STUDY OF SOMATIC AND GERMINAL MUTATIONS IN ARTERIOVENOUS MALFORMATIONS OF THE BRAIN USING WHOLE EXOME SEQUENCING

A. Bekbayeva, A. Gusmaulemova, B. Kurentay, E. Zholdybayeva

National Center for Biotechnology, Republic of Kazakhstan, 000010, Astana, Qorgalzhyn highway 13/5
e-mail: aika_bekbaeva@mail.ru

Rare vascular lesions known as brain arteriovenous malformations (bAVMs) are caused by shunts between cerebral arteries and veins that do not involve the capillary bed. The majority of bAVMs do not cause any symptoms, however some can be controlled by seizures and result in potentially fatal brain hemorrhages. Over the past ten years, significant advancements in our understanding of bAVM pathogenesis have been accomplished, primarily through the use of genome sequencing and biomolecular analysis. An autosomal dominant vascular disorder called Hereditary Hemorrhagic Telangiectasia (HHT, also known as Rendu-Osler-Weber Syndrome) affects about 1 in 5000 people globally and is associated with about 5% of brain AVMs, while the remaining 95% are due to sporadic mutations.

The major aim of the project is to investigate genetic causes of sporadic forms of AVMs in the brain by means of whole-exome sequencing of tissues with AVM lesions and blood samples. Analysis of somatic mutations will broaden the understanding of AVM pathogenesis.

This study was carried out in accordance with the Declaration of Helsinki's tenets and approved by the National Center of Biotechnology 's ethics committee (№9, 7 November 2023,) Astana). Based on clinical data, the age range of the patients at the onset of the disease also encompassed 25 to 40 years. To confirm the diagnosis, MRI and CAG data were used to validate the diagnosis. Each patient completed a specially designed questionnaire with medical information (such as age of debut and type of course) and demographic data (such as age, gender, and nationality). Three patients were classified as having a Spetzler-Martin grade II, while one patient was categorized as grade III. The sizes of arteriovenous malformations (AVMs) varied from 0 to 3 mm in one patient (grade II) to 3 to 6 mm in three patients (grades II-III). AVM tissue specimens were obtained during clinically necessary procedures. Brain AVM tissues were collected during surgery, which involved trepanation and excision of the AVM. Blood samples were also collected from these patients. Both DNA and RNA were isolated from these tissue and blood samples for subsequent exome sequencing an. Qubit 2.0 fluorometer was employed for more precise determination of DNA concentration, given that the nucleic acids will be utilized in future whole exome sequencing analysis. Whole exome sequencing was performed on six samples (blood and tissue) by Novaseq 6000 (Illumina).

The annotated results were analyzed for the presence of single-nucleotide polymorphisms (SNP) and insertion-deletion mutations (Indels) between DNAs from the AVM tissue sample and blood. The GATK workflow was used for the analysis and annotation of the raw data, and vcf files were generated. Then, the variants were filtered for a quality ≥ 30 in Excel 1st blood 117554, 1st tissue 114715; 2nd blood 112972, 2nd tissue 116022; 3rd blood 112859, 3rd tissue 117168 and genotype differences between tissue and blood DNA samples 1st blood 114512, 1st tissue 111682; 2nd blood 110614, 2nd tissue 111982; 3rd blood 110564, 3rd tissue 112805. After selecting only non-synonymous, frameshift insertions and deletions variants with different genotypes in the blood and tissue samples, 1st blood 279, 1st tissue 277; 2nd blood 279, 2nd tissue 271; 3rd blood 275, 3rd tissue 268 variations were observed. Analysis of whole-exome data continues.