Studying of epileptic encephalopathies using NimbleGen-based target panels

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A wide variety of non-specific and overlapping syndromes and non-syndromic epilepsy phenotypes dramatically complicates the clinical diagnosis and performing of genetic testing. A large number of genes are involved at epileptogenesis processes. The most cost-effective way is the study of gene targeted panels in the survey of disease for focused groups of patients. In this study, using high-throughput sequencing and NimbleGen target enrichment procedure, we studied genes that are associated with epileptic encephalopathy. Particular attention was given to patients of first years of life with epileptic encephalopathy and resistance to anticonvulsant drugs. The goals of the study were: (1) search for new mutations, associated with epileptic encephalopathy; (2) evaluation of the distribution patterns for found mutations among genes; (3) assessment of the applicability of the NimbleGen targeted enrichment for design of small gene panels comprising genes which are located on different chromosomes.

Material and methods: a total of 90 patients were tested, including control group. Genomic DNA isolation was performed using the protocol selection on magnetic particles (MagNA Pure LC 2.0 station). A qualitative and quantitative assessment of the DNA was performed using NanoDrop2000 and Promega QuntiFluor-ST, respectively. The selection of protein-coding regions were performed using a set of oligonucleotide probes NimbleGen (SeqCap EZ), for sequencing we used Roche 454 GS Junior. All stages of the sample preparation and sequencing procedure were performed according to the standard protocols. NimbleGen library design was performed so to keep the total size of the protein-coding regions for selected genes inside 100 kbp. In total we selected 34 genes (associated with epileptic encephalopathies). During every run we sequenced up to 12 samples.

Among of the all analyzed genes the most number of variants have been found for *SCN1A* gene - 10 (15 persons), including 3 nonsense-mutations; most of them are undescribed at the literature and databases. In other genes the following missense-mutations

have been found (here are presented missense-mutations with the minor allele frequency less than 0,5%): *SCN1B* - 1 (3 patients), *SCN2A* - 2 (2 patients), *SCN9A* - 1, *NRXN1* - 4 (8 patients), *ZEB2* - 1 (2 patients), *TREX* - 1, *CNTNAP2* - 2 (3 patients), *DLGAP* - 5 (6 patients), *SPTAN1* - 2 (2 patients), *GRIN2A* - 4 (4 patients), *GRIN2B* - 1, *RNASEH2A* - 1, *RNASEH2B* - 2 (3 patients), *CDKL5* - 1, *PCDH19* - 1, *UBE3A* - 1 (2 patients). For two patients we found 70 bp deletions in the *SCN1A* gene; for one patient we found an inversion about 300 bp in the *NRXN1* gene.

The every sequencing run produced 45,5-115,4 Mbp (81,6 Mbp on the average); for each sample we had 5795 to 46270 reads (16562 reads per sample on the average). The designed gene panel covers 34 genes; the gene distribution is following: one gene is located on every chromosomes 1, 3-8, 12-15, 18, 20; two genes are located on every chromosomes 9, 11, 16; three genes are located on chromosome 19, six genes are located on every chromosomes 2 and X. During every runs we studied up to 12 samples; also we vary a number of experimental parameters. A number of reads, falling on chromosomes was different (are listed only four mostly covered chromosomes): 28.5-49.3% on chromosome 2, 7.6-15.7% on chromosome 9, 5.9-12% on chromosome X, 3-7.6% on chromosome 16.

Conclusions: (1) analysis of the small-sized sets of genes could be rather informative and cost-effective, especially in case of studying the focused groups of patients even in case of such multifactorial disease as epilepsy; (2) the features of chromosomal coverages should be taken into account under design of small NimbleGen target panels.

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