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Detection of the DPY19L2 gene deletion using nanopore sequencing

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Introduction: Globozoospermia is a rare (less than 0.1 %) severe disorder in male infertility [2]. Two types of globozoospermia have been described in the literature. Type 1 or total globozoospermia is diagnosed by the presence of 100 % of the spermatozoa with a small, round, and acrosome-free head. Type 2 or partial globozoospermia is classified if the quantity of round-headed spermatozoa in a semen sample is less than 100 % [1]. Since round-headed spermatozoa cannot penetrate the zona pellucida due to the lack of an acrosome, this type of disorder causes primary male infertility.
Methods and Algorithms: A married couple came to the International Center for Reproductive Medicine (St. Petersburg, Russia) with complaints of infertility. The man was diagnosed with total globozoospermia, the semen analysis revealed the spherical heads without an acrosome in 100 % of the sperm cells. The sperm was taken for DNA extraction and genome analysis using Oxford Nanopore long read sequencing technology. For DNA extraction was used The Monarch DNA Extraction Kit by New England Biolabs. This was followed by precipitation with ethanol in the presence of 0.3 M sodium acetate. High quality DNA samples were processed using Nanopore Ligation Sequencing Kit SQK-LSK110 to prepare the libraries, which were sequenced using the MinION R9.4.1 flowcells. Obtained FAST5 files were basecalled using Guppy (Oxford Nanopore Technologies) and mapped with Minimap2 to the GRCh38/hg38 genome assembly (Human Genome Reference Consortium). Aligned file was sorted by Samtools. Finally, we used the Integrative Genomics Viewer to visualize your data with masked secondary reads.
Results: The analysis revealed a homozygous deletion of a region of about 200 kb at 12q14.2 carrying the entire gene *DPY19L2* (Fig. 1). We confirmed the deletion by amplification with the following primers: FORWARD CTCCAAGATGTCTGCTGGC and REVERSE CACACTTTCCTAAAACATATGCTG, followed by Sanger sequencing.

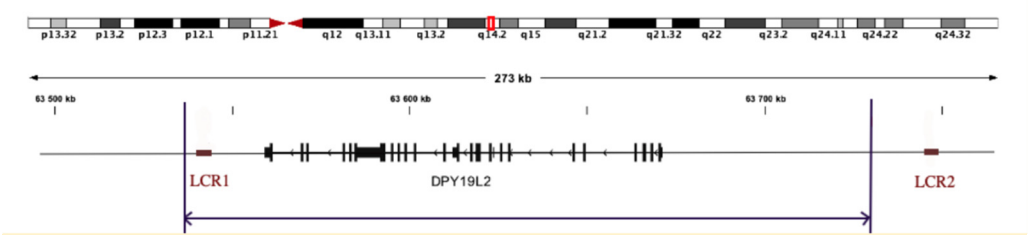

Fig. 1. Schematic representation of the analyzed region. Blue arrow represents the deletion region

Discussion: Globozoospermia has been associated with alterations in specific genes. To type I globozoospermia lead mutations in the *PICK1, DPY19L2*, and *SPATA16* genes. It has been shown that *DPY19L2* mutations can be detected in a vast majority of globozoospermic patients (66.7 %) [4]. The most common *DPY19L2* mutation is a 200 kb deletion between two very similar 28 kb low copy copy (LCR) flanking the gene. The mechanism underlying the deletion is most likely non-allelic homologous recombination (NAHR) between flanking LCRs [3]. In our case, the extreme points of the about 200-kbp deletion site are located close to the LCRs (Fig. 1).

Detecting a deletion of this size, given the presence of highly homologous sequences to the lost gene in the human genome, is difficult. The use of sequencing, which allows long reads, made it possible to do this reliably and quickly.

In this case, the homozygous form of the mutation arose as a result of closely related marriages and is explained by the region of residence of the patient. Consanguineous marriages increase homozygosity in offsprings leading to an increase in the risk of inherited disorders. This mutation was not observed in other relatives and was found *de novo*.

The identified gene *DPY19L2* deletion in this clinical case led to the proposal of an oocyte fertilisation method using calcium ionophore. Intracytoplasmic Sperm Injection (ICSI) has produced 4 developing embryos that have formed a blastocyst on day 5 of development and can develop into a healthy baby for this family. Genetic counselling based on the information we have identified will enable the family to avoid developing abnormalities in their offspring in the future.

Conclusion: Third-generation sequencing technologies give us the ability to quickly identify genomic variations that cause infertility in particular and develop a case management strategy. Since globozoospermia is considered to be a severe genetic disease, descendants of that family should be tested for deletion of this gene, which could prevent an increased risk of transmission of the disease to offspring, especially in case of consanguineous relationships. Accurate deletion mapping will allow us to develop a fast and reliable way to detect pathology.

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