The clinical utility of array-CGH and targeted NGS in idiopathic intellectual disabilities and developmental delays: a case report of *SCN2A* p.Ala263Val

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INTRODUCTION

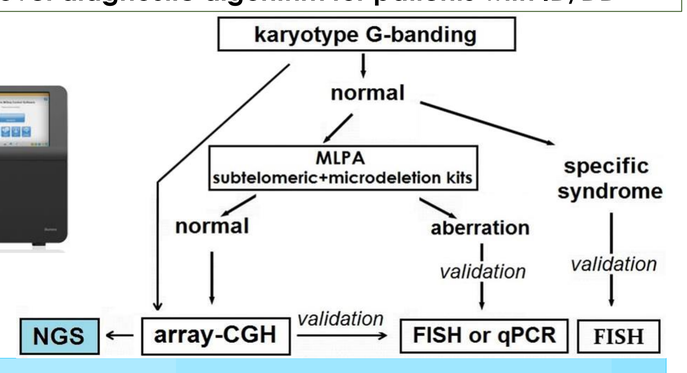
Although array comparative genomic hybridization (aCGH) is a powerful tool for detecting relative small genomic in patients with intellectual disability (ID), autism spectrum disorders and multiple congenital anomalies (MCA), next-generation sequencing (NGS) techniques have become a powerful tool for 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, $\log_2$ ratio 0.25 (Agilent Genomic Workbench + Cytogenomics)
2) **FISH**: **FISH probe RP11-866E20 (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)

Fig. 1: Novel diagnostic algorithm for patients with ID/DD



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 results 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.166166923C>T (p.Ala263Val). *De novo* origin was confirmed by Sanger sequencing analysis of parental DNA samples.

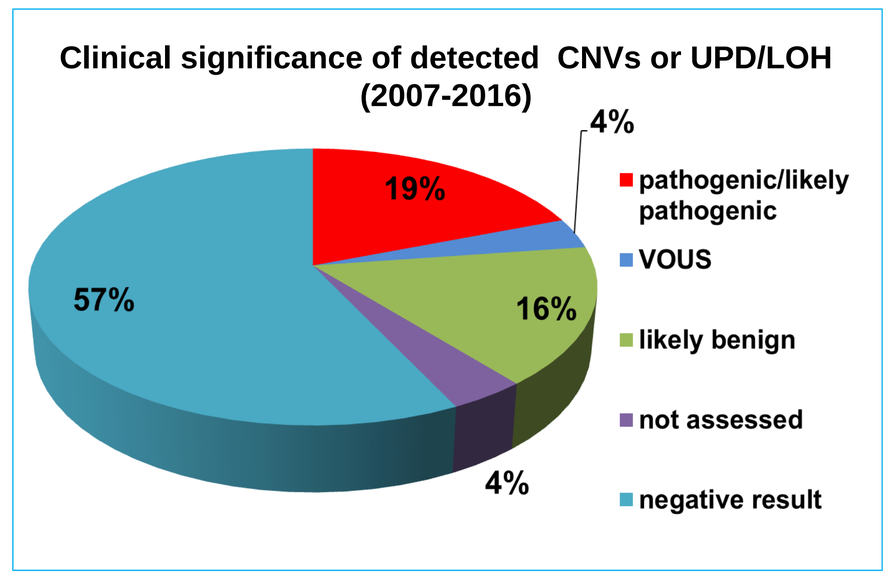


Fig. 1: Clinical significance of detected CNVs or UPD/LOH between the years 2007-2016.

patient ID	gender year of birth	gene	variant (protein level)	phenotype
1109/13	Male, *2004	***MED13L***	**p.Gln473fs**	Moderate ID, absence of speech, hearing impairment, facial stigmatization, syndactyly
935/15	female, *2013	***ASXL3***	**p.Arg1004fs**	Severe ID, growth retardation, facial stigmatization
1173/16	Female, *2016	***TSEN54***	**p.Arg54Gly**	Microbrachycephaly, severe multiple dysplasia of CNS, muscle hypertonia
			p.Ala307Ser	
1176/16	Female, *2014	***SCN2A***	**p.Met1545Val**	Severe ID, early-onset myoclonic encephalopathy, axial hypotonia, limb spasticity, facial stigmatization
2486/15	Male, *2007	***SCN2A***	**p.Ala263Val**	Severe ID, early-onset myoclonic encephalopathy

Fig. 2: The overview of pathogenic/likely pathogenic DNA sequence variants detected in 31.25% children´s patients (5/16) enrolled in our pilot project using targeted NGS.

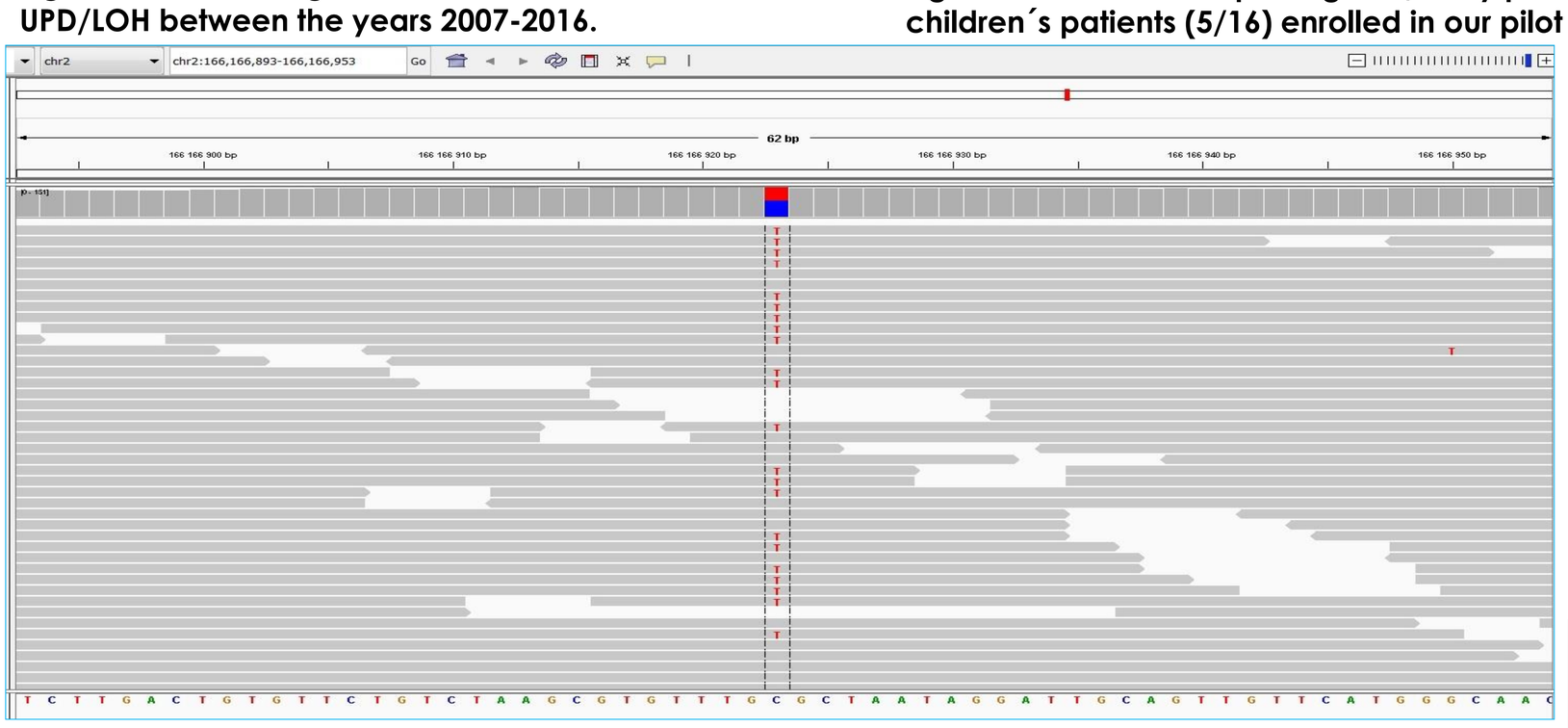


Fig. 3: *SCN2A* gene and heterozygous substitution g.166166923C>T (c.788C>T) leading to p.Ala263Val. The substitution was detected in 45% reads (55/145). *SCN2A* gene encodes one of sodium-channel alpha subunits expressed in CNS. In the literature, p.Ala263Val in *SCN2A* gene is described as a pathogenic variant in children with early-infantile epileptic encephalopathy 11/Ohtahara syndrome.

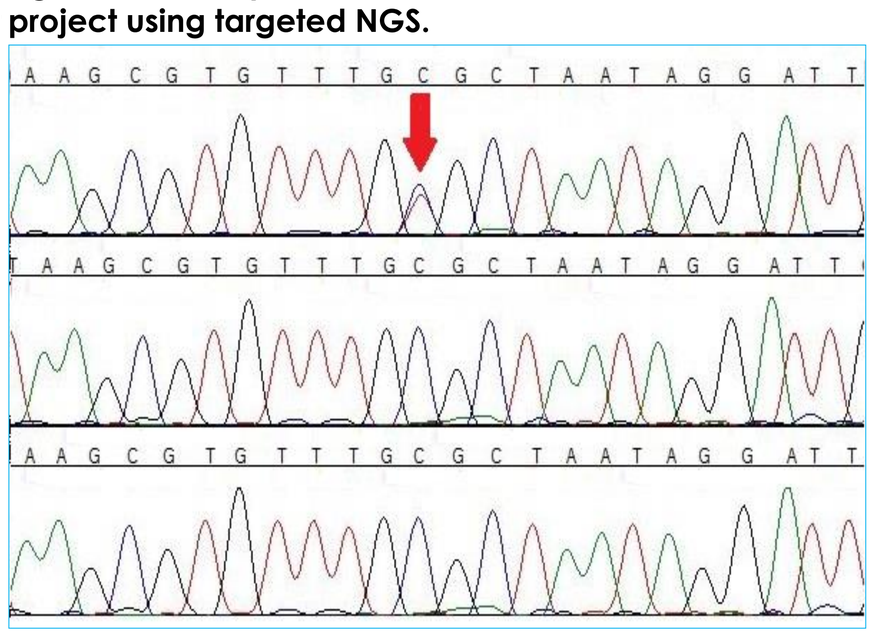


Fig. 4: The *SCN2A* gene variant g.166166923C>T (p.Ala263Val) validation and origin analysis by targeted Sanger sequencing.

We identified *de novo* origin and heterozygosity of p.Ala263Val variant in our patient.

Chromatograms: patient – father - mother

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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