) [3]. This led us to hypothesize that mutations of the *NOS1* or *MLN* gene may be causative factors of IHPS. All patients had the *NOS1* and *MLN* genes sequenced using two genes. In one patient a variant located between the 21st and 22nd exome of the first isoform of the *NOS1* gene (chr12:117,669,718C>T (GRCh37/hp19 assembly)) was detected. The mutation affected a splice site donor site. In another unrelated individual we identified a variant of the 3' untranslated region (3'UTR, chr12:117 648 733G>T) which, according to bioinformatic predictions [4], could potentially affect binding of two transcription factors: MZF1 and BEN.

Similarly, a mutation of the *MLN* gene 5'UTR region was identified (chr6:63771789). To estimate its impact on gene expression, we per-

formed real-time polymerase chain reaction in peripheral blood mononuclear cells. The patient harboring the mutation showed mRNA levels in the upper 5% of normalized expression in the whole group, which suggested that this variant was associated with strongly increased transcription of *MLN* and motilin protein synthesis. Conversely, in the patient with a mutation of *NOS1* splice site, *NOS1* mRNA expression was within the bottom 10% of normalized expression scores, which suggested that this variant lowered the expression of nitric oxide synthase, which was described as associated with IHPS [5].

None of the three novel mutations was present in a control group of 15 healthy individuals, in the parents of the affected group ($n = 90$), or in the 1000Genomes dataset (<http://www.1000genomes.org/home>).

We did not observe any deviations in the clinical course of treatment of the 3 patients with IHPS harboring the potentially pathogenic variants of *MLN* or *NOS1*, possibly due to the group's moderate size.

In conclusion, although the study did not identify any directly causative mutations affecting *NOS1* or *MLN* through disruption of the protein sequence, the unique variants could, by modifying gene expression, contribute to the development of IHPS.

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Conflict of interest

The authors declare no conflict of interest.

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