This study was supported by Ministry of Health, Czech Republic in NGS).

**INTRODUCTION**

Although array comparative genomic hybridization (aCGH) is a powerful tool for detecting relative small genomic in patients with intellectual disability (ID), autism spectrum disorders and multiple congenital anomalies (MCA), next-generation sequencing (NGS) techniques have become a powerful tool for the identification of pathogenic sequence variants. Here, we present our first experience with targeted NGS as a novel step in the diagnostic algorithm for patients with ID/MCA. In our pilot NGS project, we investigated 16 patients with using a commercially available 2742-gene panel. We illustrate the impact of targeted NGS approach on a case of 9-year-old boy with severe ID/MCA related to early-onset myoclonic encephalopathy.

**PATIENTS AND METHODS**

DNA from peripheral blood cells of 395 children with severe ID/DD/MCA, autism spectrum disorders

1) Array-CGH: SureTag Labelling Kit: Cy3/Cy5 + SurePrint 4X180K CGH Microarray (Agilent Technologies) detection filters: 5 probes, 100kb, log, ratio 0.25 (Agilent Genomic Workbench + Cytogenomics)

2) FISH: FISH probe RP11-B466E20 (Texas Red) (EmpireGenomics)

3) Relative qPCR: Power SYBR®Green (Thermofisher + custom DNA primers (IDT))

4) NGS: SureSelect Inherited Disease design (Agilent Tech.). MiSeq benchtop sequencer (Illumina)

In the course of our ten-year experience with array-CGH analysis (2007-2016) using 4X44K and 4X180K CGH & CGH+SNP microarrays we investigated 395 children’s patients with ID/DD/MCA. We detected 76 pathogenic/likely pathogenic CNVs which result in the diagnostic yield of 19.2% (76/395) (Fig. 1). In 2015/2016 in our pilot NGS project, we investigated 16 patients with severe ID/DD/MCA using a commercially available 2742-gene panel SureSelect Inherited Disease (Agilent Technologies) and detected pathogenic or likely pathogenic sequence variants in 31.25% patients (5/16) (Fig. 2).

Here we report on a 9-year-old boy (ID 2486/15) with severe ID/DD related to early-onset myoclonic encephalopathy. He was examined a normal male karyotype 46,XY. Using 180K CGH array we detected 18q21.32 539-kb copy-number gain classified as likely benign. We estimated its de novo origin by relative qPCR and R-values calculation. FISH analysis using a custom probe excluded the dispersed 18q21.32 duplication. Targeted NGS analysis identified pathogenic SCN2A gene variant g.168166923C>T (p.Ala263Val). De novo origin was confirmed by Sanger sequencing analysis of parental DNA samples.

**RESULTS**

In the course of our ten-year experience with array-CGH analysis (2007-2016) using 4X44K and 4X180K CGH & CGH+SNP microarrays we investigated 395 children’s patients with ID/DD/MCA. We detected 76 pathogenic/likely pathogenic CNVs which result in the diagnostic yield of 19.2% (76/395) (Fig. 1).

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Here we report on a 9-year-old boy (ID 2486/15) with severe ID/DD related to early-onset myoclonic encephalopathy. He was examined a normal male karyotype 46,XY. Using 180K CGH array we detected 18q21.32 539-kb copy-number gain classified as likely benign. We estimated its de novo origin by relative qPCR and R-values calculation. FISH analysis using a custom probe excluded the dispersed 18q21.32 duplication. Targeted NGS analysis identified pathogenic SCN2A gene variant g.168166923C>T (p.Ala263Val). De novo origin was confirmed by Sanger sequencing analysis of parental DNA samples.

**Conclusions**

In our pilot study and presented case, we show our first experience with NGS as a novel step in molecular diagnostic algorithm. We confirm the effectivity of combination of array-CGH and targeted NGS as robust and sensitive genomic techniques with a diagnostic yield of 19.2% (array-CGH) and 31.25% (targeted NGS). Based on our experience and worldwide studies, our novel diagnostic algorithm including targeted gene-panel NGS could lead to higher diagnostic yield in patients with heterogeneous genetic conditions.

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We declare no conflicts of interest.

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