MUTATION ANALYSIS OF THE NLRP3 GENE IN CHILDREN WITH CRYOPYRIN-ASSOCIATED FEVER

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Introduction: CAPS is a group of severe multi-system, auto-inflammatory diseases with an autosomal dominant type of inheritance associated with mutations in the cryopyrin NLRP3 gene located on the first chromosome (1q44). CAPS-syndrome is represented by three phenotypes: familial cold urticarial, Muckle – Wells syndrome, and CINCA/NOMID syndrome. Today, it is quite a lot known about CAPS-syndrome, but due to the nonspecific, chronic, remitting course of the disease, diagnosis and, consequently, treatment of the patient is hindered. The diagnosis is based on the results of molecular genetic approach.

Methods: The DNAs were extracted from the whole blood of patients with suspected periodic fever (according to the manufacturer's instructions). The presence or absence of mutations of the NLRP3 gene were determined by PCR using specific primers followed by purification of amplicons and Sanger sequencing. Results of Sanger sequencing were analyzed using Seq 6, Sequencher 2.0 software.

Results: The molecular characteristic of the NLRP3 gene includes 10 exons, 32.952base pairs. As of today, according to the register and database https://infevers.umai-montpellier.fr/web/search.php, 204 mutations of the NLRP3 gene are known, but only 19 mutations are considered pathogenic. Taking into account that the majority of pathogenic mutations are located in exon 3 of the NLRP3 gene we designed primers of the 3 exon. After optimizing the primers of 3 exons, we examined 2patients by Sanger sequencing. Analysis of the electrophoregrams using the Mutation Testing resource revealed a heterozygous carrier of c. 732G>A (p.Ala 244=; rs3806268) as prediction polymorphism. The frequency of this polymorphism is high in different populations; the frequency of alleles is about 0.555. The results of genetic testing allow us to consider the detected variant as the cause of cryopyrin-related syndrome only if there is sufficient clinical and anamnestic data to confirm the diagnosis.

Conclusion: Thus, this result does not exclude the presence of mutations in other exons, it is necessary to conduct next generation sequencing of WES. Designing primers were optimized and can use to confirm diagnosis in molecular genetic tools.