Summary

Intellectual disability (ID)/developmental delay (DD) and epilepsy are common neurodevelopmental disorders in children. The diverse mechanisms that underlie these disorders and the variability of clinical phenotypes can pose a significant challenge in uncovering their etiologies. Recent studies have shown that copy-number variants (CNVs), especially submicroscopic genomic imbalances, play an important role in the causation of neurodevelopmental disorders. Application of microarray-based technologies such as array comparative genomic hybridization (aCGH), enables molecular karyotyping and identification of submicroscopic CNVs. The ability to analyze the entire genome in a single experiment with a resolution previously unachievable by classical cytogenetic methods, has revolutionized the diagnosis of neurodevelopmental disorders. Currently, aCGH is a widely used method in genetic diagnostics of ID/DD, as well as in other neurodevelopmental disorders, including epilepsy. Because of the diagnostic and research potential of aCGH, we decided to use this method in further studies for unraveling the genetic etiology and consequently the pathomechanism(s) of neurodevelopmental disorders. The aim of our study was to identify and determine the role of submicroscopic CNVs in patients with ID/DD and various types of epilepsies using aCGH.

We have applied aCGH in 256 patients with ID/DD with or without dysmorphic features and/or congenital malformations. Moreover, we have studied 103 patients with epilepsy, 51 individuals with genetic/idiopathic generalized epilepsy (GGE/IGE) and 52 subjects with epilepsy accompanied by additional neurodevelopmental abnormalities (autism spectrum disorder or ID with/without dysmorphic features). aCGH was performed using a custom-designed whole-genome oligonucleotide microarray (V7.4 or V8.0/V8.1, Agilent Technologies) designed in the Department of Medical Genetics at the Institute of Mother and Child in a collaboration with the Medical Genetics Laboratories in the Department of Molecular & Human Genetics at Baylor College of Medicine (BCM), Houston, Texas. Array V7.4 consisted of approximately 105,000 oligonucleotides with an average resolution of 30 bp, while V8.0/V8.1 arrays had 180,000 oligonucleotides with additional exonic coverage for over 1,700 selected genes. Among the selected genes with exonic coverage were those known or candidate
in the etiology of ID/DD, epilepsy (361 genes), autism spectrum disorders (221 genes), and congenital heart disease (273 genes).

In a cohort of patients with ID/DD, we identified a total of 84 non-polymorphic CNVs in 69 subjects (26.9%). We found 18 pathogenic chromosomal aberrations greater than 5 Mb in size unrecognized by conventional karyotype analysis and 66 submicroscopic CNVs of various clinical significance. Among the submicroscopic changes, there were 23 pathogenic, 14 potentially pathogenic, and 29 CNVs of unknown clinical significance. In total, 41 pathogenic changes (23 submicroscopic CNVs and 18 large chromosomal aberrations) were identified. The detection rate of the pathogenic genome imbalances in our cohort of patients with ID/DD was 16%. Our studies demonstrate the higher efficiency of aCGH in the detection of genomic causes for ID/DD as compared to the standard karyotype analyses that detect chromosomal aberrations in ~ 4% of patients.

In a group of 51 patients with idiopathic/genetic generalized epilepsy, chromosomal microarray analysis revealed submicroscopic CNVs in 11 individuals, including three pathogenic CNVs and eight variants of unknown clinical significance. In the group of 52 patients with epilepsy accompanied by additional neurodevelopmental abnormalities, CNVs were found in 12 patients. Among 14 identified CNVs, nine were pathogenic and five were of unknown clinical significance. The detection rates of the pathogenic CNVs in both groups were 5.8% and 17.3%, respectively.

The high efficiency of aCGH in identification of genomic imbalances demonstrates its usefulness for detection of genetic causes of neurodevelopmental disorders. Our results further confirm the important role of CNVs in the etiology of ID/DD, enabling diagnosis in 14.8% of patients. Genome-wide molecular analyses using aCGH also demonstrated significance of CNVs in the etiology of epilepsy, especially when accompanied by additional neurodevelopmental abnormalities. In addition, in the selected patients with the identified chromosomal aberrations, we have attempted to identify genes responsible for those neurodevelopmental disorders and to correlate genotypes with phenotypes. In one patient with juvenile myoclonic epilepsy and moderate ID, we have detected an ~ 1.6 Mb deletion in the distal chromosomal region 7q11.23. Out of several genes mapping to this genomic interval, two potentially dosage-sensitive genes HIP1 (OMIM 601767) and YWHAG (OMIM 605356) expressed in the central nervous system are of particular interest.
We hypothesize that haploinsufficiency of those genes contribute to the described clinical features. Moreover, identification and characterization of other patients (in a collaboration with BCM) with the similar clinical diagnoses and the same recurrent deletion of the above-mentioned region has enabled description of the new distal microdeletion 7q11.23 syndrome (OMIM 613729).

Our studies further demonstrate the usefulness of array CGH as a first-tier test in the clinical diagnosis of patients with intellectual disability/developmental delay and epilepsy of unknown etiology. Furthermore, our results have allowed proposing a diagnostic algorithm in patients with these neurodevelopmental disorders.